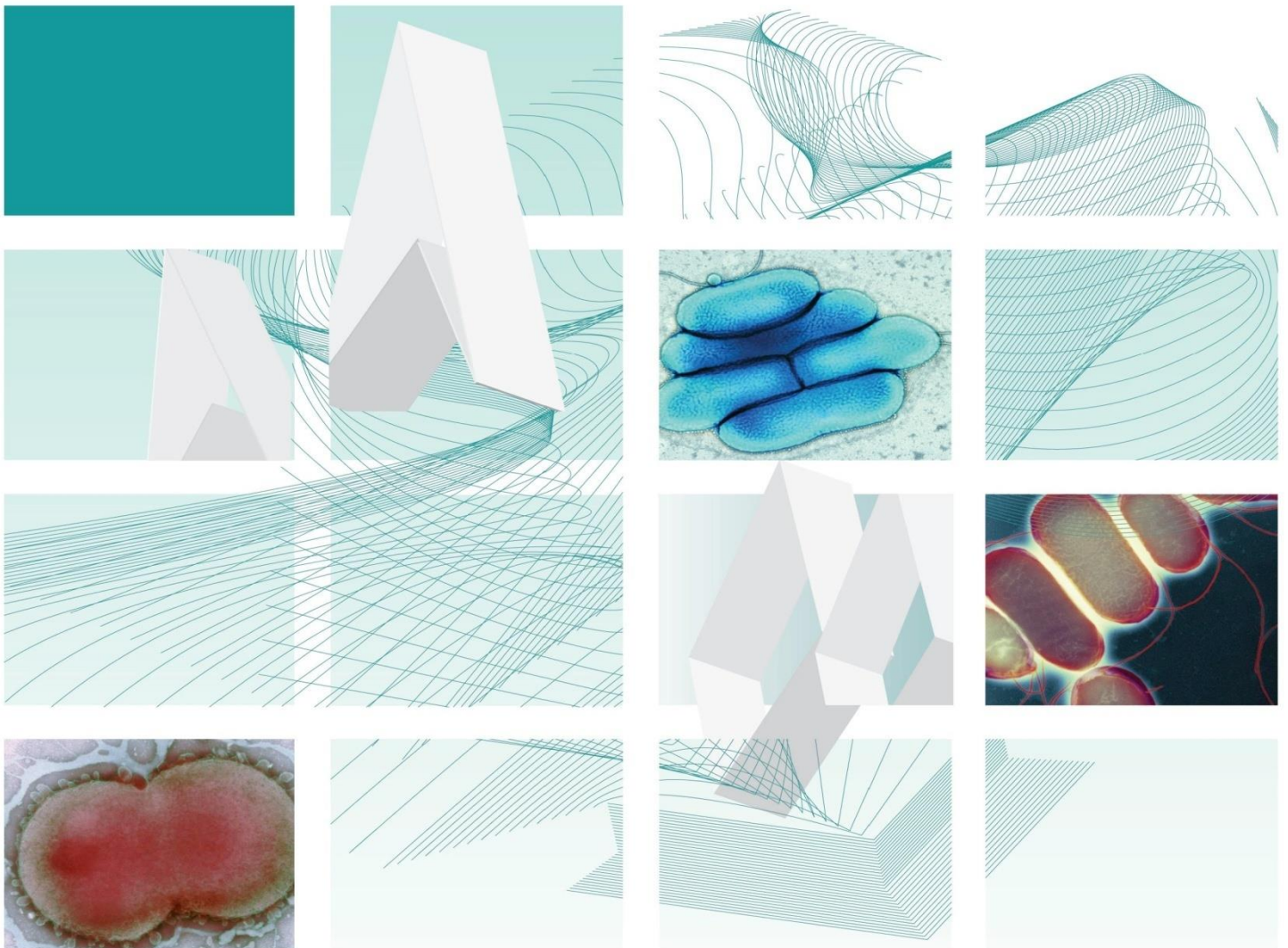




# UK Standards for Microbiology Investigations

## Processing of Faeces for *Clostridium difficile*



## Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

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

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UK Standards for Microbiology Investigations are produced in association with:



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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

## Amendment Table

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Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@phe.gov.uk](mailto:standards@phe.gov.uk).

