IDENTIFICATION OF CLOSTRIDIUM SPECIES

BSOP ID 8

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Centre for Infections
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Suggested citation for this document:
INDEX

STATUS OF NATIONAL STANDARD METHODS................................................................. 2
INDEX.......................................................................................................................... 3
AMENDMENT PROCEDURE ....................................................................................... 4
SCOPE OF DOCUMENT .............................................................................................. 5
INTRODUCTION ............................................................................................................. 5
TECHNICAL INFORMATION/LIMITATIONS ................................................................. 5
1 SAFETY CONSIDERATIONS .................................................................................... 6
2 TARGET ORGANISMS .............................................................................................. 6
3 IDENTIFICATION ....................................................................................................... 7
  3.1 MICROSCOPIC APPEARANCE .......................................................................... 7
  3.2 PRIMARY ISOLATION MEDIA ........................................................................... 7
  3.3 COLONIAL APPEARANCE .............................................................................. 7
  3.4 TEST PROCEDURES ......................................................................................... 8
  3.5 FURTHER TESTS ............................................................................................... 8
  3.6 STORAGE AND REFERRAL ............................................................................. 8
4 IDENTIFICATION OF CLOSTRIDIUM SPECIES - FLOW CHART ......................... 9
5 REPORTING ............................................................................................................. 10
  5.1 PRESUMPTIVE IDENTIFICATION .................................................................. 10
  5.2 CONFIRMATION OF IDENTIFICATION ......................................................... 10
  5.3 MEDICAL MICROBIOLOGIST ...................................................................... 10
  5.3 CCDC .............................................................................................................. 10
  5.5 CENTRE FOR INFECTIONS .......................................................................... 10
  5.6 INFECTION CONTROL STAFF ....................................................................... 10
6 REFERRALS ............................................................................................................ 11
7 ACKNOWLEDGEMENTS AND CONTACTS ......................................................... 12
REFERENCES ............................................................................................................ 13
**AMENDMENT PROCEDURE**

<table>
<thead>
<tr>
<th>Controlled document reference</th>
<th>BSOP ID 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlled document title</td>
<td>Identification of Clostridium species</td>
</tr>
</tbody>
</table>

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

<table>
<thead>
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<th>Amendment Number/ Date</th>
<th>Issue no. Discarded</th>
<th>Insert Issue no.</th>
<th>Page</th>
<th>Section(s) involved</th>
<th>Amendment</th>
</tr>
</thead>
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<td>2.1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>13</td>
<td>References</td>
<td>References reviewed and updated</td>
</tr>
</tbody>
</table>
IDENTIFICATION OF CLOSTRIDIUM SPECIES

SCOPE OF DOCUMENT

This National Standard Method (NSM) 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 NSM.

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.

Characteristics of Clostridium species

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 A 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 CO₂.

Virtually all of the members of the genus, except Clostridium perfringens, are motile with peritrichous flagellae 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 exotoxins.

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 man. Clostridial toxins are biologically active proteins that are antigenic in nature and can therefore be neutralised with specific antiserum. 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 produce.

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 ß-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 Manual. 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
1 SAFETY CONSIDERATIONS

Hazard Group 2 organisms

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

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 disease

Commonly isolated
- C. perfringens
- C. septicum
- C. tertium
- C. difficile

Rarely isolated
- C. novyi 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. paraputreficium
- C. cadaveris
- C. bifermantans
- C. fallax
- C. clostridioforme
3 IDENTIFICATION

3.1 MICROSCOPIC APPEARANCE
(See BSOPTP 39 - Staining Procedures)

Gram stain
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).

