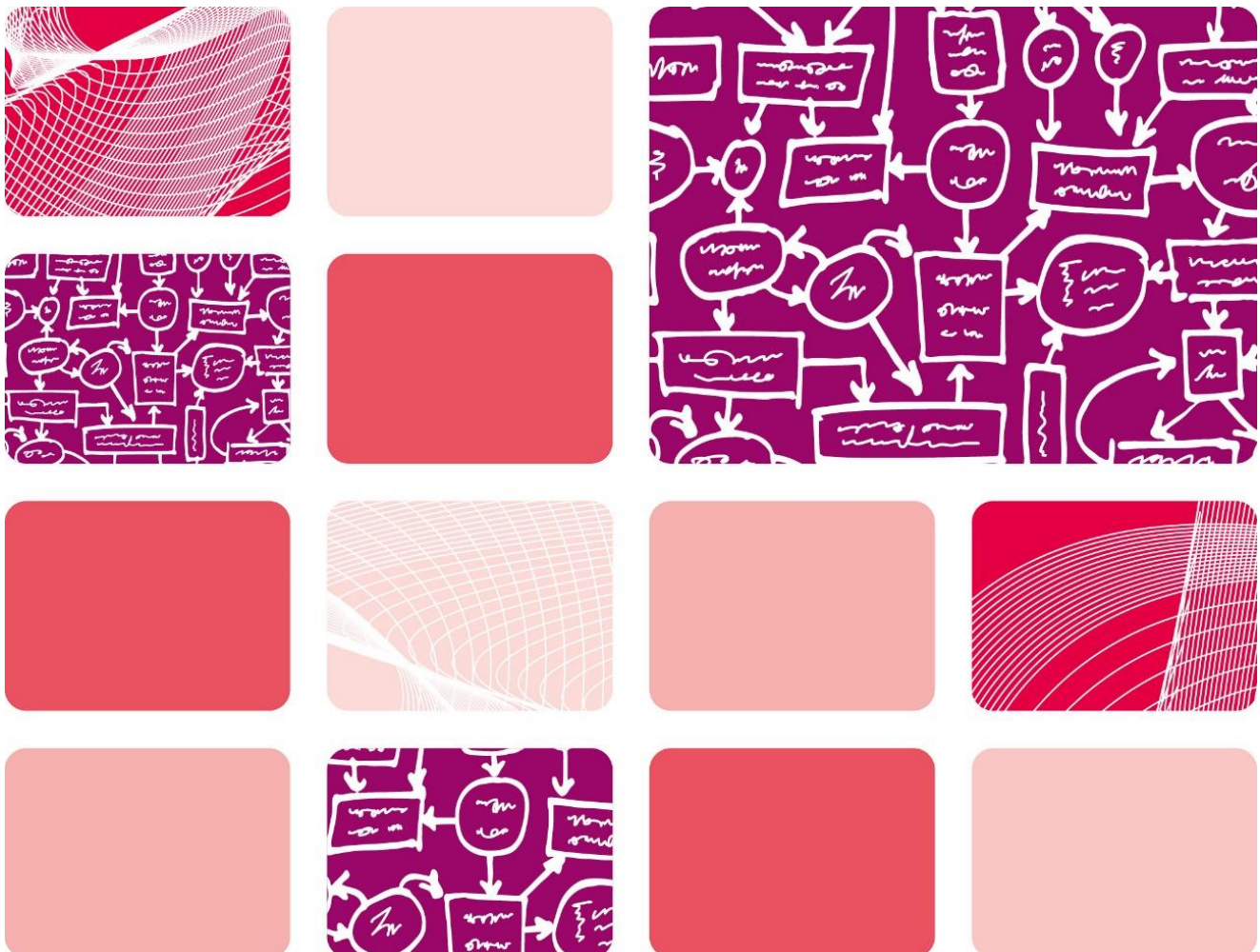




UK Health
Security
Agency

UK Standards for Microbiology Investigations

Gastroenteritis



Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on [the UK SMI website](#). UK SMIs are developed, reviewed and revised by various working groups which are overseen by a [steering committee](#).

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Pathology: the science behind the cure

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Amendment table

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

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	5/21.01.26
Issue number discarded	2.2
Insert issue number	2.3
Section(s) involved	Amendment
Whole document.	<p>Administrative changes include minor spacing edits.</p> <p>Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms</p> <p>Partner organisation logos updated.</p> <p>Broken links to devolved administrations replaced.</p> <p>References to NICE accreditation removed.</p> <p>'Public health responsibilities of diagnostic laboratories' section added.</p>
Antimicrobial susceptibility testing	Removal of erroneous mention of EUCAST topical antibiotics breakpoints.

Amendment number/date	4/08.10.24
Issue number discarded	2.1
Insert issue number	2.2
Section(s) involved	Amendment
Algorithm	Duplicated algorithm 5.3 Gastroenteritis in hospital (in-patients) setting has been replaced with 5.2 Gastroenteritis in community setting.

Amendment number/date	3/29.07.24
Issue number discarded	2
Insert issue number	2.1
Section(s) involved	Amendment
Whole document	Recommendations for the primary isolation of non-sorbitol fermenting non-O157 serogroups O26 updated to include chromogenic selective agar in line with UK SMI ID 22: <i>Identification of Shiga toxin-producing Escherichia coli (STEC) using conventional methods</i> . Document has been transferred to new template.
Flowcharts and tables	Updated to include STEC non-O157 serogroups
References	Addition of two scientific papers to support the recommendation to use chromogenic selective agar for STEC non-O157 serogroups.
Hyperlinks	Updated.

Amendment number/date	2/19.10.2020
Issue number discarded	1
Insert issue number	2
Anticipated next review date*	19.10.2023
Section(s) involved	Amendment
Whole Document	This new syndromic document supersedes the previous UK SMI S 7 document. The content and layout has been changed to widen the scope of the document and include the UK SMI B 30.

*Reviews can be extended up to 5 years where appropriate

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 (1). 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. 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.

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 (2).

This document does not cover *Helicobacter pylori* infection, hepatitis viruses, non-protozoal parasitic presentations as well as infections not transmitted through the enteric route. Testing algorithm for the investigation of *Clostridioides difficile* is also not covered. Please refer to the [UK SMI B 10: Investigation of faecal specimens for *Clostridioides difficile*](#) for more detail.

Refer to UKHSA, 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 intestines, characterised by diarrhoea and/or vomiting (2) or it can be described as a transient disorder characterised by the sudden onset of diarrhoea with or without vomiting (1). This enteric infection can be caused by viruses, bacteria, or parasites (1). 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 (typically classed as 5 to 7 on the Bristol Stool Form Scale (3) occurring more frequently than is normal for the individual (4). 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.

Note: Frequently passed formed stools are not considered to be diarrhoea.

A wide range of bacterial pathogens, viruses and parasites are capable of causing diarrhoea by a number of mechanisms such as but not limited to:

- multiplication of pathogens in the gut
- ingestion of pre-formed toxins in food, produced by bacteria
- toxin production in the large intestine by toxin producing bacteria

4.3 Other infections transmitted through the enteric route

There are several food or water pathogens that do not necessarily give rise to symptoms of gastroenteritis such as botulism, *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 (2).

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.

Many organisms that cause gastroenteritis are notifiable causative agents under the Health Protection (Notification) Regulations 2010.

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

5 Clinical presentations of gastrointestinal infections

Gastrointestinal infections present as sporadic and outbreak cases occurring in community or hospital settings (4,5).

Sporadic cases: also known as 'non-outbreak' cases. They are cases that occur at irregular intervals, in scattered or isolated instances.

