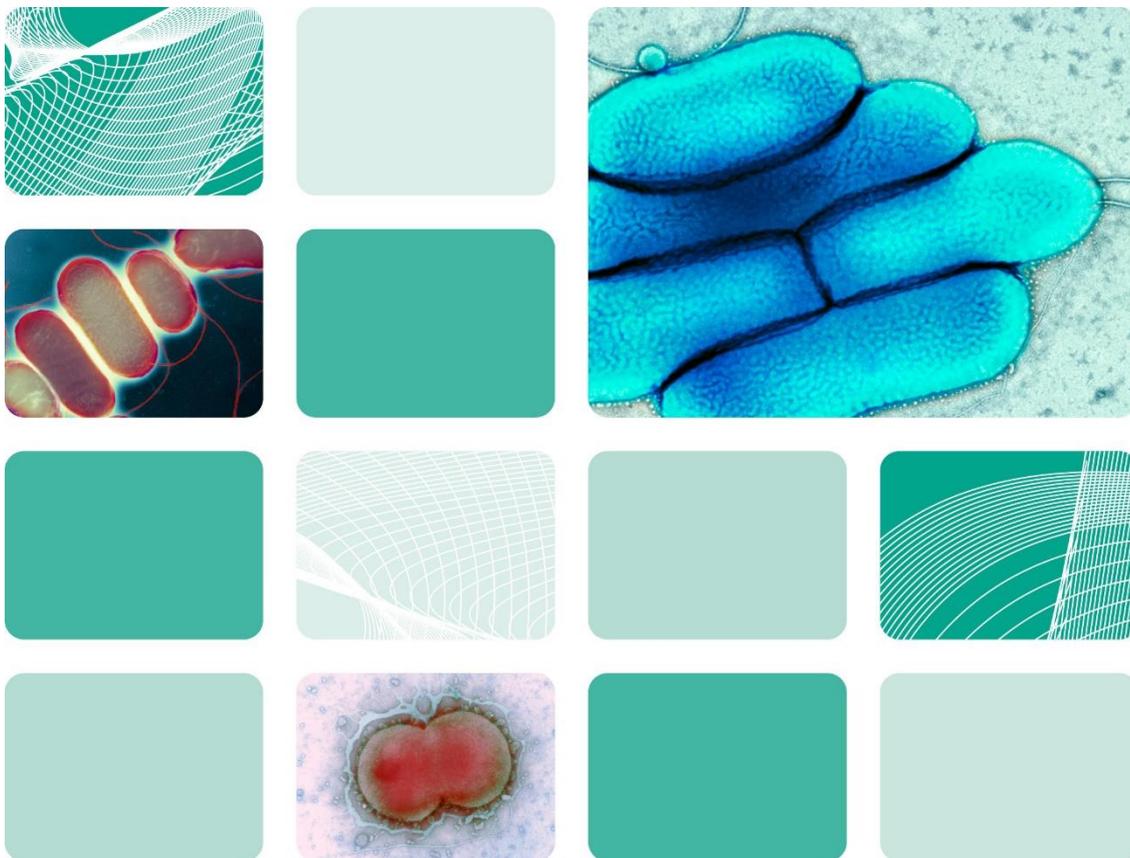




UK Health
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UK Standards for Microbiology Investigations

Investigation of faecal specimens for *Clostridioides difficile*



Acknowledgments

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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	8/24.03.25
Issue number discarded	1.7
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Anticipated next review date*	24.03.28
Section(s) involved	Amendment
Title	Title has been changed from 'Processing of Faeces for <i>Clostridium difficile</i> ' to 'Investigation of faecal specimens for <i>Clostridioides difficile</i> '
Whole document	<p>Document has been transferred to a new template and restructured.</p> <p>Hyperlinks updated to direct reader to UK SMI webpages on the RCPATH website.</p> <p><i>Clostridium</i> replaced with <i>Clostridioides</i> throughout.</p> <p>All sections have been updated with current information and relevant information and supported with recent literature where available.</p> <p>Some sections have been restructured as appropriate to align with current laboratory practices.</p>
Scope of document	<p>Types of specimens have been moved to Section 8: Pre-laboratory processes (pre analytical stage).</p> <p>Scope has been extended to include the recommended two stage testing approach, with a third test in the cases of inconsistent results.</p> <p>Topics that are outside the scope of this document have been mentioned and links to relevant UK SMIs were provided if available.</p> <p>Definitions section has been added.</p>
Section 4: Introduction	Information updated and supported with recent literature where available.

Section 5: <i>C. difficile</i> infection diagnostic tests	This section has been added as an introduction to the tests recommended throughout the document.
Section 6: Technical information and/or limitations	This section has been expanded to cover general considerations and test limitations.
Section 7: Safety considerations	Information reviewed and references have been updated where applicable.
Section 8: Pre-laboratory processes (pre analytical stage)	This section has been added with essential information for consideration prior to testing, and further expanding on details from the previous version.
Section 9: Laboratory processes (analytical stage)	This section replaces the previous 'Specimen processing/procedure' section. All information regarding <i>Clostridioides difficile</i> culture and identification has been moved to the appendix. Information has been updated based on current laboratory testing procedure.
Section 10: Post-Laboratory processes (post analytical stage)	This section has been added to assist laboratories when reporting results and provides information regarding repeat testing of specimens.
Section 11: Mandatory reporting of <i>C. difficile</i> infection	This section includes links to information regarding mandatory reporting schemes of devolved administrations.
Section 12: Antimicrobial susceptibility testing	Information and hyperlinks reviewed and updated as appropriate. Hyperlink to British Society for Antimicrobial Chemotherapy (BSAC) removed.
Section 13: Referral to reference or specialist testing laboratories	Hyperlinks have been updated.
Appendix	This section includes updated information and references regarding <i>Clostridioides difficile</i> culture and identification.
References	References reviewed and updated.

*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 UK SMI describes the investigation of faeces for *Clostridioides difficile*, the laboratory interpretation of results, and the reporting of *C. difficile* infection (CDI) cases for the purposes of mandatory surveillance.