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

|  |  |
|--|--|
| Amendment No/Date.                               | 7/19.09.18   |
| Issue no. discarded.                             | 1.6  |
| Insert Issue no.                                 | 1.7  |
| <b>Section(s) involved</b>                       | <b>Amendment</b>   |
| Section 4.8 Referral for Outbreak Investigations | This section has been updated to cover the arrangements within Northern Ireland for referring outbreak investigations. |
| Whole document.                                  | Scientific content remains unchanged.  |

|                                |   |
|--------------------------------|---|
| Amendment No/Date.             | 6/03.09.18  |
| Issue no. discarded.           | 1.5   |
| Insert Issue no.               | 1.6   |
| <b>Section(s) involved</b>     | <b>Amendment</b>  |
| Specimen processing/procedure. | <p>In section 4. 3.1, the alcohol shock method has been clarified and made easier to understand for users.</p> <p>The timing for incubation has been updated from 30min to 60min and references added to support this.</p> <p>Section 4.6 updated with information on the minimum level of identification in the laboratory.</p> <p>Some information formerly in section 4. 5 moved up to section 4.3.1 as it reads better.</p> |
| Whole document.                | Scientific content remains unchanged.   |

|                      |            |
|----------------------|------------|
| Amendment No/Date.   | 5/02.04.14 |
| Issue no. discarded. | 1.4        |
| Insert Issue no.     | 1.5        |

| Section(s) involved | Amendment  |
|---------------------|--|
| Whole document.     | <p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Standard safety and notification references have been reviewed and updated.</p> <p>Scientific content remains unchanged.</p> |

| Amendment No/Date.  | 4/29.03.12   |
|---|--|
| Issue no. discarded.  | 1.3  |
| Insert Issue no.  | 1.4  |
| Section(s) involved   | Amendment  |
| Whole document.   | <p>Document presented in a new format.</p> <p>The term "CE marked leak proof container" replaces "sterile leak proof container" (where appropriate) and is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to Directive itself EC<sup>1,2</sup>.</p> <p>Edited for clarity.</p> <p>Reorganisation of some text.</p> <p>Minor textual changes.</p> |
| Technical Information / Limitations.                                | Text to describe the use of the term CE marked leak proof container added.   |
| Sections on specimen collection, transport, storage and processing. | Reorganised. Previous numbering changed.   |
| Notification to HPA.  | Standard text added.   |
| References.   | Some references and hyperlinks updated.  |

## UK SMI<sup>#</sup>: Scope and Purpose

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### Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

### Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

### Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at <http://www.hpa.org.uk/SMI/Partnerships>. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

### Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent

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<sup>#</sup> Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

## Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

## Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs are subject to PHE Equality objectives

[http://www.hpa.org.uk/webc/HPAwebFile/HPAweb\\_C/1317133470313](http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313).

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

## Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

## Suggested Citation for this Document

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<http://www.hpa.org.uk/SMI/pdf>

## Scope of Document

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### Type of Specimen

Faeces

## Scope

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This SMI describes the culture and identification of *Clostridium difficile* from faeces. It advises that all samples selected for investigation should be tested in the first instance, with a toxin detection kit or cell cytoxin assay. The laboratory can then either store toxin-positive faeces for culture at a later time if required or, if from a laboratory in England, they can request access to the *C. difficile* Ribotyping Network for England (CDRNE) through the Regional Microbiologists. Isolates may be referred to the Anaerobe Reference Laboratory from elsewhere in the UK (except Scotland) for outbreak investigation, in conjunction with the Regional PHE laboratories under the DH/PHE surveillance scheme, or to the PHE Centre for Infection (Laboratory of HealthCare Associated Infection). In Scotland isolates may be submitted to the Scottish *C. difficile* Reference Service according to criteria developed in conjunction with Health Protection Scotland (HPS) as part of the mandatory surveillance programme:

<http://www.documents.hps.scot.nhs.uk/hai/sshaip/guidelines/clostridium-difficile/protocol-scottish-surveillance-programme-cdad-2009-03.pdf>

[B 30 - Investigation of Faeces Specimens for Enteric Pathogens](#) and [ID 8 - Identification of Clostridium Species](#) are recommended for additional background information.

## Introduction

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### ***Clostridium difficile* Infection (CDI) and Antibiotic Associated Diarrhoea (AAD)**

*C. difficile* is a Gram positive, spore forming, strictly anaerobic rod, so named because of the difficulty in original culture and characterisation<sup>3</sup>. Toxigenic strains produce large protein toxins A and B, both being major virulence factors<sup>4</sup>. Most disease associated with *C. difficile* is intestinal, though *C. difficile* may be isolated from blood or tissues<sup>5-9</sup>.

Changes in the gut flora associated with broad spectrum antibiotics and chemotherapeutic agents can result in colonisation by *C. difficile*; it is the commonest identifiable cause of AAD<sup>10</sup>. Almost all drugs with an antibacterial spectrum of activity have been implicated causally in AAD. The most frequently implicated drugs are those which have a marked effect on the microflora of the colon. These include broad spectrum beta lactams, cephalosporins, clindamycin and fluoroquinolones<sup>11</sup>. The incidence of *C. difficile* infection has been shown to decrease once antibiotic therapy is controlled.

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



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

The organism has been associated with outbreaks in hospitals and in extended care facilities for the elderly<sup>12</sup>. It represents an important cause of hospital-acquired infection. *C. difficile* can be isolated from soil, hospital environments and both human and animal faeces<sup>13</sup>. It is rarely found in the flora of normal adults, but up to 50% of infants may be colonised in the first few months, although disease is rarely present at this age<sup>14,15</sup>. *C. difficile* infection is more common in the elderly. The reasons for this are not clear, although there is some evidence to suggest that these patients have a less effective natural barrier to infection<sup>16</sup>. The importance of age can be demonstrated by figures from CDSC which show that 81% of cases (in which age was reported) were from patients >65 years old<sup>17</sup>.

Elderly medical patients, those undergoing general surgery, oncology patients and those with chronic renal disease are at particular risk of infection by *C. difficile*<sup>18-21</sup>.

### **Clostridium difficile Toxins and Toxin Detection**

Demonstration of *C. difficile* toxins in diarrhoeal stools is generally regarded as being suggestive of CDI, in the absence of any other recognised cause for gastrointestinal disturbance. In outbreaks it is suggested that primary culture of the organism is undertaken in tandem with toxin detection<sup>22</sup>. The culture of toxin negative faeces followed by toxin testing of the isolate may increase the number of patients diagnosed<sup>23</sup>.

Although considered by some to be the 'gold standard', use of tissue culture for the detection of *C. difficile* toxins by virtue of its cytopathic effect (neutralisable with *C. sordellii* antitoxin) requires technical expertise, and involves usually a 24 (up to 48) hour delay for the final result<sup>24</sup>. Tissue culture, especially with Vero cells, may detect other faecal cytotoxins that are associated with diarrhoea, eg, *C. perfringens* enterotoxin<sup>22</sup>. Cytopathic effect (CPE) that is not neutralised by *C. sordellii* antitoxin may indicate that another pathogen is present.

There are numerous commercially available EIA tests intended to detect the toxins of *C. difficile*. Some detect Toxin A, others A and B, although the sensitivity and specificity of these are variable<sup>25-29</sup>. Commercial EIAs that detect both toxins A and B are considered more appropriate than those which detect A alone, because infection due to A- B+ strains has been recorded<sup>30</sup>.