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 2 or more cases of a similar illness resulting from the ingestion of a common food ingredient or cross contamination.

Water borne outbreaks are defined as 2 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 the outbreak response lead, or infection control team (hospital) or the public health team (community), and the microbiologist to agree appropriate tests based on the clinical and epidemiological information available.

Acute watery diarrhoea (6,7): 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.

Organisms implicated include norovirus, rotavirus, adenovirus, *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).

Acute bloody diarrhoea: This is a sudden onset of diarrhoea where frank blood is present. Dysentery is an acute infectious gastroenteritis characterized by loose stools with blood and mucus (8), often accompanied by pyrexia and abdominal cramps.

Organisms implicated in acute bloody diarrhoea include *Campylobacter* species, *Shigella* species, Shiga toxin producing *Escherichia coli* (STEC) including serogroup O157, *Salmonella* species and *Entamoeba histolytica*.

Patients with bloody diarrhoea and a suspected ulcerative colitis would benefit from rapid PCR testing. In patients who have travelled, consider PCR for *E. histolytica* as microscopy has poor sensitivity and cannot be used to distinguish *E. dispar* (non-pathogenic) from *E. histolytica* (pathogenic) (9,10).

Persistent and chronic diarrhoea: persistent diarrhoea is defined as diarrhoea of >14 days but fewer than 30 days in duration (11,12). 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. Diarrhoea that lasts >30 days is referred to as “chronic” (13). Chronic diarrhoea is a major clinical feature and a leading cause of morbidity and mortality in uncontrolled HIV infection. Organisms implicated are predominantly parasites – *Giardia* species, *Cryptosporidium* species, *Cyclospora cayetanensis* and *Microsporidia* species.

Chlamydia trachomatis infection including lymphogranuloma venereum (LGV) and *Neisseria gonorrhoeae* infection can present atypically as sexually transmitted infectious colitis in individuals who have unprotected anorectal sex (14).

Vomiting: this 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. Organisms implicated include *Bacillus cereus*, norovirus, *Vibrio* species, *Staphylococcus aureus* and *Clostridium perfringens*.

5.1 Testing pathway for community and hospital settings

The following general information is provided to guide users to navigate the algorithms in sections 5.2. and 5.3

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* species, STEC (including O157) and norovirus. This UK SMI recommends inclusion of *Giardia* species and *Cryptosporidium* species in the primary test set.

Testing for Rotavirus should be undertaken depending on local policy and clinical presentation. Testing for additional target organisms 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, 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.

Public health surveillance systems have identified an increase in the number of reported infections of STEC O26:H11 involving bloody diarrhoea, linked to the consumption of prepacked sandwiches (15).

Traditional culture media used for the detection of STEC O157 is not selective for STEC serotypes other than O157. Chromogenic selective agar, can detect non O157 serogroups O26, O45, O103, O111, O121 and O145 (16).

When STEC is suspected as the aetiological agent faecal specimens should be referred following the applicable National Reference Laboratory guidelines (5,17).

5.1.1 Gastroenteritis in community setting:

This algorithm recognises that testing for viral causes of gastrointestinal infections is not normally undertaken except 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 with additional testing added as described in the algorithm (for instance, if overseas travel has occurred).

C. difficile is an important cause of community acquired diarrhoea.

All symptomatic patients should be tested for *Cryptosporidium* species and *Giardia* species irrespective of whether the stool specimens presented take the shape of the container or not (18,19).

In the case of persistent diarrhoea, *Giardia* species and *Cryptosporidium* species should always be included in primary testing whilst other parasites

including amoebae (*Entamoeba histolytica*) and *Cyclospora cayetanensis* should be considered as secondary testing.

b) Outbreak cases

Acute diarrhoea with or without vomiting:

This usually occurs at >24 hours incubation period. In addition to primary testing, additional tests should be undertaken dependent on clinical presentation. Referral of stool samples to the local public health laboratories is necessary if testing is not available in house, and if requested by Public Health for additional in-depth typing as part of outbreak investigations. In addition to primary testing, *S. aureus*, *Bacillus* species, *C. perfringens* and norovirus testing should be performed.

Acute vomiting with or without diarrhoea:

This usually occurs at <24 hours incubation period. 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 (20-22). Laboratories may opt to test only during the seasonal increase. Testing for *C. perfringens* should be considered after discussion with the laboratory.

5.1.2 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), adenovirus, sapovirus and astrovirus as they may also present with gastrointestinal symptoms.

Sporadic cases (> 72 hours)

- Gastrointestinal symptoms presenting >3 days after admission are rarely caused by *Salmonella*, *Shigella*, *Campylobacter* and STEC (including O157). The UK SMI endorses the '3-day' rule which does not recommend routine testing for these pathogens (23). However, laboratories considering applying the "3-day rule" should perform analysis of their submission and positivity data and undertake a risk assessment. Clusters of diarrhoea cases must be investigated.

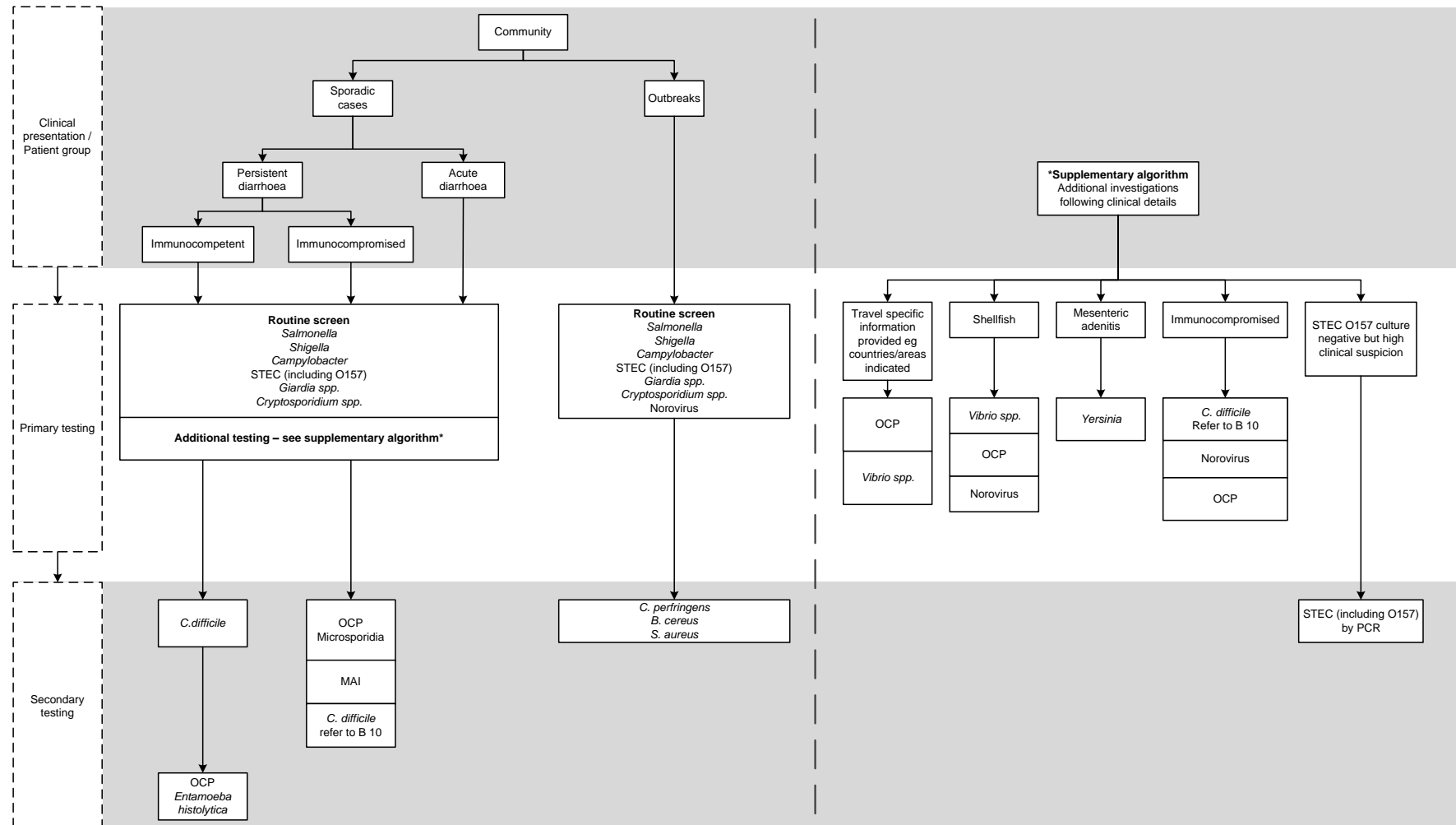
The “3-day rule” suggests that faecal samples from patients should undergo full screen for all important gastrointestinal pathogens in the following circumstances (23,24):