The diagnosis of CDI is based on both clinical presentation and laboratory testing. Clinical correlation is also required to ensure accurate interpretation of results and appropriate patient management by taking factors such as recent antibiotic use into consideration (1-4). No single assay is reliable as a stand-alone test for the laboratory diagnosis of CDI (1-5).

This document describes why a two-stage testing approach is required followed by a third test in cases of inconsistent GDH and toxin A and B results. (1-4,6,7). A sensitive *C. difficile* glutamate dehydrogenase (GDH) immunoassay or Nucleic Acid Amplification Test (NAAT) is recommended as a primary test followed by a highly specific toxin A and B immunoassay (1-4,6,7).

For the management and treatment of CDI, clinicians should assess the patient's current clinical condition and refer to the relevant guidance. For example, the National Institute for Health and Care Excellence (NICE), the Healthcare Infection Society (HIS) and the Scottish Antimicrobial Prescribing Guidance (SAPG) guidelines (8-10).

Please refer to [UK SMI S 07: Gastroenteritis](#) and [UK SMI ID 8 - Identification of Clostridium species](#) for additional information.

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

Definitions

Key terms	Definition
Diarrhoea	<p>Diarrhoea is defined as the passage of 3 or more loose or liquid stools per day (or more frequent passage than is normal for the individual). Frequent passing of formed stools is not diarrhoea, nor is the passing of loose, pasty stools by breastfed babies (11).</p> <p>Stools of a loose or liquid consistency are typically classed as 5 to 7 on the Bristol Stool Form Scale, taking the form of the container.</p> <p>Diarrhoea may be associated with symptoms such as abdominal cramps, nausea, malaise, vomiting, fever and dehydration.</p> <p>Note: Severe CDI is not always associated with loose stool or diarrhoea such as in the case of ileus.</p>

Colonisation/Carriage	Both terms are used interchangeably and generally mean the presence of <i>C. difficile</i> in the absence of CDI symptoms.
Toxigenic	<p><i>C. difficile</i> strain containing toxin genes (<i>tcdA</i> and/or <i>tcdB</i>) and is therefore capable of producing toxins that cause <i>C. difficile</i> infection.</p> <p>Note: The term toxigenic should not be used when reporting/interpreting results to avoid confusion.</p>
<i>C. difficile</i> infection	<p><u>England, Wales, and Northern Ireland:</u></p> <p>One episode of diarrhoea* that is not attributable to any other cause, including medicines, and that occurs at the same time as a positive toxin assay (with or without a positive <i>C. difficile</i> culture) and/or endoscopic evidence of pseudomembranous colitis (PMC) (2).</p> <p><u>Scotland:</u></p> <p>A case of CDI is someone in whose stool <i>C. difficile</i> toxin has been identified at the same time as they have experienced diarrhoea* not attributable to any other cause, or from whose stool <i>C. difficile</i> has been cultured at the same time as they have been diagnosed with pseudomembranous colitis (PMC) (4).</p> <p>* Refer to the definition of diarrhoea above.</p> <p>Note: It is not recommended to wait for at least 3 episodes of diarrhoea to instigate testing for <i>C. difficile</i> infection (1).</p>

4 Introduction

Clostridioides difficile is a Gram positive, spore forming, strictly anaerobic rod, so named because of the difficulty in original culture and characterisation (12,13). The taxonomic differences between this species and other members of the *Clostridium* genus is reflected by the change in nomenclature to *Clostridioides* (14-16). Toxigenic *C. difficile* strains produce two large, structurally similar protein toxins (A and B), which are the main virulence factors in *C. difficile* Infection (CDI) (14). *C. difficile* can colonise the colon and may cause a spectrum of disease in humans, from a self-limiting mild diarrhoea to the advance and severe illness characterised by pseudomembranous colitis (PMC).

Transmission of *C. difficile* occurs primarily through the faeco-oral route. *C. difficile* spores can contaminate the environment and in healthcare-settings, where infection prevention and control are sub-optimal, the environment can act as a reservoir for cross-infection. *C. difficile* can be isolated from soil, water, domiciliary environments as well as animal faeces, all of which can act as a reservoir for cross-infection (17).

CDI is predominantly healthcare-associated but the burden of community-onset cases has risen in recent years, reflecting the change in epidemiology and transmission patterns (18). CDI cases are subject to mandatory reporting which assists in monitoring the spread of CDI and guide infection prevention and control efforts.

4.1 Pathogenicity

The enterotoxin, toxin A (TcdA), and cytotoxin, toxin B (TcdB) produced by *C. difficile* cause cell death (13). These type III exotoxins glycosylate and inactivate intracellular Rho-family GTPases result in changes in the actin cytoskeleton. They cause the characteristic intestinal mucosal damage consisting of plaque-like lesions which may, in some cases, result in the formation of pseudomembranes in the colon (13).

In strains which possess the toxin gene, toxin production is controlled by specific genes and can occur in response to various conditions, such as the presence of antibiotics or specific nutrients. Only strains that possess the pathogenic locus (PaLoc) carry the genes for the toxins A (TcdA) and B (TcdA) and can cause CDI (13). Most pathogenic strains produce both TcdA and TcdB, however, clinically significant strains causing diarrhoea and colitis have been isolated which are TcdA negative and TcdB positive (19). Outbreaks caused by these strains have been documented and although TcdA positive and TcdB negative strains have not been recorded, it is essential to use diagnostic toxin immunoassays capable of detecting both toxins (20). Some NAATs only target either toxin A (*tcdA*) or toxin B (*tcdB*) genes. This is suitable if those that only detect toxin A genes target the conserved portion of the *tcdA* gene and not the truncated region, which is absent in *C. difficile* strains that do not produce TcdA enterotoxin.

Deletions in the toxin regulatory gene *tcdC* are associated with greater production of TcdA and TcdB as described in the hypervirulent *C. difficile* strain 027 (21,22). In addition, around one third of strains of *C. difficile* produce a binary toxin (CDT) which is not part of PaLoc and therefore may be present in the absence of TcdA and TcdB. This toxin may contribute to the severity of infection in certain hypervirulent strains, although its exact role in the pathogenesis of CDI remains unclear (23).