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

3.2 PRIMARY ISOLATION MEDIA
Agar containing blood incubated anaerobically at 35°C - 37°C for 40 – 48 h.

3.3 COLONIAL APPEARANCE
Colonial appearance varies with species and brief descriptions of the most common species are given here

<table>
<thead>
<tr>
<th>Organism</th>
<th>Characteristics of growth on agar containing blood after anaerobic incubation at 35°C – 37°C for 40 – 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. perfringens</td>
<td>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</td>
</tr>
<tr>
<td>C. botulinum/sporogenes</td>
<td>Large (3 mm), irregularly circular, smooth, greyish, translucent with a fibrillar edge that may spread. Most strains are β-haemolytic; produces lipase</td>
</tr>
<tr>
<td>C. difficile</td>
<td>Glossy, grey, circular colonies with a rough edge; fluoresce green-yellow under UV light. They are usually non-haemolytic, with a characteristic farmyard smell.</td>
</tr>
<tr>
<td>C. novyi</td>
<td>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</td>
</tr>
<tr>
<td>C. sordellii/bifermentans</td>
<td>Grey-white, convex, circular colonies with crenated edges, which may spread. They may be β-haemolytic; produce lecithinase; indole positive</td>
</tr>
<tr>
<td>C. septicum</td>
<td>Usually produce a thick swarming growth with a narrow zone of β-haemolysis</td>
</tr>
<tr>
<td>C. tetani</td>
<td>Fine swarming growth (may be difficult to see) which may appear β-haemolytic</td>
</tr>
</tbody>
</table>
Other Clostridium species | Colonial appearances vary, but may produce a spreading growth which may or may not be β-haemolytic

3.4 TEST PROCEDURES

Nagler (see BSOPTP 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 can be used for differentiation of *C. perfringens* from other *Clostridium* species\(^\text{19}\).

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 TESTS

N/A

3.6 STORAGE AND REFERRAL

If required save the pure isolate in fastidious anaerobe broth or Robinson’s cooked meat broth for referral to the Anaerobe Reference Laboratory.
4 IDENTIFICATION OF CLOSTRIDIUM SPECIES - FLOW CHART

Clinical specimens
Primary isolation plate

Blood-containing agar incubated anaerobically

B- or non-haemolytic colonies which may spread

Gram stain on pure culture
It is important to ensure the culture is pure, as the fine spreading growth of some Clostridium species may mask contaminating organisms

Gram-positive rods, which may have a single endospore
Some species may appear Gram-negative

Spore stain/phase contrast microscopy
Used to determine the shape and position of the spore

Subculture to egg yolk agar

Lecithinase

Indole
Positive
Negative

Lipase

UV fluorescence
Positive
Negative

No reaction

Swarming growth

Urease
Positive
Negative

C. sordellii
C. bifermentans
C. novyi, B & L

C. novyi

C. sordellii (C. botulinum)

β-haemolytic
Positive
Negative

C. septicum
C. tetani
C. difficile
C. tertium
C. novyi
C. histolyticum
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 a presumptive and confirmed Clostridium species. When the request card bears 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) myofascitis, 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

5.3 CCDC
Refer to local Memorandum of Understanding.

5.5 CENTRE FOR INFECTIONS
Refer to current guidelines on CDSC and COSURV reporting.

5.6 INFECTION CONTROL STAFF
Inform the infection control team of presumptive and confirmed isolates of C. botulinum and C. difficile.
6 REFERRALS

6.1 REFERENCE LABORATORY

For identification and for information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory refer to:

Anaerobe Reference Laboratory
NPHS Microbiology Cardiff
University Hospital of Wales
Heath Park
Cardiff CF14 4XW

Telephone +44 (0) 29 2074 2171 or 2378

http://www.hpa.org.uk/cfi/arl/default.htm

For toxin detection and for information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory refer to:

Food Safety Microbiology Laboratory
Centre for Infections
Health Protection Agency
61 Colindale Avenue
London NW9 5HT

http://www.hpa.org.uk/cfi/fsml/default.htm

Contact CfI main switchboard: Tel. +44 (0) 20 8200 4400
7 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

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For further information please contact us at:

Standards Unit
Evaluations and Standards Laboratory
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ

E-mail: standards@hpa.org.uk
REFERENCES


8. Health and Safety Executive, editor. Biological Agents: Managing the risks in laboratories and healthcare premises. 5 A.D.