- Inpatients developing diarrhoea within 3 days of hospital 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 immunocompromised
 - Patients with neutropenia
 - Suspected nosocomial outbreak (eg norovirus, *Salmonella*)
 - Those with suspected non-diarrhoeal manifestations of enteric infections.
- All samples for patients over 2 years of age should be tested for *C. difficile* (25). For patients less than 2 years of age, testing for *C. difficile* is not generally advised.
- Routine testing for norovirus is recommended (22). 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.

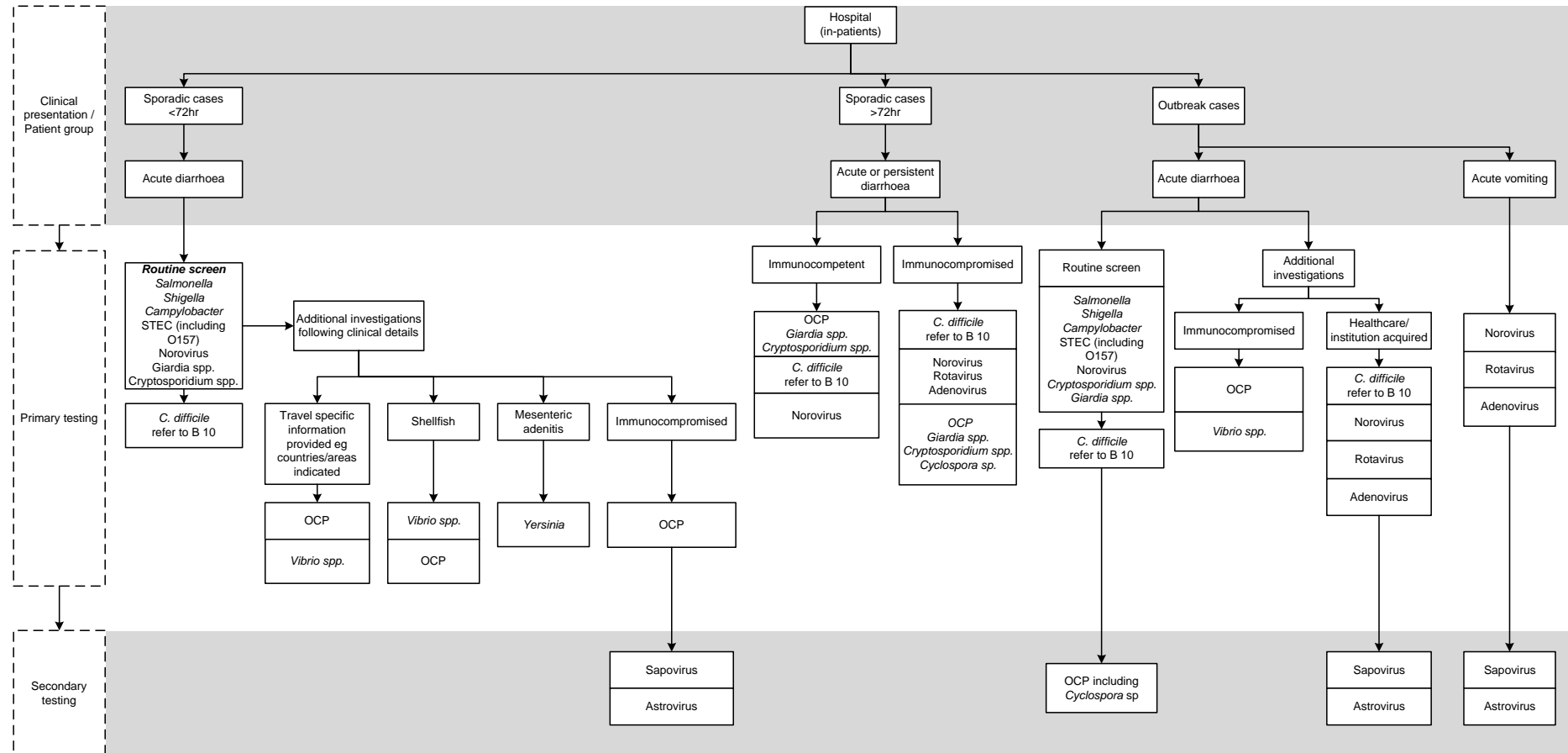
b) Outbreak cases (< 48 hours)

Testing should include routine examination with *C. difficile*, norovirus and other viruses depending on clinical features.

5.2 Algorithm: Gastroenteritis in community setting

MAI – *Mycobacterium avium-intracellulare* complexOCP - Ova, cysts and parasites including *Cyclospora cayetanensis*

5.3 Algorithm: Gastroenteritis in hospital (in-patients) setting



MAI – *Mycobacterium avium-intracellulare* complex

OCP - Ova, cysts and parasites including *Cyclospora cayetanensis*

6 Pre-laboratory processes (pre-analytical stage)

6.1 Specimen type

The types of specimens include faeces and 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 (22). Rectal swab may be used where it is not possible to collect a faecal specimen (26).

Blood cultures are recommended if the patient presents with features of sepsis or is systemically unwell, such as clinical suspicion of enteric fever (27,28). 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 (29).

Faecal 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 *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 (30). 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 (7,31).

If faeces are liquid or soft, approximately 5 mL should be collected, and if formed, 1 to 2g is adequate for culture (29).

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 a public health situation requires sampling to be carried out. For example:
 - 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 Specimen transport and storage

Faeces should be submitted in a suitable and appropriate transport container.

Specimens should be transported and processed as soon as possible (29).

Important pathogens such as *Shigella* species may not survive the pH changes that occur in faecal specimens if not promptly delivered to the laboratory, even if refrigerated (32).

If processing is delayed, refrigeration is preferable to storage at ambient temperature (29).

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.

6.4 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 (33):

- 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 and dates
- Recreational/untreated 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

6.5 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 (34). 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 (34).

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 (35). Typhoid vaccine is indicated for active immunisation against typhoid fever and is recommended for laboratory personnel who may handle *S. Typhi* in the course of their work (28,36).

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 (37). Use of a class 1 microbiological safety cabinet should be considered, if possible, subject to local risk assessment.