4.2 Risk factors and course of *C. difficile* infection

Acquisition of *C. difficile* alone does not induce CDI. Individuals may be *C. difficile* carriers (i.e., have asymptomatic colonisation) of either non-toxigenic or toxigenic strains of *C. difficile*. Colonisation with toxigenic strains of *C. difficile* highlights two significant risks: the potential for transmission, particularly if the individual becomes symptomatic with or without CDI and the increased likelihood of progressing to CDI, typically in the presence of other contributing factors.

The incidence of *C. difficile* colonisation varies in the literature, influenced by factors such as study population and epidemiological differences. Some studies report that around 2% of the general population is colonised with toxigenic *C. difficile* strains. In hospitalised patients, this rate can be as high as 7 - 25% (24-26). However, even when CDI is ruled out, it is worth applying infection prevention and control measures to carriers, especially when the patient has loose stools, given the potential for contamination of the environment with *C. difficile*. Infants may become colonised with *C. difficile* the first few months of life, although disease is rarely present (27-30).

The main risk factor for CDI is the repeated use of broad-spectrum antibiotics, such as broad-spectrum beta lactams, cephalosporins, clindamycin and fluoroquinolones, that alter or distort the normal microbiota (12,31,32). Changes in the human gut microbiota, associated with antibiotic therapy or other treatments such as chemotherapeutic and radiotherapeutic agents permits asymptomatic colonisation by *C. difficile* (33). The over-growth of toxin-producing strains of *C. difficile* can damage the lining of the colon leading to the development of CDI.

Other risk factors for the development of CDI include immunosuppression, advanced age, patients undergoing general surgery and underlying co-morbidities such as chronic renal disease, inflammatory bowel disease and cancer (34-36). The importance of age can be demonstrated by figures from the UKHSA annual summary report on CDIs which show that the highest proportion of cases (in which age was reported) were from patients > 65 years old (18).

The spectrum of *C. difficile* disease ranges from asymptomatic carriage, and self-limiting mild diarrhoea through to the advanced and severe illness characterised by pseudomembranous colitis (37). Severe colitis, with or without pseudomembranes, can result in partial or complete ileus or toxic megacolon, perforation, and death (13). If present, pseudomembranous colitis is seen on endoscopy.

Endoscopy is not routinely indicated but is diagnostic in, for example, suspected cases of 'silent' CDI, such as ileus, toxic megacolon, or pseudomembranous colitis without diarrhoea such that submission of a stool specimen for laboratory testing is impossible (37). In suspected cases of 'silent' CDI, rectal swabs can be submitted for testing and when CDI is suspected but laboratory results are consistently negative, blood white cell count (WCC), serum creatinine and abdominal imaging such as computerised tomography (CT) scanning can also aid diagnosis and management (38).

4.3 Treatment and management

Treatment and management guidelines for CDI are produced in the United Kingdom by the National Institute for Health and Care Excellence (NICE), the Healthcare Infection Society (HIS), and the Scottish Antimicrobial Prescribing

Group (SAPG) (8-10). Outside of the United Kingdom, they are produced by societies such as the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Infectious Disease Society of America (IDSA) (8,9,39,40).

Scotland and Northern Ireland follow their respective National Infection, Prevention and Control Manuals (41,42). While England and Wales have adopted the Scottish National Infection, Prevention and Control Manual (43).

5 *C. difficile* infection diagnostic tests

This section describes the tests available for CDI diagnosis. Refer to Section 9 and 10 for the order of CDI testing.

5.1 Glutamate dehydrogenase antigen detection assays

GDHs are a group of enzymes abundantly present in all bacteria, including toxigenic and non-toxigenic strains of *C. difficile*. The GDH of *C. difficile* can therefore be used as an accurate marker for the presence of *C. difficile* in faecal samples. GDH assays are commercially available as membrane assays and enzyme immunoassays (EIAs) and are quick to perform and comparatively inexpensive.

The GDH antigen test has a high clinical sensitivity but is less specific than *C. difficile* toxin-based assays as it detects both toxigenic and non-toxigenic strains, with variation between kits available. In addition, GDH antigen test may show cross-reactivity with *Clostridium* species or other organisms such as *Staphylococcus aureus*. Therefore, GDH antigen test is used as a primary test in conjunction with more specific additional test(s). Same day reporting of negative samples is achievable with this method as GDH negative results do not need further testing for *C. difficile*. The role of GDH in the detection of *C. difficile* has been the focus of several studies which have recognised its effective use in a dual test approach to testing (7,44-46).

5.2 Molecular methods

NAATs, particularly PCR assays, have been used to target *C. difficile* toxin A and B genes in faeces (47). These assays detect the presence of strains with potential to produce toxins (48). The detection of these genes by NAAT (NAAT detected result) in a sample where no toxin proteins are detectable by toxin immunoassay is well-described. This is because, like GDH tests, the NAAT assays are more sensitive than the toxin A and B immunoassays. Also, similar to GDH assays, NAATs have lower clinical specificity, but results can be available within an hour.

In addition, there are several multiplex molecular gastrointestinal pathogen panels commercially available that include *C. difficile*. These have the advantage of being both rapid and analytically sensitive with results for gastrointestinal pathogen panels available in less than a day (49,50). Some of these multiplex PCR assays detect not only the toxin B gene, but also other conserved genes indicative of the 'hypervirulent' strain ribotype 027, other related strains, and binary toxin (51,52). Due to the lower clinical specificity of these tests for CDI, in comparison with toxin A and B immunoassays, these tests should not be used alone as a diagnostic test for

C. difficile (50,53). NAAT testing, as with GDH detection, may detect asymptomatic carriage of *C. difficile* (53,54).

5.3 Toxin A & B antigen detection assays

There are numerous commercially available toxin A and B immunoassay tests intended to detect *C. difficile* toxins. Use of immunoassays that detect both toxins A and B is essential, as infection due to A negative but B positive strains have been recorded (19,55). *C. difficile* toxin assays are commercially available as membrane assays and well-based EIAs and are quick to perform and relatively inexpensive. As with all assays, the analytical and clinical sensitivity/specificity of these kits vary. However, in general *C. difficile* toxin immunoassays lack clinical sensitivity, if used alone, but are highly specific for CDI. For this reason, they should be used in a two-stage testing approach followed by a third test in cases of inconsistent results (56-61).

