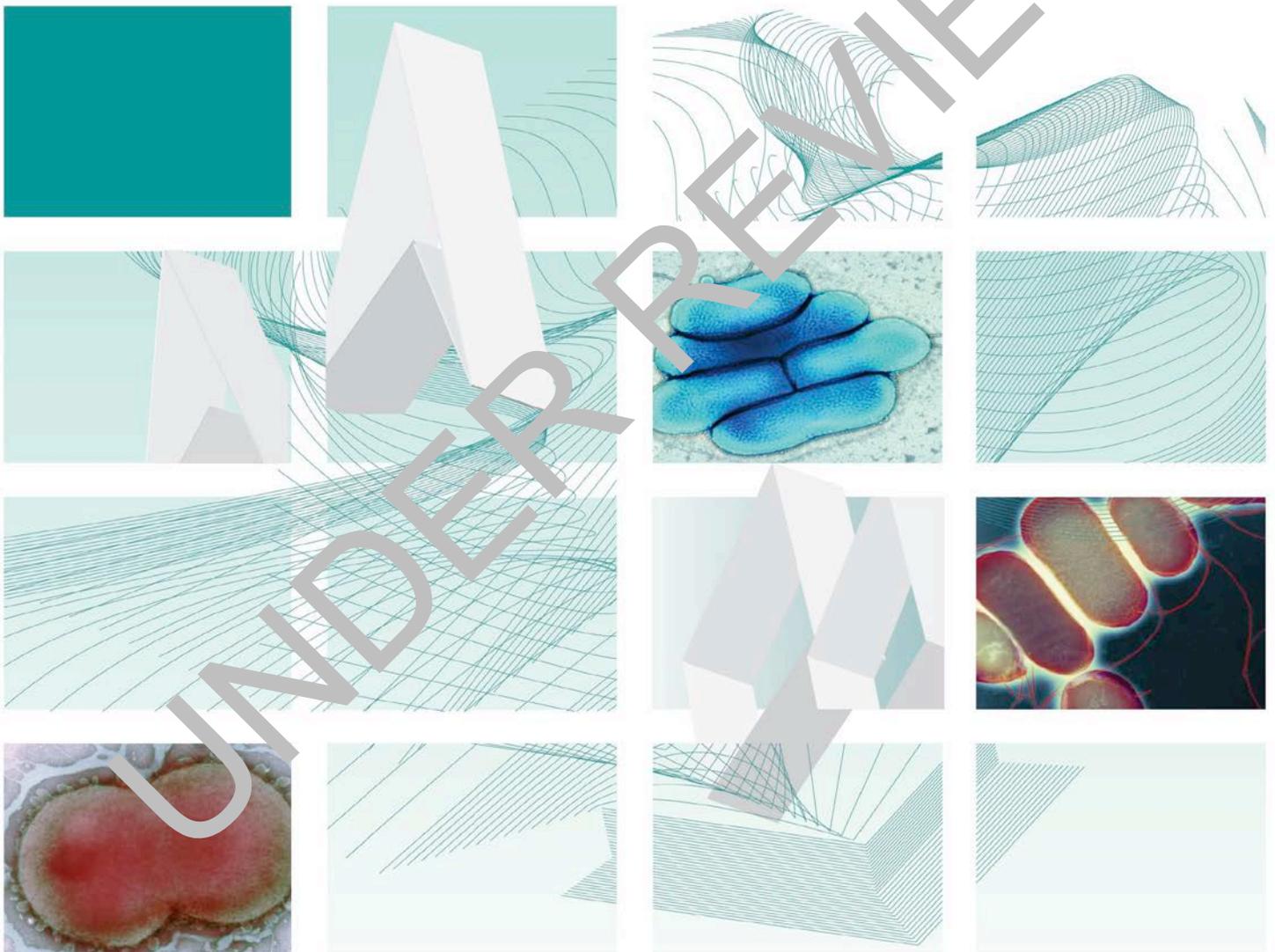




UK Standards for Microbiology Investigations

Identification of *Clostridium* species



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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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.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

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.

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

Amendment No/Date.	5/10.03.14
Issue no. discarded.	3.1
Insert Issue no.	3.2
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.	4/21.10.11
Issue no. discarded.	3
Insert Issue no.	3.1
Section(s) involved	Amendment
Whole document.	Document presented in a new format.
References.	Some references updated.

UK Standards for Microbiology Investigations[#]: Scope and Purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs 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.
- SMIs 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.