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 stage)

The testing pathway incorporates a range of tests including:

7.1 Microscopy

For parasites, routine testing for *Cryptosporidium* species and *Giardia* species is recommended nationally. Microscopy is of low sensitivity when compared to NAAT (including PCR) and EIA testing, and evidence suggests a doubling of detection rates

using EIA (38,39). 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.

Many laboratories utilise molecular techniques for the detection of gastrointestinal pathogens for primary testing. However, pathogens outside of the molecular panels in use locally should be detected using the microscopy techniques described here.

Ensure a request for OCP is submitted if parasitology testing is required.

7.1.1 Sample preparation

For generic safety considerations, refer to the link in Section 2 of this 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 (18,19), 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 except specific screens, for example *Salmonella* species screens on known positives (refer to [UK SMI TP 39 - Staining techniques](#)) (18,19).

Supplementary

For information on wet preparations for microscopy for ova, cysts and parasites and faecal concentrations of parasites, refer to [UK SMI 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 the parts of faecal samples containing blood, pus, or mucus for direct examination as wet preparations or for staining.

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

For microscopic examination of protozoa please refer to [UK SMI B 31: investigation of specimens other than blood for parasites](#).

7.1.3 Technical limitations

Concentration methods

Faecal specimens should be submitted fresh, without formalin, and a concentration 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 microscopic expertise that is required. Consideration should be given towards implementing more sensitive techniques (e.g. NAAT/PCR testing, EIA testing).

7.1.4 Identification

Minimum level

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

7.2 Reporting other tests including molecular testing

7.2.1 Nucleic Acid Amplification Tests (NAAT including PCR)

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 (42-44). 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 testing (45,46).

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 parasites (47-49). Generally, PCR-based assays offer greater sensitivity and specificity than EIAs and culture-based techniques. They are highly accurate for viruses, *Salmonella* species, *Shigella* species, *Campylobacter* species, 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*) (37,50). It should be noted that for *Salmonella* species there have been reports of enrichment culture techniques providing superior sensitivity to NAATs (51); the joint Public Health England and Chartered Institute of Environmental Health operational guidelines on Typhoid and Paratyphoid (enteric fever) (28) still endorse culture methods for contact tracing and clearance samples. This should be considered during the validation process of any assay.

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

Note:

1. Laboratories should note that molecular methods do not provide information on antimicrobial susceptibility used to guide clinical management (27).
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 NAAT/PCR test for that organism rather than an enteric PCR panel.
5. If there is a strong clinical suspicion of a pathogen targeted by the GI multiplex PCR screening but the assay is negative consider the need for additional testing such as, but not necessarily limited to, the following:
 - Enrichment culture based methods for *Salmonella* species (eg in vulnerable patient groups, where enteric fever is suspected, or for *Salmonella Typhi/Paratyphi* clearance)
 - Enrichment culture with PCR for STEC

7.2.1 Sample preparation

Molecular Tests

For safety considerations, refer to the [scientific information](#).

Follow manufacturers' instructions on how to prepare and process the clinical specimens using the manufacturers' test kit inserts.

7.2.2 Technical limitations

Rapid diagnostic test kits/systems

EIA and NAAT (including 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. The clinical significance of results obtained by commercial kits and molecular platforms should be considered 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 in the misidentification of *Shigella* species as *E. coli* by MALDI-TOF MS (52-54).

Molecular Assays

Laboratories should be aware of the limitations of molecular assays, and of emerging strains. Surveillance should be conducted for emerging strains by the assay manufacturer, or locally.

Preservation of specimens

Preservation of faeces in 10% formalin is no longer recommended by the UK SMIs. 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 (30).

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) may be lost during the procedure. Most EIAs require the use of fresh or frozen stool specimens (30). 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.

7.2.3 Investigation

Follow manufacturer's 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 verified prior to use.

If using in-house tests/kits, laboratories should ensure that these have been validated prior to routine use.

7.2.4 Actions for NAAT (including PCR) positive specimens from sporadic cases

Pathogen	Local confirmation (e.g. by culture)	Referral to reference laboratory
Parasites		
<i>Giardia</i> species	No	No, unless giardiasis is suspected and microscopy is negative.
<i>Cryptosporidium</i> species	No	Refer for speciation and typing following the local protocols in Scotland, England and Wales.
<i>Entamoeba histolytica</i>	No	Required for speciation if molecular speciation assays are not available locally.

Pathogen	Local confirmation (e.g. by culture)	Referral to reference laboratory
<i>Cyclospora cayetanensis</i>	No	No. Only refer in suspected outbreaks if advised by reference laboratory. Positive specimens are recommended to be stored for 3 weeks
Bacteria		
<i>Salmonella</i> species	Culture	Send isolate for confirmation and typing
<i>Campylobacter</i> species	Culture if treatment is indicated	No
STEC O157	Culture for O157	Send O157 isolate for confirmation and typing
Non-O157 STEC	Culture using selective chromogenic agar	Send non-O157 STEC isolate for confirmation and typing
<i>Shigella</i> species	Culture	Send isolate for speciation (if not performed locally) and typing
<i>Vibrio</i> species	Culture	Refer for typing
<i>Plesiomonas</i> species	Optional	Optional
<i>Aeromonas</i> species	Optional	Optional
<i>Yersinia</i> species	Culture	Refer for typing

For information on clearance and screening of contacts refer to current guidance (55).

7.3 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.

7.3.1 Primary culture

Many laboratories utilise molecular techniques for the detection of gastrointestinal pathogens for primary testing. However, pathogens outside of the molecular panels in use locally should be detected using the culture techniques described here.

For culture and isolation, refer to the table in section 7.3.4 in this document.

Culture is important for typing in cases of increased incidence, in outbreak detection and investigation, 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.

7.3.2 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) (56). 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 (57)
- 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 ([UK SMI 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 (57). 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

7.3.3 Technical limitations

Sample Dilution

Sample dilution prior to inoculation may be useful; a study by Nye KJ et al (56) 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 of culture media. A 1:4 dilution was shown to significantly reduce the amount of competing flora without compromising isolation of low numbers of pathogens (56); fewer subcultures for *Campylobacter* species may therefore be required when using a dilute inoculum, reducing labour costs (56).

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 (58). Recovery of organisms such as *Arcobacter* species and *Helicobacter cinaedi* may however be compromised.

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

- organisms may be sensitive to selective agents incorporated to campylobacter selective agars (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 SMI are primarily aimed at detecting *C. jejuni*, *C. coli* and *C. lari*.

Salmonella species

A study comparing xylose lysine deoxycholate (XLD), deoxycholate citrate (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 (59). 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 (60).

Chromogenic agar has also been evaluated and has been shown to be comparable to traditional plated media (61).

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 (62):

- 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 cases of suspected STEC from whom STEC O157 has not been isolated should be referred to a reference laboratory for detection of non-O157 STEC

Chromogenic media

Chromogenic identification plates are commercially available and have been evaluated for certain clinical samples (61,63,64). 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 (61,63-65).

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 verified prior to use.

7.3.4 Investigation

Using a sterile pipette or sterile swab inoculate each agar plate with specimen (refer to [UK SMI Q 5 – Inoculation of culture media for bacteriology](#)).