5.4 Other methods

5.4.1 Cell-Cytotoxicity neutralisation assay (Cell-culture)

Although regarded as the “gold standard”, use of tissue culture for the detection of *C. difficile* toxins by virtue of its cytopathic effect (CPE) has now been surpassed by other methods. The unavailability of the *C. sordellii* antitoxin, required to neutralise the CPE, along with the required technical expertise and 24 to 48-hour delay for the final result have made this method no longer viable (62,63).

5.4.2 Faecal calprotectin

Some studies have investigated faecal calprotectin (an indicator of general intestinal inflammation) as an adjunctive method to diagnose CDI. Although the sensitivity of this test is high, it lacks specificity and is therefore not recommended (64).

5.5 Identification

5.5.1 *C. difficile* culture

Refer to the Appendix for information regarding the isolation of *C. difficile*, confirmation of presumptive isolates and reporting of results.

Culture has limited clinical utility for the diagnosis of CDI due to its long turnaround time and the need for confirmatory testing. Therefore, it is more widely used by laboratories for epidemiological surveillance where typing and/or susceptibility testing is required and used as a reference method to validate newer diagnostic tests.

In Scotland, laboratory culture of faeces is recommended in cases of severe disease, suspected outbreaks, in recurrent cases (> 28 days and < 56 days) and as part of the [C. difficile snapshot programme](#). In England and Northern Ireland culture may be undertaken by the CDRN. In Wales, all GDH/NAAT positive samples should be referred to the UKARU for *C. difficile* culture.

5.6 Typing of *C. difficile*

Typing of isolates of *C. difficile* is useful in the investigation of increased frequency of CDI, to determine potential transmission, as well as in cases of increased severity, complication, recurrence, or the death rate presumptively associated with CDI (65). Historically, many typing techniques have been used in the investigation of outbreaks of CDI. Those in recent use are exclusively DNA-based (66-68).

PCR-ribotyping in conjunction with multilocus variable-number tandem-repeat analysis (MLVA) and more recently, analysis of whole genome sequencing (WGS) data, are techniques used routinely to distinguish isolates of *C. difficile* in the UK (66).

The culture and identification of *C. difficile* is important for typing in cases of increased incidence, in outbreak situations and for general surveillance, including drug resistance and virulence determinant detection.

A pure culture or an isolate of *C. difficile* is required for typing. Please refer to Section 13 for the appropriate UK *C. difficile* typing service and specific requirements.

5.6.1 PCR Ribotyping and MLVA

PCR ribotyping is an internationally recognised method for typing of *C. difficile* isolates, based on 16S - 23S intergenic spacer region diversity. More than 1,100 PCR ribotypes have now been identified (69-71).

The MLVA technique offers enhanced fingerprinting of *C. difficile* isolates and can be used to determine closely related isolates (and help identify transmission) in settings where PCR-ribotyping data are insufficient (66,67).

5.6.2 Whole Genome sequencing

Whole genome sequencing allows the comparison of entire microbial genomes and has emerged as an important tool in the epidemiological study of *C. difficile*. This technique can simultaneously detect sequences that:

- (i) encode toxin genes and other virulence factors.
- (ii) potentially confer resistance to antibiotics.
- (iii) facilitate the measurement of relatedness between *C. difficile* strains with high resolution.

WGS-based typing can be undertaken using traditional, core- or whole-genome MLST (cgMLST/wgMLST). Alternatively, single-nucleotide polymorphism (SNP) typing offers ultimate discriminatory power (66). Therefore, analysis of whole genome sequence data may allow surveillance of emerging lineages with higher transmissibility and important genetic markers.

6 Technical information and/or limitations

As with all laboratory tests, *C. difficile* assays may vary in their analytic and clinical sensitivity and specificity, with some performing particularly poorly. Therefore, it is crucial for laboratories to understand the strengths and limitations of any method they employ for detection of *C. difficile*, and its toxins.

Laboratories should also incorporate these assays within the recommended two-stage testing approach followed by a third test in cases of inconsistent GDH and toxin A and B results (see section 9). This is because the detection of *C. difficile* by culture, GDH test or by NAAT alone has a poor specificity for the diagnosis of CDI (6). These methods do not detect toxin produced by *C. difficile* but instead detect the presence of the bacterium or the genes for toxin production.

Manufacturer's instructions should be followed and all test kits and NAAT platforms should be validated and verified prior to use. In addition, rectal swabs require local validation prior to molecular testing.

Laboratories should ensure the following:

- availability of two-stage/three-stage testing (including toxin testing) seven days a week
- the turnaround time from sample requests (for hospital in-patients) to delivery for laboratory testing is reduced
- efficient and prompt communication of results

Audit of practice in these areas is essential to ensure optimal laboratory performance.

7 Safety considerations

This section covers specific safety considerations related to this UK SMI and should be read in conjunction with the general [safety considerations](#) (69-89)

7.1 Specimen Collection, Transport and Storage

Collect specimens using aseptic technique.

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

Compliance with postal, transport and storage regulations are essential.

7.2 Specimen Processing

Processing of specimens should be carried out in a containment level 2 laboratory, as a minimum.

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

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

8 Pre-laboratory processes (pre-analytical stage)

8.1 Eligibility criteria for testing:

In all UK nations, faecal specimens that meet the following testing criteria should be tested for CDI: all faecal specimens from a patient who has diarrhoea* that is not attributable to an underlying condition such as inflammatory colitis or therapy such as laxatives. In all UK nations, screening of symptom-free patients and clearance testing is not recommended.

*Please refer to the Scope of the document and Section 8.3.1 for the definition of diarrhoea and the correct specimen type, respectively.

In the UK (except Scotland) faecal specimens that meet the testing criteria should be tested for *C. difficile* from:

- Ideally, all patients aged ≥ 2 years old.

Whilst some older literature suggests that community testing may not be required, more recently, studies are emerging suggesting that it is beneficial (90-95). Therefore, it is recommended that laboratories consider testing all eligible patients aged ≥ 2 years.

However, acknowledging the variation in laboratory context such as regional epidemiological data, a local decision should be made in liaison with relevant stakeholders, including health authorities and commissioning bodies. This decision should also consider the impact on end users, including clinical and public health professionals and patients.