Latex agglutination kits are available, but are not as accurate as EIA due to poor sensitivity<sup>31</sup>. Detection by counter immuno-electrophoresis (CIE) has been suggested, but this method lacks sensitivity and specificity, and is not recommended<sup>32,33</sup>.

These and other testing procedures are reviewed in a recent report presented to the Department of Health<sup>34</sup>.

### **Typing of *C. difficile***

Typing of isolates of *C. difficile* is sometimes useful in the investigation of multiple cases of infection. Typing methods that have been used include bacteriophage/bacteriocin typing and serotyping<sup>35,36</sup>. PCR ribotyping is gaining

acceptance as an internationally recognised method and within England a PCR ribotyping network (CDRNE) has been established by the PHE for use when there is an increase in frequency of CDI, or increased severity, complication, recurrence or death rate associated with CDI<sup>37</sup>. The Anaerobe Reference Laboratory in Cardiff currently provides the same service for Wales and the rest of the UK (except Scotland) and performs the typing for the DH/PHE surveillance scheme. In England. In Scotland this service is provided by the Scottish *C. difficile* Reference Service, which is based at the Scottish *Salmonella* Reference Laboratory in Glasgow. Ribotyping and other more refined, molecular methods of strain differentiation are performed at the Centre for Infection. Other methods include cell surface protein profiles and other DNA-based methods of analysis<sup>38-40</sup>.

### Other Organisms Associated with AAD

In addition to *C. difficile*, infection with *C. perfringens*, *Staphylococcus aureus*, *Klebsiella oxytoca*, *Candida* species and *Salmonella* species have been implicated with AAD<sup>41,42</sup>.

## Technical Information/Limitations

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### Limitations of UK SMIs

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

### Specimen Containers<sup>1,2</sup>

SMIs use the term, “CE marked leak proof container,” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes.

# 1 Safety Considerations<sup>1,2,43-57</sup>

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## 1.1 Specimen Collection, Transport and Storage<sup>1,2,43-46</sup>

Use aseptic technique.

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

Compliance with postal, transport and storage regulations is essential.

## 1.2 Specimen Processing<sup>1,2,43-57</sup>

Containment Level 2.

Containment Level 3 if the following organisms are suspected from clinical information or laboratory findings:

- *Salmonella* Typhi
- *Salmonella* Paratyphi A, B and C
- Vero cytotoxin producing *E. coli* O157 (VTEC)
- *Shigella dysenteriae*

Under normal circumstances, a culture for *C. difficile* would not be requested from patients suspected of having any of the above organisms. However, if clinical details or routine culture indicate any of the above, then all specimen preparation and culture for *C. difficile* should be performed in the cabinet in Containment Level 3.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet<sup>49</sup>.

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

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

# 2 Specimen Collection

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## 2.1 Type of Specimens

Faeces

## 2.2 Optimal Time and Method of Collection<sup>58</sup>

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible<sup>58</sup>.

Specimen 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.

Formed stools are unsuitable for investigation for *C. difficile*. These should be rejected with the appropriate comment appended to the report.

## 2.3 Adequate Quantity and Appropriate Number of Specimens<sup>58</sup>

A liquid specimen of 1-2ml is sufficient for culture and toxin detection. Repeat testing of samples if there is no indication within a 28 day period. This applies to repeat testing of positive results. On the contrary, a negative test, if symptoms persist, should be re-tested as it is known that a one-off negative can occur.

In suspected outbreaks, samples should be stored at 4°C or frozen at -20°C for culture.

Isolates confirmed as *C. difficile* may be referred for ribotyping in accordance with the Anaerobe Reference Laboratory (ARL: guidelines for typing investigations listed online at: [www.hpa.org.uk/cfi/arl](http://www.hpa.org.uk/cfi/arl)) or the relevant Scottish guidance:

<http://www.documents.hps.scot.nhs.uk/hai/sshaip/guidelines/clostridium-difficile/smf-recommended-protocol-testing-for-cdiff-2009-12.pdf>

In general sending 10 isolates from each outbreak should be sufficient.