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

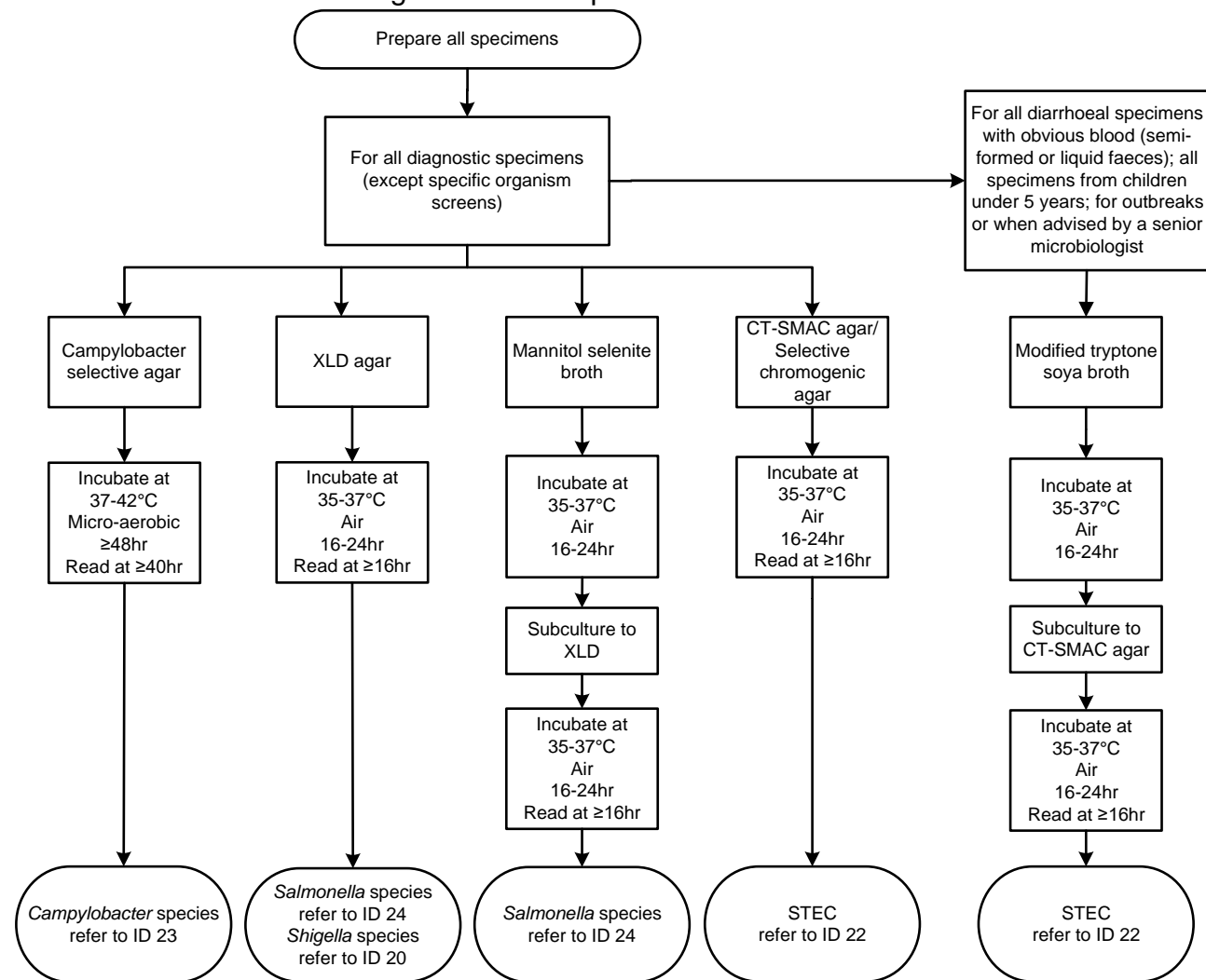
Table 1: Investigation of faeces by culture

Clinical details/ Conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
For all diagnostic specimens (except specific organism screens)	Faeces	Campylobacter selective agar (66,67)	37-42	micro-aerobic	≥48hr	≥40hr	Campylobacter species
		Xylose lysine deoxycholate (XLD) agar	35-37	air	16-24hr	≥16hr	Salmonella species Shigella species
		Mannitol selenite broth then subculture to:	35-37	air	16-24hr	N/A	Salmonella species
		XLD	35-37	air	16-24hr	≥16hr	
		Cefixime tellurite sorbitol MacConkey (CT-SMAC) agar / Selective chromogenic agar (68)	35-37	air	16-24hr	≥16hr	STEC O157
All diarrhoeal specimens with obvious blood (semi-formed or liquid faeces) (69) All diarrhoeal specimens from children under 5 years For outbreaks or when advised by a senior microbiologist	Faeces	Modified tryptone soya broth (MTSB) and then subculture to:	35-37	air	16-24hr	N/A	
		CT-SMAC agar	35-37	air	16-24hr	≥16hr	STEC O157
For these situations, add the following:							
Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
If a more rapid result is required (eg for non-enteric fever Salmonella outbreaks)	Faeces	Mannitol lysine crystal violet brilliant green agar	35-37	air	16-24hr	≥16hr	Salmonella species (except S. Typhi and S. Paratyphi A and B)

For these situations, add the following:							
Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Food poisoning (according to clinical details and advice from senior microbiologist)	Faeces	<i>B. cereus</i> selective agar (PEMBA)	35-37 then RT	air air	16-24hr then 16-24hr	16-24hr then 40-48hr	<i>B. cereus</i> <i>B. subtilis</i> <i>B. licheniformis</i>
	Faeces	Neomycin fastidious anaerobe agar	35-37	anaerobic	16-24hr	≥16hr	<i>C. perfringens</i>
Food poisoning (according to clinical details and advice from senior microbiologist)	Faeces	Mannitol salt agar or Baird Parker agar	35-37	air	40-48hr	16-24hr	<i>S. aureus</i>
Suspected Cholera or suspected infection with <i>V. parahaemolyticus</i> , seafood consumption, and/or recent travel (2-3 weeks) to known cholera area	Faeces	Thiosulphate citrate bile salts sucrose agar (TCBS) agar	35-37	air	16-24hr	≥16hr	<i>V. cholerae</i> <i>V. parahaemolyticus</i>
For suspected <i>Vibrio</i> outbreaks or when advised by a senior microbiologist.	Faeces	Alkaline peptone water then subculture to:	35-37	air	5-8hr	N/A	<i>V. cholerae</i> <i>V. parahaemolyticus</i>
		TCBS agar	35-37	air	16-24hr	≥16hr	
Appendicitis Mesenteric lymphadenitis Terminal ileitis Reactive arthritis (70)	Faeces	Cefsulodin irgasan (triclosan) novobiocin agar (CIN) agar	28-30	air	24-48hr	≥24hr	<i>Y. enterocolitica</i> <i>Y. pseudo-tuberculosis</i> <i>Yersinia</i> species
When advised by a senior microbiologist: Appendicitis Mesenteric lymphadenitis Terminal ileitis Reactive arthritis (70)	Faeces	Tris-buffered 1% peptone (petone (pH 8.0)) then subculture to: CIN agar	8-10 or 28-30 28-30	air air	7d or 24-48hr 24-48hr	 ≥24hr	
Other organisms for consideration - <i>Mycobacterium</i> species (UK SMI B 40 - Investigation of specimens for Mycobacterium species), toxin of <i>C. botulinum</i> and parasites.							
Laboratories should follow manufacturer's instructions when using selective chromogenic agar.							