Resources or financial constraints should not be considered a justifiable reason for excluding testing in the recommended age groups.

As a minimum, the following patients should be tested:

- All hospital patients aged ≥ 2 years old.
- All community patients ≥ 65 years old.
- Community patients < 65 years old when specifically requested and in situations specific to the following, but not limited to them (96):
 - If *C. difficile* is mentioned in request clinical details
 - If the patient is recognised by the testing laboratory, microbiologists or IPC team as previously having *C. difficile*
 - If current or prior antibiotic therapy is mentioned
 - If a hospital admission is mentioned
 - If residence at a care home or other community care facility is indicated by the patient address, or mentioned on the request form
 - Immunocompromised (e.g., transplant patients, patients on chemotherapy)
 - Inflammatory bowel disease (IBD, e.g., Crohn's disease or Ulcerative Colitis)

- Recent abdominal surgery
- Chronic renal/kidney disease (CKD)
- If clinical details indicate patient has been prescribed a proton pump inhibitor (PPI; e.g. lansoprazole, omeprazole)

Note: CDI is not confined to the groups above and may not have been considered by the requestor. Half of community CDI cases may be undetected because of absence of clinical suspicion, accounting for three times more undiagnosed adults in the community compared with hospitals in one study (95).

In Scotland, faecal specimens that meet the testing criteria should be tested for *C. difficile* in:

- All patients \geq 3 years old.

8.2 Specimen type:

Faeces

Note: Rectal swabs should only be collected when 'silent' CDI is suspected such as paralytic ileus, toxic megacolon, or pseudomembranous colitis without diarrhoea or in cases where a stool sample is not feasible. Rectal swabs are an exception to the routine use of stool samples for *C. difficile* testing and can be used alongside other diagnostic methods such as colonoscopy or clinical indicators (3,38,97-99).

Rectal swabs should be analysed using molecular methods and requires validation prior to use to ensure reliable results (3,38,97-99).

8.3 Specimen Collection

For safety considerations refer to Section 7.

Faecal samples from symptomatic patients should be taken at the earliest point *C. difficile* is suspected.

A liquid specimen of 1 - 2mL is sufficient for culture and toxin detection.

Frequency of specimen collection is dependent on the clinical condition of patient.

8.3.1 Correct specimen type and method of collection

Specimens may be passed into a clean, dry, disposable bedpan or similar container and transferred into a CE marked leak proof container. The specimen is unsatisfactory if any residual soap, detergent, or disinfectant remains in the pan. Samples mixed with large amounts of urine are also unsuitable.

Ideally, the sample container should be one quarter full (to indicate the patient has diarrhoea) and the sample should take the shape of the container. Formed stools are usually unsuitable for investigation for *C. difficile*. These should be rejected with the appropriate comment appended to the report, unless specifically requested by a consultant microbiologist or equivalent.

8.4 Transport and Storage

Specimens should be transported and processed as soon as possible.

If processing is delayed, refrigeration at 4°C is preferable to storage at ambient temperature. Refrigerate for up to the time indicated in the manufacturer's recommendations if unable to process within two hours. Freezing samples at -20°C compromises faecal toxin testing (100). Therefore, it is essential to conduct toxin tests before long-term freezing and thawing of samples for subsequent culturing and typing (2,4,100).

All *C. difficile* toxin immunoassay positive faecal samples should be kept refrigerated or frozen, for culture and subsequent typing, which may be required in certain situations e.g., an outbreak or as part of surveillance (101).

Where capacity allows, storage of toxin immunoassay negative but NAAT positive samples may be useful, as transmission may only be apparent when these strains are typed. It is not necessary to keep the whole specimen, a small aliquot will suffice (102). The duration of storage needs to be determined locally but should allow for appropriate outbreak investigation, where necessary (87).

9 Laboratory processes (analytical stage)

9.1 *C. difficile* infection diagnostic procedure

A two-stage testing approach is required consisting of a GDH immunoassay or NAAT followed by a toxin A and B immunoassay. An additional third test is to be performed in cases of inconsistent GDH and toxin A and B results (1-4). In this case, NAAT should be performed as a reflex test to distinguish between samples containing toxigenic and non-toxigenic strains (1,3,6). The requirements for further *C. difficile* culture and identification vary by nation, refer to Section 5.5.1 *C. difficile* culture.

In Scotland, laboratory culture of faeces is recommended in cases of severe disease, suspected outbreaks and as part of [C. difficile snapshot programme](#) (4).

In England and Northern Ireland, culture may be undertaken by the CDRN.

In Wales, all GDH/NAAT positive samples should be referred to the UKARU for *C. difficile* culture.

10 Post-laboratory processes (post analytical stage)

10.1 Interpreting and reporting laboratory results

No single test is reliable as a stand-alone test for diagnosing *C. difficile* infection. A GDH immunoassay, NAAT or multiplex molecular gastrointestinal pathogen panels positive test is not sufficient for the diagnosis of CDI (see sections 5 and 6). A two-stage testing approach followed by a third test in cases of inconsistent GDH and toxin A/B results (see footnote*) is advised for the diagnosis of CDI as outlined in section 9.

Results require clinical correlation, particularly for toxin immunoassay negative or inconsistent results. These should be discussed with the clinical team to ensure accurate interpretation and appropriate patient management. Appropriate IPC measures should be applied to symptomatic patients with a positive immunoassay or NAAT.

Tables 1 and 2 address a testing algorithm that incorporates reflex testing. Table 3 addresses a scenario where all tests are conducted simultaneously.

Whilst the reporting process may vary according to local procedures, it is crucial that results are conveyed accurately and in a timely manner to the relevant healthcare teams to facilitate appropriate decision-making and care.

Table 1: Interpretation and reporting GDH immunoassay followed by Toxin A and B immunoassay (followed by a 3rd test if required, see footnote ^a)

	1st Assay	2nd Assay	3rd Assay (if required ^a)	Interpretation ^c	Result interpretive comment ^d	Clinical comments for inclusion on laboratory reports
	GDH immunoassay	Toxin A and B immunoassay	NAAT (toxin DNA detection) ^b			
1	Negative	Not required	Not required	<i>Clostridioides difficile</i> not detected	No laboratory evidence of <i>Clostridioides difficile</i> infection	Consider other infectious and non-infectious causes of diarrhoea. If symptoms persist without another established cause, <i>C. difficile</i> re-testing should be considered.