## 3 Specimen Transport and Storage<sup>1,2</sup>

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### 3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible<sup>58</sup>.

If processing is delayed, refrigeration is preferable to storage at ambient temperature<sup>44</sup>. Refrigerate for up to two days if unable to process within two hours. Freeze at -20°C or below if unable to process within two days of collection<sup>59</sup>.

All *C. difficile* toxin-positive faecal samples should be kept refrigerated, or frozen, so that culture can be performed to recover isolates for typing<sup>60</sup>. It is not necessary to keep the whole specimen; an aliquot in a small eppendorf would suffice. The duration of storage needs to be determined locally, but should allow appropriate outbreak investigation.

## 4 Specimen Processing/Procedure<sup>1,2</sup>

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### 4.1 Test Selection

If clinically indicated, patients who fulfil any of the following criteria should be screened: antibiotic-associated diarrhoea (everyone over the age of two); pseudomembranous colitis; and post-antibiotic treatment on all patients over 65 years old (in Scotland, all patients over 65 years old with diarrhoea)<sup>61</sup>.

Manufacturers' instructions must be followed when using toxin detection kits. It is recommended that a kit that is capable of detecting both A and B toxins is used. In order to ensure that strain type monitoring can be carried out in the event of an outbreak, samples of all positive stool samples should be at 4°C or -20°C for later culture<sup>60</sup>.

The culture and identification of *C. difficile* from faeces is intended to be followed in outbreaks, or as part of enhanced surveillance. This method is described in section 4.5 Culture and Investigation.

## 4.2 Appearance

N/A

## 4.3 Sample Preparation

For safety considerations refer to Section 1.2.

### 4.3.1 Standard

#### Alcohol shock method<sup>62-64</sup>

The advantage of using alcohol shock for 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 are usually based on cefoxitin and cycloserine (although others have been described), and these are usually inhibitory to most other clostridial species. 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.

- Make a suspension of approximate equal parts faeces and absolute alcohol (that is for example, 1g of stool sample added to 1ml of absolute alcohol. If stool sample is liquid, the same applies too.) in a screw capped glass bijoux.
- Vortex gently and leave at room temperature for 60min.
- With a disposable pastette, inoculate two drops (approx. 50 -75 µl) of the deposit to cefoxitin-cycloserine egg yolk agar\* (CCEY) selective agar and streak for single colonies. At the same time, inoculate the control organisms on CCEY from their spore suspension and incubate as outlined below
- Incubate anaerobically at 35°C - 37°C for 48 - 72hr.

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

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

### 4.3.2 Supplementary

N/A

## 4.4 Microscopy

### 4.4.1 Standard

N/A

### 4.4.2 Supplementary

N/A

## 4.5 Culture and Investigation

### 4.5.1 Culture media, conditions and organisms

| Clinical details/<br>conditions   | Specimen | Standard<br>media | Incubation |           |         | Cultures<br>read | Target<br>organism(s)        |
|---|----------|-------------------|------------|-----------|---------|------------------|------------------------------|
|   |          |                   | Temp<br>°C | Atmos     | Time    |                  |                              |
| As clinically indicated on patients who fulfil the following criteria:<br><br>antibiotic-associated diarrhoea (≥2yrs)<br><br>pseudomembranous colitis<br><br>post antibiotic treatment on all patients over 65 years old  | Faeces   | CCEY              | 35°C-37°C  | Anaerobic | 48-72hr | >48hr*           | <i>Clostridium difficile</i> |
| * Cultures may be examined after overnight incubation, but should not be removed from the anaerobic cabinet because sporulation is inhibited on selective media, and young cultures may die on exposure to air. If using anaerobic jars, cultures must not be examined before 48 hours incubation |          |                   |            |           |         |                  |                              |