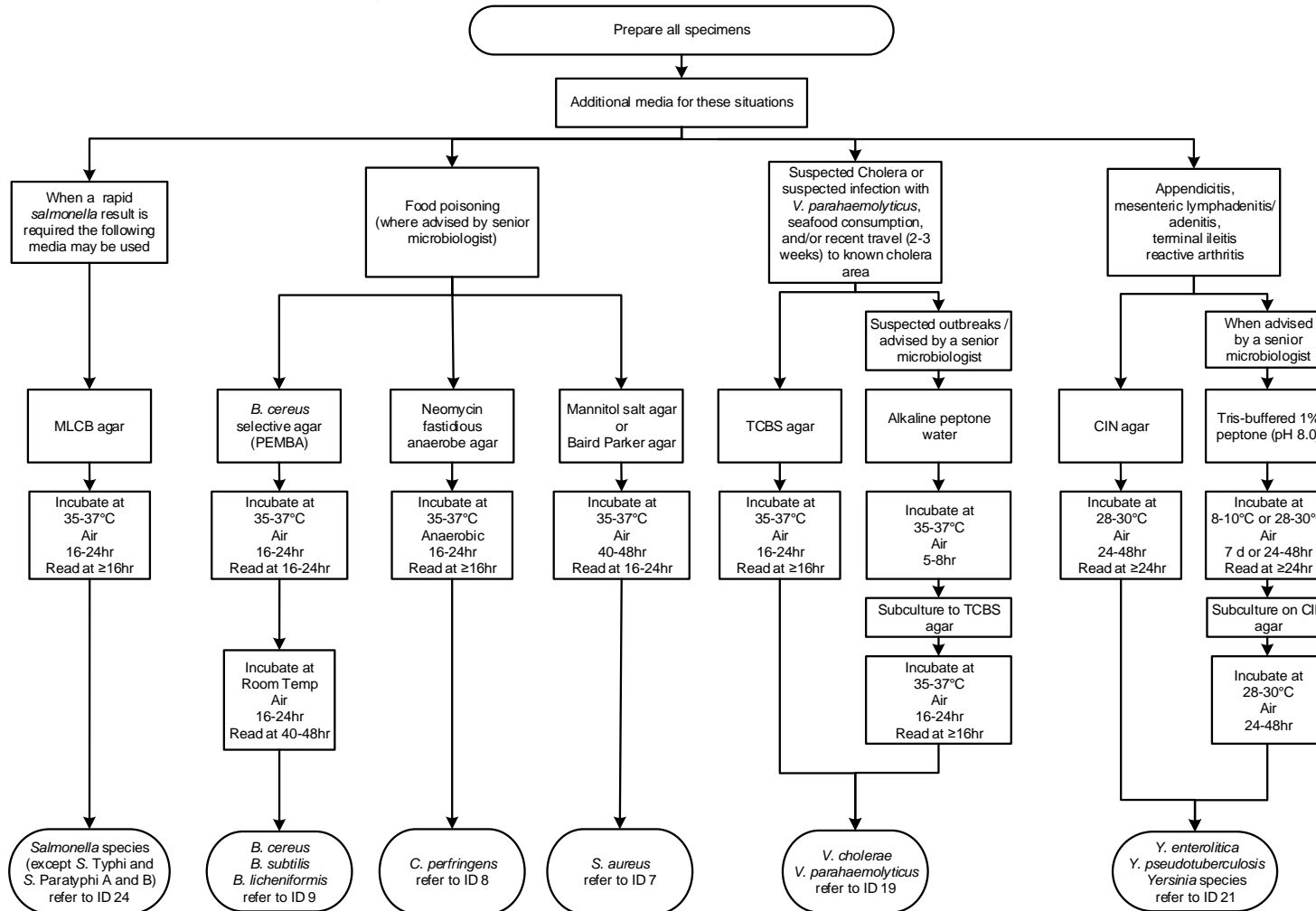
7.3.4.1 Flowchart for Investigation of faecal specimens for routine bacterial pathogens

The information within this algorithm is also presented in table 7.3.4



7.3.4.2 Flowchart for investigation of faecal specimens for additional bacterial pathogens

The information within this algorithm is also presented in table 7.3.4



To view associated UK SMI documents please access from: [UK Standards for Microbiology Investigations \(rcpath.org\)](https://rcpath.org)

7.4 Identification

This is the minimum level of identification for microorganisms by culture (or microscopy for parasites). 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

<i>Bacillus</i> species	genus level
<i>Campylobacter</i> species	genus level
<i>C. perfringens</i>	species level
STEC	species level + serogroup
<i>Salmonella</i> species	genus level
<i>S. Typhi/Paratyphi</i>	serovar level
<i>Shigella</i> species	species level
<i>S. aureus</i>	species level
<i>Vibrio</i> species	species level For <i>V. cholerae</i> , to consider whether O1, O139 or non-O1, non-O139
<i>Yersinia</i> species	species level
Parasites	species level and their stages where possible
<i>Cryptosporidium</i> species	genus level
<i>Giardia</i> species	genus level
<i>Cyclospora cayetanensis</i>	species level

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

See section 9. Refer to [EUCAST](#) guidelines.

7.5 Rapid methods

7.5.1 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 few minutes/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 Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry \(MALDI-TOF MS\) test procedure](#) for further information.

7.5.2 Enzyme immunoassays (EIA)

These rapid tests detect pathogen antigens 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. Laboratories should follow manufacturers' instructions when using EIA.

However, EIA have reduced sensitivity for detecting viruses and therefore it is advised where possible (71), to use NAAT instead. It is also advised that where EIA is used to detect viruses and the EIA result is negative, a NAAT/PCR is performed for confirmation.

7.5.3 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 cassettes 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* species and *Cryptosporidium* species from clinical specimens (faeces) which is usually confirmed using a qualitative test method (72).

8 Post-laboratory processes (post analytical stage)

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. For those which are non-pathogenic, include a comment to state that.

8.1.2 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 results:

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

8.2.2 Culture reporting time:

Interim or preliminary results should be issued on detection of clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer-generated reports should follow preliminary and verbal reports as soon as possible.

8.3 Reporting other tests including molecular testing

8.3.1 NAAT (including PCR) reporting

Positives

A positive result indicates the presence of the nucleic acid (DNA or RNA) of an organism.

Bacterial, parasite or viral nucleic acid (DNA or RNA) 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 nucleic acid (DNA or RNA) not detected.

Report result as for example, “*Norovirus* RNA not detected”.

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

8.3.1.1 NAATs/PCR reporting time

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

8.3.2 Enzyme immunoassays (EIA)

8.3.2.1 Interpreting and Reporting Laboratory Results

A positive reaction provides evidence of the presence of the organism in the faecal specimen. However, interpretation should take into consideration manufacturers' instructions, results of all other tests and the clinical picture.

Positive reactions from *Cryptosporidium* / *Giardia* kits need to be confirmed.

Negative results should be interpreted considering other test results.

Refer to the table in Appendix 1 of this document for further information regarding clinical presentations of gastrointestinal infections.

8.3.2.2 Enzyme Immunoassays assays reporting

Report results following manufacturers' interpretation instructions.

8.3.3 Immunochromatographic lateral flow (ILFA) assays

8.3.3.1 Interpreting and Reporting Laboratory Results

A positive reaction provides evidence of the presence of the organism in the faecal specimen. However, interpretation should take into consideration, results of all other tests and the clinical picture.

Negative results should be interpreted considering other test results.

Positive reactions from *Cryptosporidium* / *Giardia* kits need to be confirmed.

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

Report results following manufacturers' interpretation instructions.

9 Antimicrobial susceptibility testing

For interpretation of susceptibility testing results, laboratories should test and interpret according to the relevant EUCAST breakpoint, refer to [EUCAST guidelines for breakpoint information](#).

Isolates can also be sent to an appropriate specialist or reference laboratory.