Investigation of faecal specimens for *Clostridioides difficile*

	1st Assay	2nd Assay	3rd Assay (if required ^a)		Result interpretive comment ^d	Clinical comments for inclusion on laboratory reports
	GDH immunoassay	Toxin A and B immunoassay	NAAT (toxin DNA detection) ^b	Interpretation ^c		
2	Positive	Positive	Not required	<i>Clostridioides difficile</i> toxin detected	Laboratory evidence of <i>Clostridioides difficile</i> infection.	Consistent with <i>Clostridioides difficile</i> infection. Please clinically evaluate the patient and liaise with microbiology for advice. Refer to local management guidelines for treatment and IPC precautions to reduce risk of further transmission.
3	Positive	Negative	Negative	<i>Clostridioides difficile</i> toxin and DNA not detected	No laboratory evidence of <i>Clostridioides difficile</i> infection.	Consider other infectious and non-infectious causes of diarrhoea. If symptoms persist without another established cause, <i>C. difficile</i> re-testing should be considered.
4	Positive	Negative	Positive	<i>Clostridioides difficile</i> DNA detected.	May indicate <i>Clostridioides difficile</i> infection requiring treatment.	Please clinically evaluate the patient and liaise with microbiology for advice. Refer to local management guidelines for IPC precautions to reduce risk of further transmission.
5	Positive	Negative	Invalid/inhibitory ^e	Invalid/inhibitory result	Repeat specimen testing required.	Please clinically evaluate the patient and liaise with microbiology for advice. Refer to local management guidelines for treatment and IPC precautions to reduce risk of further transmission. Please send a repeat sample.
6	Positive	Negative	Test not available in-house and referred to external laboratory*(see footnotes)	Interim report	Specimen referred for additional testing.	Please clinically evaluate the patient and liaise with microbiology for advice. Refer to local management guidelines for IPC precautions to reduce risk of further transmission.

Table 2: Interpretation and reporting NAAT followed by Toxin A and B immunoassay.

	1st Assay	2nd Assay	Interpretation ^c	Result interpretive comment ^d	Clinical comments for inclusion on laboratory reports
	NAAT (toxin DNA detection) ^b	Toxin A and B immunoassay			
1	Negative	Not required	<i>Clostridioides difficile</i> DNA not detected	No laboratory evidence of <i>Clostridioides difficile</i> infection.	Consider other infectious and non-infectious causes of diarrhoea. If symptoms persist without another established cause, <i>C. difficile</i> re-testing should be considered.
2	Positive	Positive	<i>Clostridioides difficile</i> DNA and toxin detected	Laboratory evidence of <i>Clostridioides difficile</i> infection.	Consistent with <i>Clostridioides difficile</i> infection. Please clinically evaluate the patient and liaise with microbiology for advice. Refer to local management guidelines for treatment and IPC precautions to reduce risk of further transmission.
3	Positive	Negative	<i>Clostridioides difficile</i> DNA detected.	May indicate <i>Clostridioides difficile</i> infection requiring treatment.	Please clinically evaluate the patient and liaise with microbiology for advice. Refer to local management guidelines for IPC precautions to reduce risk of further transmission.
4	Invalid/inhibitory	Not required	Invalid/inhibitory result ^e	Repeat specimen testing required.	Please clinically evaluate the patient and liaise with microbiology for advice. Refer to local management guidelines for treatment and IPC precautions to reduce risk of further transmission. Please send a repeat sample.

Table 3: Interpretation and reporting GDH immunoassay performed at the same time as Toxin A and B immunoassay (followed by a 3rd test if required, see footnote ^a).

	Combined Assays		3 rd Assay (if required ^a)	Interpretation ^c	Result interpretive comment ^d	Clinical comments for inclusion on laboratory reports
	GDH immunoassay	Toxin A and B immunoassay	NAAT (toxin DNA detection) ^b			
1	Negative	Negative	Not required	<i>Clostridioides difficile</i> not detected.	No laboratory evidence of <i>Clostridioides difficile</i> infection.	Consider other infectious and non-infectious causes of diarrhoea. If symptoms persist without another established cause, <i>C. difficile</i> re-testing should be considered.
2	Positive	Positive	Not required	<i>Clostridioides difficile</i> toxin detected	Laboratory evidence of <i>Clostridioides difficile</i> infection.	Consistent with <i>Clostridioides difficile</i> infection. Please clinically evaluate the patient and liaise with microbiology for advice. Refer to local management guidelines for treatment and IPC precautions to reduce risk of further transmission.
3	Positive	Negative	Negative	<i>Clostridioides difficile</i> toxin and DNA not detected	No laboratory evidence of <i>Clostridioides difficile</i> infection.	Consider other infectious and non-infectious causes of diarrhoea. If symptoms persist without another established cause, <i>C. difficile</i> re-testing should be considered.
4	Positive	Negative	Positive	<i>Clostridioides difficile</i> DNA detected.	May indicate <i>Clostridioides difficile</i> infection requiring treatment.	Please clinically evaluate the patient and liaise with microbiology for advice. Refer to local management guidelines for IPC precautions to reduce risk of further transmission.

	Combined Assays		3 rd Assay (if required ^a)	Interpretation ^c	Result interpretive comment ^d	Clinical comments for inclusion on laboratory reports
	GDH immunoassay	Toxin A and B immunoassay	NAAT (toxin DNA detection) ^b			
5	Negative	Positive ^f (see footnotes. Report as invalid toxin result)	Negative	<i>Clostridioides difficile</i> DNA not detected	No laboratory evidence of <i>Clostridioides difficile</i> infection.	Consider other infectious and non-infectious causes of diarrhoea. If symptoms persist without another established cause, <i>C. difficile</i> re-testing should be considered.
6	Negative	Positive	Positive	<i>Clostridioides difficile</i> toxin and DNA detected	Laboratory evidence of <i>Clostridioides difficile</i> infection.	Consistent with <i>Clostridioides difficile</i> infection. Please clinically evaluate the patient and liaise with microbiology for advice. Refer to local management guidelines for treatment and IPC precautions to reduce risk of further transmission.
7	Positive	Negative	Invalid/inhibitory ^e	Invalid/inhibitory result	Repeat specimen testing required.	Please clinically evaluate the patient and liaise with microbiology for advice. Refer to local management guidelines for treatment and IPC precautions to reduce risk of further transmission. Please send a repeat sample.