## 4.6 Identification

### 4.6.1 Minimum level of identification in the laboratory

|                              |                 |
|------------------------------|-----------------|
| <i>Clostridium difficile</i> | 'Species' level |
|------------------------------|-----------------|

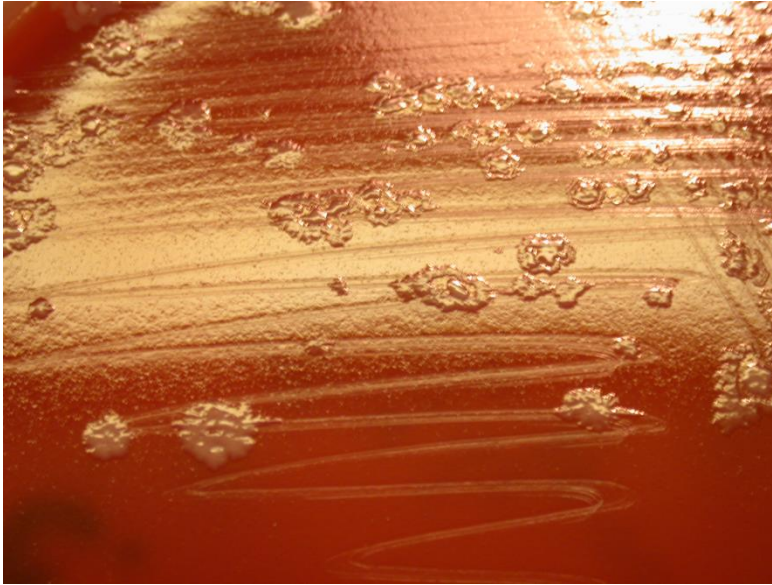
Organisms may be further identified if this is clinically or epidemiologically indicated. Colonies of *C. difficile* can be recognised by the following characteristics:

- If using egg-yolk based agar, a lack of opacity surrounding the colonies due to non-production of lecithinase (unlike *C. bifermentans*, *C. perfringens* or *C. 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)

For ease of identification, it is useful to sub-culture a putative *C. difficile* colony on FAA blood agar.

### 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 Fastidious Anaerobe Agar (See Plate 1):



Courtesy of Dr. Jon Brazier of the Anaerobic Reference Laboratory

Plate 1. Colonies of *C. difficile* on Fastidious Anaerobe Agar

### Colonial Fluorescence

- Remove the test and control plates from the incubator and examine the colonies for fluorescence. Expose the colonies to long wave ultra-violet light (365nm) in a darkened room or light box held closely to the UV source and view by reflection

**Note:** UV protective goggles must be worn.

- 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 >48hrs old on non-selective agars will diminish due to increased sporulation
- Mark any suspect (fluorescent) colonies on the underside of the plate with a felt tip pen. Sub-culture to a Fastidious Anaerobe Blood Agar (FAA) plate and incubate anaerobically for 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 species. Routine Gram staining is not recommended in this SMI.

### 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:

- *C. bifermentans*

- *C. sordellii*
- *C. glycolicum*

### Controls

Set up controls alongside test cultures and on each new batch of medium (see 2.5).

Control organisms required:

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

Other clostridial species are commonly mistaken for *C. difficile*. These include *C. innocuum*, *C. glycolicum*, *C. bifermentans* and *C. sordellii*. However, these may be differentiated according to the criteria listed in Table 1.

**Table 1. Differential tests for recognition of colonies of *C. difficile***

|                                      | <i>C. difficile</i> | <i>C. bifermentans</i> | <i>C. sordellii</i> | <i>C. 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 SMIs for organism identification.