Bacteria	Examples of agents to be included within primary test panel	Examples of agents to be considered for supplementary testing	Notes
<i>Shigella</i> species	Ciprofloxacin Azithromycin Amoxicillin Co-trimoxazole	Ceftriaxone Ceftazidime Meropenem Pefloxacin	Resistance enzyme determination if indicated
<i>Salmonella</i> species	Azithromycin Ceftriaxone Amoxicillin Co-trimoxazole	Ceftriaxone Ceftazidime Meropenem Pefloxacin	Resistance enzyme determination if indicated.
<i>Campylobacter</i> species	Ciprofloxacin Erythromycin	Tetracycline Gentamicin*	(*use ECOFF data with MIC)

Note: conventionally these antimicrobials are not reported in gastroenteritis cases

9.1 Reporting of antimicrobial susceptibility testing

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

10 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.

For further information on reporting, see the [scientific information related to UK SMLs](#).

Additionally, in England, hospital outbreaks of suspected or confirmed norovirus are reported to the Hospital Norovirus Outbreak Reporting System (HNORS) - [Norovirus Reporting \(phe.gov.uk\)](#).

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

11 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

Appendix

Table 1: Overview of pathogens associated with gastroenteritis (73-77)

Organism	Incubation Period/Infectious dose (ID)	Clinical presentations/features	Mode of transmission/risk factors	Frequency of infections caused by the organism
Common bacteria				
<i>Campylobacter</i> species (<i>C. jejuni</i> and <i>C. coli</i>)	2 to 10 days. The ID is as low as 500 - 800 organisms.	Self-limiting 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.
<i>Clostridioides difficile</i>	< 1 week but can be up to 4 weeks.	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.	Outbreaks occur in hospitals and in extended care facilities for the elderly, associated with antibiotic therapy (25). Transmission is via person to person by the faecal-oral route and by environmental contamination.	

Gastroenteritis

Organism	Incubation Period/Infectious dose (ID)	Clinical presentations/features	Mode of transmission/risk factors	Frequency of infections caused by the organism
Shiga toxin producing <i>Escherichia coli</i> (STEC) including serotype O157	3 to 4 days. The incubation period may depend on the number of organisms ingested. ID is <100 organisms or even <10 organisms for STEC O157.	Mild to bloody diarrhoea, pain in abdomen, nausea, and vomiting. Note: STEC is associated with haemorrhagic colitis and haemolytic uraemic syndrome (HUS) (5,78). Blood is not always present in faeces in STEC infections.	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.
Enteraggregative <i>E. coli</i> (EAEC)	20 to 48hrs. ID is $10^6 - 10^8$ organisms	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 >14 days.	This organism has been detected in travel-associated infections.	
Enteroinvasive <i>E. coli</i> (EIEC)	12 to 72hrs. ID is <100 organisms.	Fever, abdominal cramps, and watery diarrhoea with blood and mucus which generally contains leucocytes. EIEC can cause dysentery in humans. The condition may closely resemble shigellosis.	Foodborne and waterborne outbreaks.	

Gastroenteritis

Organism	Incubation Period/Infectious dose (ID)	Clinical presentations/features	Mode of transmission/risk factors	Frequency of infections caused by the organism
Enteropathogenic <i>E. coli</i> (EPEC)	12 to 72hrs. ID is 10^6 – 10^8 organisms.	Severe, prolonged non-bloody diarrhoea usually with passage of mucus. Vomiting and fever are also common. EPEC is known to cause sporadic cases and outbreaks of diarrhoea among children <2 years of age.	EPEC may be associated with travellers' diarrhoea. Transmitted from person-to-person by the faecal-oral route or via contaminated food.	Enteropathogenic <i>E. coli</i> (EPEC)
Enterotoxigenic <i>E. coli</i> (ETEC)	12 to 72hr ID is 10^6 to 10^8 organisms.	Travellers' Diarrhoea (mild, watery diarrhoea with abdominal cramps, nausea and low-grade fever). Dehydration and vomiting may occur in some cases.	Travel – associated: visitors to developing tropical/semi-tropical countries	Peak incidence is especially during the warm, wet season.
<i>Salmonella</i> species (28,36)	The usual incubation period is 12 to 72hr. ID is usually 10^3 organisms. Incubation period depends on host factors and the size of the infecting dose.	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.	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 (79) have been implicated in outbreaks of salmonellosis.	There is marked seasonal variation in occurrence of infection with peaks of incidence during summer and autumn.

Gastroenteritis

Organism	Incubation Period/Infectious dose (ID)	Clinical presentations/features	Mode of transmission/risk factors	Frequency of infections caused by the organism
Enteric fever caused by <i>Salmonella enterica</i> serovar Typhi (36)	8 to 14 days, although can be significantly longer or shorter. ID for <i>S. Typhi</i> is 10^3 organisms.	Acutely, fever, vomiting and constipation, followed by fever with diarrhoea (may be bloody)	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.	There is marked seasonal variation in occurrence of infection with peaks of incidence during summer and autumn.
Enteric fever caused by <i>Salmonella enterica</i> serovar Paratyphi types A, B and C) (36) Note: Most paratyphoid cases reported in UK travellers are caused by <i>S. Paratyphi A</i> .	Incubation is 1 to 10 days. ID for <i>S. Paratyphi</i> species is 10^6 organisms.	Diarrhoea (may be bloody), accompanied by fever and vomiting as well as constipation.	Human faecal contamination of food or water.	There is marked seasonal variation in occurrence of infection with peaks of incidence during summer and autumn.
<i>Shigella</i> species (80) (<i>S. flexneri</i> , <i>S. dysenteriae</i> , <i>S. boydii</i> , and <i>S. sonnei</i>)	1 to 3 days. As few as 10 to 100 organisms are required for an ID (35). 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. <i>S. dysenteriae</i> serotype 1 infection can very rarely be complicated by haemolytic uraemic syndrome, most commonly in children <5 years of age.	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.

Gastroenteritis

Organism	Incubation Period/Infectious dose (ID)	Clinical presentations/features	Mode of transmission/risk factors	Frequency of infections caused by the organism
Common parasites				
<i>Cryptosporidium</i> species	1 to 14 days, median 5 to 7 days ID is as low as 1 oocyst.	<p>Acute onset, persistent (sometimes chronic) diarrhoeal disease with signs of malabsorption, abdominal pain, low grade fever, nausea and vomiting.</p> <p>Other more rare presentations in severely immunocompromised patients include atypical gastrointestinal disease such as cholangitis, cholecystitis, pancreatitis and hepatitis. Asymptomatic carriage possible.</p> <p>Note:</p> <p>Symptoms can be particularly severe in those who are immunocompromised (81) .</p>	<p>Predominant species infecting humans in the UK are <i>C. parvum</i> (zoonotic) and <i>C. hominis</i> (anthroponotic)</p> <p>Primarily spread from person to person via the faecal-oral route, or zoonotically.</p> <p>Causes waterborne and foodborne outbreaks e.g. swimming pools, drinking water, fresh produce, unpasteurised milk. Seasonal outbreaks linked to petting farms.</p> <p>Risk groups include MSM, children attending nurseries, farm visitors, the elderly in care homes.</p>	Infection shows seasonal variation with peak incidence in the spring (largely <i>C. parvum</i>) and especially the autumn (largely <i>C. hominis</i>).
<i>Giardia</i> species	3 to 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 (81).	Waterborne and foodborne outbreaks have been reported. Outbreaks have also been reported in MSM. The parasite is primarily spread from person to person via the faecal-oral route, or zoonotically (81).	Shows little seasonality throughout the year with small increase in summer months.