Footnotes:

- a** UK SMI recommends the use of a NAAT for all samples where GDH and toxin A and B produce inconsistent results. If NAAT is not available in-house, an interim report should be issued, and specimens should be referred to a laboratory capable of testing using a *C. difficile* NAAT. It should be recognised that not performing an in-house NAAT severely compromises the clinical utility of laboratory results.

- b** Some multiplex NAAT detect not only toxin A and B genes, but also other virulence genes that may contribute to the severity of CDI such as binary toxins and markers associated with hypervirulent strains e.g., ribotype 027.
- c** Laboratories are not expected to report out what is in the 'Interpretation' column. The purpose of this column is it to help with results interpretation.
- d** The 'Results interpretive comment' column is recommended. The use of the terms 'Detected' and 'Not detected' is recommended over the terms 'Positive' and 'Negative' to reflect the test results more accurately without implying clinical significance and to avoid misinterpretation. In addition, GDH is suppressed in the results section as it is not considered helpful. Please refer to local laboratory's standard practice.
- e** Platforms may use different terminology, such as 'invalid' instead of 'inhibitory'.
- f** Where GDH and toxin A and B immunoassay testing is undertaken simultaneously, a toxin A and B immunoassay positive result in the absence of GDH and NAAT positivity should be reported as 'invalid' as there is an extremely high probability that it represents a false positive toxin A and B immunoassay result.

10.2 Repeat Testing of Specimens

In the event of negative test results for CDI, where patient symptoms persist in the absence of an alternative diagnosis, re-testing is recommended as it is known that false negatives can occur (103). In the event of a positive test result, subsequent testing is not necessary for test of cure as *C. difficile* may persist in the bowel without causing disease (104).

If a patient recovers from CDI diarrhoea and then has a recurrence of symptoms, develops other symptoms consistent with CDI, or symptoms persist after completing a treatment course, new faecal samples should be tested for CDI.

When repeat testing is required within 28 days of a positive test, laboratories should adhere to a strict clinical testing criteria and interpret results with caution within 1 to 2 months of the CDI diagnosis to avoid misdiagnosis due to prolonged shedding of *C. difficile* toxin (105,106). Please note that not all samples would require re-testing within 28 days and teams are advised to seek microbiology advice.

For CDI symptoms, diarrhoea should meet the specific definition provided in the document for repeat testing. For definitions of key terms used in the document, refer to the scope of the document.

11 Mandatory reporting of *C. difficile* infection

In the UK there is a mandatory reporting scheme for CDI. The public health agencies within the UK, namely UKHSA (England), the Public Health Agency (Northern Ireland), Public Health Scotland and Public Health Wales capture cases of CDI that meet specific criteria. Please ensure that these cases are entered onto the relevant data capture system, where required. Details can be found below:

England:

https://hcaidcs.phe.org.uk/ContentManagement/LinksAndAnnouncements/HCAIDCS_Mandatory_Surveillance_Protocol_v4.4.pdf

In England there is also a collection of quarterly aggregated totals of laboratory results. For more information, please see the following link:

https://hcaidcs.phe.org.uk/ContentManagement/LinksAndAnnouncements/HCAIDCS_Case_Capture_QMLR_UserGuide_V2.0.pdf

Wales:

Data is extracted from LIMS and patient administration systems by PHW Healthcare and associated infection Antimicrobial Resistance and Prescribing Programme (HARP). Please refer to the following link for the Welsh HCAI Surveillance Monthly Dashboard:

<https://public.tableau.com/app/profile/victoria6405/viz/WalesHCAISurveillanceMonthlyDashboard/HBMonthlyDashboard>

Scotland:

[Protocol for the Scottish Surveillance Programme for *Clostridioides difficile* infection: user manual | National Services Scotland](#)

Northern Ireland:

Data of CDI cases are aggregated into a central repository via SharePoint. This data is validated against laboratory downloads to ensure accuracy and included in surveillance reports on a quarterly basis.

12 Antimicrobial susceptibility testing

Routine susceptibility testing is not generally carried out in the UK, however CDRN, UKARU and EBIS monitor antimicrobial susceptibility of isolates of *C. difficile* submitted as part of the voluntary surveillance schemes. Continuing surveillance of susceptibility is required to detect the emergence of resistance.

Vancomycin and fidaxomicin are currently the antibiotics of choice for treatment of CDI and as the choice depends upon the clinical scenario, it is recommended that local treatment guidelines are referred to (8). Oral metronidazole is no longer recommended to treat *C. difficile* infection. Refer to [EUCAST](#) and [NICE](#) guidelines for information.

Prudent use of antimicrobials according to local and national protocols is recommended.

13 Referral to reference or specialist testing laboratories

When sending away isolates to reference or specialist testing laboratories for processing, ensure that the specimen is placed in the appropriate package and transported accordingly. Follow local regulations and instructions provided by the reference or specialist testing laboratories for sending isolates/samples.

Contact the appropriate reference laboratory (refer to the links provided below) for information on the tests available, turnaround times, transport procedure and any other requirements for isolate/sample submission.

[England](#)

[Wales](#)

[Scotland](#)

[Northern Ireland](#)

13.1 Referral for Outbreak Investigations

Isolates or faeces for typing should be referred to the appropriate reference laboratory. The reference laboratory should be contacted prior to sample submission, where required.