[#]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.

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 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/web/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

Public Health England. (2014). Identification of *Clostridium* species. UK Standards for Microbiology Investigations. ID 8 Issue 3.2. <http://www.hpa.org.uk/SMI/pdf>.

UNDER REVIEW

Scope of Document

This SMI describes the identification of *Clostridium* species.

There are many species of clostridia, which may be found naturally in animal faeces and the environment. Only species associated with humans will be discussed in this SMI.

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

The genus *Clostridium* currently contains approximately 100 species. In 1994 the heterogeneity of this species was confirmed by 16S rRNA gene sequencing. As a result five new genera and eleven new species were proposed, none of which appear to be relevant to human infections^{1,2}.

Characteristics

Clostridium species are Gram positive rods (some are Gram variable), often arranged in pairs or short chains, with rounded or sometimes pointed or square end. They are often pleomorphic. *Clostridium* species vary considerably in their oxygen tolerance. Some species such as *Clostridium novyi* type I and *Clostridium haemolyticum* are among the strictest of obligate anaerobes and may require extended incubation on pre-reduced or freshly prepared plates and total handling in an anaerobic chamber. Conversely, *Clostridium tertium*, *Clostridium histolyticum* and *Clostridium carnis* are aerotolerant and will form colonies on blood agar plates incubated in an atmosphere of air with 5-10% added CO₂².

Virtually all of the members of the genus, except *Clostridium perfringens*, are motile with peritrichous flagella and form oval or spherical endospores that may distend the cell. They may be saccharolytic or proteolytic and are usually catalase negative. Many species produce potent exotoxins³.

Toxins of *Clostridium* species

Clinically significant *Clostridium* species produce a variety of toxins. It is the production of these toxins which leads to the distinctive clinical features of the diseases they cause, eg tetanus and botulism result from the production of neurotoxins that are amongst the most lethal substances known to man⁴. Clostridial toxins are biologically active proteins that are antigenic in nature and can therefore be neutralised with specific antisera. Detection of a particular toxin in a patient sample may be diagnostic and therefore render isolation of the organism unnecessary (eg *Clostridium difficile*).

Clostridium perfringens is the most commonly isolated *Clostridium* species. Five types (A-E) may be distinguished by the combinations of major lethal toxins they produce².

Principles of Identification

Clues to the identity of certain pathogenic species may be obtained by observing characteristics such as colonial appearance, Gram stain appearances and the presence or absence of β-haemolysis. Other phenotypic tests may also be applied to

obtain a presumptive identification in conjunction with the use of a good laboratory manual such as the Wadsworth-KTL Anaerobe Laboratory Manual⁵. It is important to ensure the culture is pure, as the fine spreading growth of some *Clostridium* species may mask contaminating organisms. If confirmation of identity is required, isolates should be referred to the Anaerobe Reference Laboratory, Cardiff.

If *Clostridium botulinum* is suspected, samples of patient's serum, faeces and implicated foodstuff should be referred directly to the Food Safety Microbiology Laboratory, Colindale.

Technical Information/Limitations

N/A

UNDER REVIEW

1 Safety Considerations⁶⁻²²

Hazard Group 2 organisms

Refer to current guidance on the safe handling of all Hazard Group 2 organisms documented in this SMI.

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

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

Compliance with postal and transport regulations is essential.

2 Target Organisms

***Clostridium* species Reported to Have Caused Human Disease³**

Commonly isolated

C. perfringens

C. septicum

C. tertium

C. difficile

Rarely isolated

C. novyii type A

C. sordellii

Very rarely isolated

C. tetani

C. histolyticum

C. botulinum

Commonly Isolated “Non-Pathogenic” Clostridia

C. sporogenes

C. ramosum

C. innocuum

C. paraputrificum

C. cadaveris

C. bifermentans

C. fallax

C. clostridioforme

3 Identification

3.1 Microscopic Appearance

Gram stain ([TP 39 - Staining Procedures](#))

Gram positive rods, which may possess a single endospore. Some species may be Gram variable.

Spore stain

Used to determine the shape and position of the spore (phase contrast microscopy is an alternative option).

<i>C. perfringens</i>	(Does not sporulate on ordinary media)
<i>C. botulinum</i>	Oval, subterminal
<i>C. difficile</i>	Oval, subterminal
<i>C. novyi</i>	Oval, subterminal
<i>C. sordellii</i>	Oval, subterminal
<i>C. septicum</i>	Oval, subterminal
<i>C. tetani</i>	Round, terminal

3.2 Primary Isolation Media

Agar containing blood incubated anaerobically at 25–37°C for 40–48hr.