## 4.7 Antimicrobial Susceptibility Testing

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

## 4.8 Referral for Outbreak Investigations

### Isolates

Isolates from outside England (except Scotland) from situations that warrant typing investigations should be referred to the Anaerobe Reference Laboratory (ARL) for PCR ribotyping as detailed below. Isolates should only be referred for ribotyping following discussion and agreement with the designated laboratory.

With a charcoal transport swab, swab all the growth from a 48hr anaerobic culture of *C. difficile* on non-selective media such as FAA and replace swab in tube of transport medium.

**Note:** It is important to send growth from a 48 – 72hr culture to ensure sporulation.

Anaerobe Reference Laboratory (ARL)



NPHS Microbiology Cardiff  
University Hospital of Wales  
Cardiff  
CF14 4XW

<http://www.hpa.org.uk/cfi/arl/>

Within Scotland, isolates from situations that warrant typing investigations should be referred to the Scottish *C. difficile* Reference Service for PCR ribotyping. Full contact details, referral criteria and isolate submission details are given on the website:

<http://www.ssrl.scot.nhs.uk/cdiffservices.asp>

### **Retention of isolates**

When harvesting the growth from the FAA purity plate take a sweep of the growth and mix this in a fresh bijou of alcohol/saline (2:1) labelled appropriately. Store at -20°C. Alternatively, make a heavy suspension of the colonies in alcohol/saline (2:1) and store at -70°C.

### **Faeces**

Within England there is now a *C. difficile* Ribotyping Network (CDRNE) consisting of six laboratories (in Leeds – reference centre, Birmingham, London, Manchester, Newcastle and Southampton) which is accessed in agreement with the relevant Regional Microbiologist. Access to this network should occur if a laboratory believes they have, or could have, a problem with an increased frequency or severity of cases of *C. difficile* infection; including increases in mortality, complications or recurrence rates. A standardised request form has been widely circulated in electronic format, which must be completed to access the service. Further details are available via the PHE website.

### **DH/PHE National *C. difficile* Surveillance Scheme**

Referrals submitted as part of DH/PHE national *C. difficile* surveillance scheme ('designated week') should not use the Outbreak Investigation form but the separate one labelled "DH/PHE National *C. difficile* Surveillance Scheme", PHE Regional Laboratories will request a pre-determined number of toxin positive stool samples within a given week from hospitals in their region rotation.

### **Northern Ireland *C. difficile* surveillance referral**

Within Northern Ireland, the HSC Public Health Agency is responsible for routine *C. difficile* surveillance. Toxin-positive faeces for routine PCR ribotyping and outbreak investigations should be referred to the Belfast Health & Social Care Trust laboratory service. Contact details are given on the website: <http://www.rvl-belfast.hscni.net/contact-us>.

### **Antimicrobial susceptibility testing**

The ARL is monitoring the antimicrobial susceptibilities of all isolates submitted under the DH/PHE surveillance scheme using the E test method for MIC determination to eight antibiotics. It is important that regular testing is done by all CDRNE laboratories to screen for any emerging resistance to the drugs of choice for treatment, namely metronidazole and vancomycin. Similar surveillance is performed in Scotland by the Scottish *C. difficile* Reference Service in conjunction with Health Protection Scotland (HPS).

## 4.9 Referral to Reference Laboratories

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

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

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

<https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services>

Scotland

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

Northern Ireland

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

## 5 Reporting Procedure

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### 5.1 Microscopy

N/A

### 5.2 Culture

Isolates of *C. difficile* submitted for typing investigations. Further report to follow.

### 5.3 Toxin Testing

*C. difficile* toxin detected **or**

*C. difficile* toxin not detected

### 5.4 Antimicrobial Susceptibility Testing

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

## 6 Notification to PHE<sup>65,66</sup> or Equivalent in the Devolved Administrations<sup>67-70</sup>

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The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

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

Other arrangements exist in [Scotland](#)<sup>67,68</sup>, [Wales](#)<sup>69</sup> and [Northern Ireland](#)<sup>70</sup>.

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