Contact details for the reference laboratories can be found at:

- The *Clostridioides difficile* Ribotyping Network (CDRN) for England and Northern Ireland – regional microbiologists:
<https://www.gov.uk/guidance/clostridium-difficile-ribotyping-network-cdrn-guide-to-services>
- The UK Anaerobe Reference Unit (UKARU) in Cardiff:
<https://phw.nhs.wales/services-and-teams/reference-laboratories-and-specialist-services/uk-anaerobe-reference-unit-ukaru/>
- The Enteric Bacterial Infections Service (EBIS); formerly known as the Scottish Salmonella, Shigella and *Clostridioides difficile* Reference Laboratory; in Glasgow: <https://www.nhsggc.scot/staff-recruitment/staff-resources/laboratory-medicine/scottish-microbiology-reference-laboratories/enteric-bacterial-infections-service/>

Appendix: *C. difficile* culture and Identification

Culture

Alcohol shock method for culture of *C. difficile*

The advantage of using alcohol shock (or alternatively heat shock method) for the selection of *C. difficile* is that only spores should survive this process, and it eliminates the growth of other non-sporing faecal organisms. The selective agents within the culture medium are usually based on cefoxitin and cycloserine (although others have been described), and these are usually inhibitory to most other clostridial, and related, species and other gut microbiota bacteria. The resulting growth from an active case of infection is often a pure culture of *C. difficile*.

It should be noted that the same medium from different suppliers may give different colonial appearances and the descriptions given here are not absolute.

1. Make an approximate 1:1 suspension of stool sample and 70% methanol, or 95% ethanol; in a screw capped glass bijou.
2. Mix by vortexing and leave to settle at room temperature for 30 min.
3. Inoculate the pellet into a selective enrichment broth such as Cycloserine-Cefoxitin-Fructose Broth (CCFB) and incubate anaerobically at 35°C - 37°C, to enhance the recovery of *C. difficile* (107).
4. With a disposable pastette, inoculate two drops (approx 50 -75 µl) of the deposit to cefoxitin-cycloserine egg yolk* (CCEY) selective agar and streak for single colonies. At the same time, culture the control organisms on CCEY from their spore suspension and incubate as outlined below.
5. Incubate anaerobically at 35°C - 37°C for 48 - 72hr. Cultures may be examined after overnight incubation but should not be removed from the anaerobic workstation because sporulation is inhibited on selective media and young cultures may die on exposure to air. If using anaerobic jars, cultures must not be examined before 48 hours incubation.

* Egg-yolk supplement is optional.

Note: Subsequent to culture, a toxin test may be performed to determine whether or not the isolate is a toxin producing strain (46). There are also commercially available chromogenic or fluorogenic media which can be used to eliminate the need for shock treatment in *C. difficile* recovery as they are highly selective and allow for direct detection without pre-treatment (108-110). It should be noted that chromogenic agar may not identify some ribotypes (111,112). As with all media, chromogenic and selective agar should be verified prior to use.

Identification

Colonies of *C. difficile* can be recognised by the following characteristics:

- If using egg-yolk based agar, a lack of opacity surrounding the colonies due to non-production of lecithinase (unlike *Paraclostridium bifermentans* [previously *Clostridium bifermentans*], *C. perfringens* or *Paraclostridium sordellii* [previously *Clostridium sordellii*]). Follow individual media manufacturer's guidelines on colonial morphology.
- Green-yellow fluorescence under long-wave UV light (see below).
- Agglutination with *C. difficile* latex reagent for somatic antigen (see below).
- MALDI-TOF MS identification. This should be performed after 18-24hr subculture to FAA or blood agar. Older cultures may lead to poor identification due to spore formation.
- NAAT. Use of a method with high analytical specificity is essential.

For ease of identification, it is useful to sub-culture a putative *C. difficile* colony on Fastidious Anaerobe Agar (FAA) or blood agar (BA).

Refer to individual UK SMIs for organism identification.

Examination of plates

Colonies of *C. difficile* may be smooth or rough and may vary considerably in size. Typical colonies may be seen after sub-culture of suspect colonies from selective media on FAA or BA.

Colonial Fluorescence

- Remove the test and control plates from the anaerobic workstation/jar and examine the colonies for fluorescence. Expose the colonies to long wave ultra-violet light (365 nm) in a darkened room, or light box held closely to the UV source, and view by reflection.
- UV protective goggles must be worn, where required.
- Colonies of *C. difficile* may vary in the intensity of fluorescence, but this will appear as a green-yellow or chartreuse colour. Fluorescence is poorly developed on some agar bases and is strongest on FAA. It is important to compare fluorescence of the test colonies with that of the control organisms to clarify positive and negative results. The colonial fluorescence of cultures >48 hrs old on non-selective agars will diminish due to increased sporulation.
- Mark any suspect (fluorescent) colonies on the underside of the plate with a marker pen. Sub-culture to a FAA or BA plate and incubate anaerobically for 24-48hrs.
- **Note:** Gram staining is rarely useful directly from selective agars, but from blood agar plates sub-terminal spores should be visible with most vegetative rods staining as Gram positive with some Gram variable forms in common with many other clostridial, and related, species. Routine Gram staining is not recommended.

Latex agglutination test for somatic antigen

Use *C. difficile* somatic antigen latex agglutination and follow the instructions in the kit insert.

Limitations of the test

Cross-reactions with this reagent are known to occur with:

- *P. bifermentans*
- *P. sordellii*
- *T. glycolicus*

Controls

Set up controls alongside test cultures and on each new batch of medium.

Control organisms required:

- *P. bifermentans*.
- *P. sordellii*.
- *C. difficile*.

Other clostridial, and related, species are commonly mistaken for *C. difficile*. These include *C. innocuum*, *T. glycolicum*, *P. bifermentans* and *P. sordellii*. However, these may be differentiated according to the criteria listed in Table A.

Interpretation of results

Table A. Differential tests for recognition of colonies of *C. difficile*

	<i>C. difficile</i>	<i>P. bifermentans</i>	<i>P. sordellii</i>	<i>T. glycolicum</i>	<i>C. innocuum</i>
UV (Fluorescence) at 365nm	+	-	-	-	+
Latex agglutination	+	+	+	+	-
Lecithinase on Brazier's CCEY medium	-	+	+	-	-

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

Refer to individual UK SMIs for organism identification.

Culture reporting

C. difficile isolated/not isolated.

Where *C. difficile* is confirmed - isolates of *C. difficile* submitted for typing investigations. Further report to follow.

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An explanation of the reference assessment used is available in the [scientific information section on the UK SMI website](#).

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