3.3 Colonial Appearance

Colonial appearance varies with species, and brief descriptions of the most common species are given here:

Organism	Characteristic of growth on agar containing blood after anaerobic incubation at 35–37°C for 40–48hr
<i>C. botulinum/sporogenes</i>	Large (2mm), irregularly circular, smooth, greyish, translucent with a fibrillar edge that may spread. Most strains are β -haemolytic; produces lipase
<i>C. difficile</i>	Glossy, grey, circular colonies with a rough edge; fluoresce green-yellow under UV light. They are usually non-haemolytic, with a characteristic farmyard smell.
<i>C. novyi</i>	Raised, circular colonies, which become flattened and irregular in old cultures. Colonies tend to fuse forming a spreading growth with a double zone of β -haemolysis. Type A produces lecithinase and lipase
<i>C. perfringens</i>	Large, smooth, regular convex colonies, but may be rough and flat with an irregular edge. Usually has a double zone of β -haemolysis; produces lecithinase
<i>C. septicum</i>	Usually produce a thick swarming growth with a narrow zone of β -haemolysis
<i>C. sordellii/bifermentans</i>	Grey-white, convex, circular colonies with crenated edges, which may spread. They may be β -haemolytic; produce lecithinase; indole positive
<i>C. tetani</i>	Fine swarming growth (may be difficult to see) which may appear β -haemolytic

Other <i>Clostridium</i> species	Colonial appearances vary, but may produce a spreading growth which may or may not be β -haemolytic
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3.4 Test Procedures

Nagler ([TP 22 - Nagler test](#)) with *C. perfringens* antitoxin

C. perfringens lecithinase is inhibited by the antitoxin as is that produced by *C. bifermentans* and *C. sordellii*.

Species other than *C. perfringens* may produce lecithinase.

Also examine for the production of lipase (pearly layer) on egg yolk agar.

Reverse CAMP test

Reverse CAMP test can be used for differentiation of *C. perfringens* from other *Clostridium* species²³.

Commercial identification kits

Results should be interpreted with caution in conjunction with other test results.

If clinically indicated refer to the Anaerobe Reference Laboratory for further identification.