Gastroenteritis

Organism	Incubation Period/Infectious dose (ID)	Clinical presentations/features	Mode of transmission/risk factors	Frequency of infections caused by the organism
Common viruses				
Adenovirus (Adenovirus Group F types 40/41)	5 to 9 days	Acute diarrhoea - prolonged diarrhoea and low-grade fever are commonly seen.	Person to person contact via faecal-oral route. Outbreaks have been recognised in nurseries and paediatric units.	Increased incidence in the late winter, spring, and early summer; however, infections can occur throughout the year.
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.
Norovirus (Norovirus GI/GII) Note: Norovirus Genogroup II genotype 4 is the commonest cause of gastroenteritis in the UK and worldwide.	Typically, 12 to 48hr. Shorter incubation periods have been reported (82). Approximately 10 virus particles are needed to cause an infection (37).	Vomiting (often projectile), diarrhoea, headaches, fever, myalgia and abdominal cramps. Increased severity may be observed in the elderly and young children. Recovery is usually within 24hr. Note: During the acute phase of the illness virus is excreted in faeces and vomitus at concentrations of approximately 10^7 particles per gram or mL.	Outbreaks are common within the community and institutions such as hospitals and elderly care homes (83). 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.	Year round, predominantly in winter with secondary peak (much lower) in the summer months.

Gastroenteritis

Organism	Incubation Period/Infectious dose (ID)	Clinical presentations/features	Mode of transmission/risk factors	Frequency of infections caused by the organism
Rotavirus (Group A and B)	24 to 72hr. ID is estimated at 10 to 100 virus particles.	Diarrhoea and vomiting occasionally, gastroenteritis in the elderly. Other symptoms may include loss of appetite and dehydration in children. Illness duration is 2-8 days.	Person to person contact via faecal-oral route, fomites or vomit where the aerosol droplets are disseminated in the environment.	Peak incidence occurs in the winter months but substantially decreasing during the summer months.
Sapovirus (Sapovirus GI, GII and GIV)	12 to 48hr and has an ID of as few as 10 virus particles.	Diarrhoea and vomiting, generally without accompanying fever. Illness duration is 1 to 4 days.	Foodborne outbreaks via consumption of raw or undercooked shellfish, such as oysters and clams have been documented.	Increased incidence in the winter months.
Other Bacteria				
<i>Bacillus</i> species (<i>Bacillus cereus</i>) <i>B. subtilis</i> and <i>B. licheniformis</i> may also be involved in food poisoning episodes.	Emetic syndrome is 1 to 6hr. Food poisoning results from the ingestion of large numbers ($>10^5$ cfu/g of food) of toxigenic bacteria or preformed emetic toxin. Diarrhoeal syndrome is 8 to 16hr.	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 24hr. It includes abdominal pain, watery diarrhoea and occasionally nausea	Ingestion of foods like rice, pasta, desserts, meat, and dairy products.	Particularly prevalent in warmer months

Gastroenteritis

Organism	Incubation Period/Infectious dose (ID)	Clinical presentations/features	Mode of transmission/risk factors	Frequency of infections caused by the organism
<i>Clostridium botulinum</i>	2hrs to 8 days (usually 12 to 36hrs)	Botulism - this include slurred speech, double vision, difficulty in swallowing, descending flaccid paralysis, typically with cranial nerve involvement, sometimes culminating in respiratory arrest (84).	Consumption of inadequately processed stored foods. Person to person spread does not occur.	
<i>Clostridium perfringens</i> (Type A)	4 to 24hr and symptoms usually subside within 10 to 24hrs. ID is $>10^5$ of organisms.	Watery diarrhoea with severe abdominal pains and vomiting. Fatalities although rare, can occur, especially in the elderly and other compromised persons.	Ingestion of improperly cooked, stored and reheated foods	Particularly prevalent in warmer months
<i>Listeria monocytogenes</i>	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 (eg patients undergoing treatment for cancer, pregnant women or even have serious underlying health conditions), symptoms are more severe and include fever, myalgia, sepsis, meningitis or even death.	Consumption of contaminated refrigerated foods like milk, soft cheeses, prepacked sandwiches, pates, etc Rarely, outbreaks have been reported in the community and hospital settings resulting from contaminated food	

Gastroenteritis

Organism	Incubation Period/Infectious dose (ID)	Clinical presentations/features	Mode of transmission/risk factors	Frequency of infections caused by the organism
<i>Staphylococcus aureus</i>	1 to 6hr and usually resolves in 12hr	General malaise, vomiting, nausea and abdominal cramps, often followed by diarrhoea. Severe dehydration can occur in children and the elderly.	Ingestion of foods - milk products such as cream-filled pastries, cream pies, butter, cheese, and sandwich fillings.	Particularly prevalent in warmer months
<i>Vibrio</i> species excluding <i>V. cholerae</i> and <i>V. parahaemolyticus</i>	2 to 3 days.	Cholera. Symptoms vary from mild diarrhoea 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, lack of availability of clean water and overcrowding (85,86).	Particularly prevalent in warmer months
<i>Vibrio cholerae</i>	2 to 3 days. Toxigenic <i>V. cholerae</i> (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 diarrhoea 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 (87). 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, lack of availability of clean water and overcrowding (85,86).	Particularly prevalent in endemic areas during warmer months (during the summer and early autumn).

Gastroenteritis

Organism	Incubation Period/Infectious dose (ID)	Clinical presentations/features	Mode of transmission/risk factors	Frequency of infections caused by the organism
<i>Vibrio parahaemolyticus</i>	2 to 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, lack of availability of clean water and overcrowding (85,86).	Particularly prevalent in warmer months
<i>Yersinia</i> species (<i>Y. enterocolitica</i> and <i>Y. pseudotuberculosis</i>)	3 to 7 days. It has a high ID of 10^6 to 10^9 organisms.	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) (88,89).	Contaminated food, milk or water, and pigs are a frequently identified source of infection.	Particularly prevalent in winter months
Other parasites				
<i>Cyclospora cayetanensis</i>	2 to 14 days ID considered to be low (<100 oocysts)	Watery diarrhoea which may be prolonged, with weight loss, anorexia, severe fatigue, nausea, vomiting and abdominal pain. Relapsing and remitting course.	Consumption of contaminated soft fruits, vegetables, herbs, and drinking recreational water. Oocysts require a period of maturation in the environment before they become infectious meaning that direct person-to-person transmission does not occur. No confirmed animal host identified.	Almost exclusively related to travel to endemic countries (mainly in the tropics) e.g. Mexico. Indigenous cases likely to be related to foods imported from endemic areas.

Gastroenteritis

Organism	Incubation Period/Infectious dose (ID)	Clinical presentations/features	Mode of transmission/risk factors	Frequency of infections caused by the organism
<i>Entamoeba histolytica</i>	2 to 4 weeks. ID is as low as 1 cyst.	<i>E. histolytica</i> 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 (41). Invasive spread to major organs is also possible.	This organism can cause diarrhoea, mostly but not exclusively 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				
Microsporidia (<i>Enterocytozoon bienersi</i> , <i>Encephalitozoon cuniculi</i> and <i>Encephalitozoon intestinalis</i>)	May be dependent on the number of organisms ingested.	Chronic diarrhoea and wasting syndrome observed in immunocompromised patients. Fever is not observed.	Contaminated water consumption.	

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, *Yersinia* species, toxin producers (*Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens* and *Clostridium botulinum*). Testing for purative pathogens *Plesiomonas shigelloides* and *Aeromonas* species may be sought under certain circumstances.

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An explanation of the reference assessment used is available in the [scientific information](#).

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