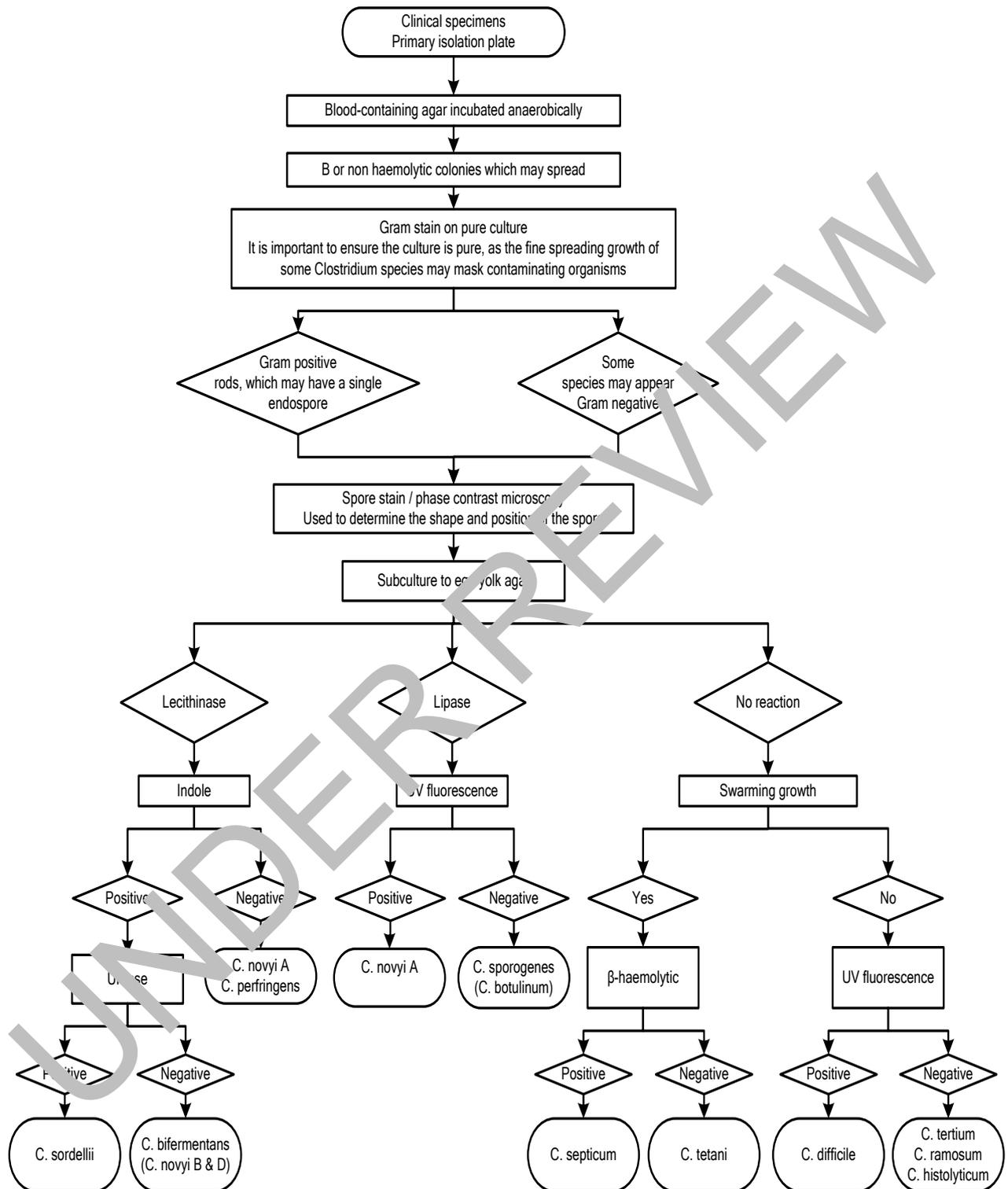
3.5 Further Identification

N/A

3.6 Storage and Referral

If required, save the pure isolate in a sterile anaerobe broth, or Robinson's cooked meat broth, for referral to the Anaerobe Reference Laboratory.

4 Identification of *Clostridium* species



The flowchart is for guidance only

5 Reporting

5.1 Presumptive Identification

If appropriate growth characteristics, colonial appearances and Gram stain of the culture are demonstrated, and the isolate is metronidazole susceptible.

5.2 Confirmation of Identification

Following Nagler plate, or Reverse CAMP test for *C. perfringens*, commercial identification kit results and/or Reference Laboratory report.

5.3 Medical Microbiologist

Inform the medical microbiologist of all positive cultures from normally sterile sites.

According to local protocols, the medical microbiologist should also be informed of presumed and confirmed *Clostridium* species. When the requestor has relevant information eg:

- Cases of trauma, penetrating injury, compound fracture or retained foreign body, or known injecting drug abuse (especially heroin)
- Septic abortion
- Suspicion of clostridial myonecrosis, (necrotising) myofasciitis, surgical wound infection (especially in cases with occlusive peripheral vascular disease and/or diabetes mellitus)
- Other serious medical conditions, eg alcohol or substance abuse, immunodeficiency, cancer, or persons receiving treatment for cancer (including neutropenia and/or mucositis)
- Food poisoning (especially involving descending paralysis with cranial nerve involvement) and/or consumption of unusual or imported foods (suspicion of botulism)
- Investigation of outbreaks
- Pseudomembranous colitis or antibiotic related diarrhoea
- Suspicion of tetanus

Follow local protocols for reporting to clinician

5.4 CDC

Refer to local Memorandum of Understanding.

5.5 Public Health England²⁴

Refer to current guidelines on CDSC and COSURV reporting.

5.6 Infection Control Team

Inform the infection control team of presumed and confirmed isolates of *C. botulinum* and *C. difficile*.

6 Referrals

6.1 Reference Laboratory

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory refer to the appropriate reference laboratory.

<http://www.hpa.org.uk/ProductsServices/MicrobiologyPathology/LaboratoriesAndReferenceFacilities/>

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

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

England and Wales

<http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370>

Scotland

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

Northern Ireland

<http://www.belfasttrust.hscni.net/Laboratory-Mutual-Services.htm>

7 Notification to PHE^{24,25} or Equivalent in the Devolved Administrations²⁶⁻²⁹

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'.

Other arrangements exist in Scotland^{26,27}, Wales²⁸ and Northern Ireland²⁹.

UNDER REVIEW

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