National Public Health Service for Wales
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NHS

Health Protection Agency

NATIONAL STANDARD METHOD

IDENTIFICATION OF *CLOSTRIDIUM* SPECIES

BSOP ID 8

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections

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Suggested citation for this document:
Health Protection Agency (2008). *Identification of Clostridium species*. National Standard Method BSOP ID 8 Issue 3. http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

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AMENDMENT PROCEDURE

Controlled document reference	**BSOP ID 8**
Controlled document title	**Identification of C*lostridium* species**

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.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
3/ 14.07.08	2.1	3	1	**Front Page**	NIMAG logo added
			All	**All**	PDF links amended to read reference document title
			13	**References**	References reviewed and updated

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[2], none of which appear to be relevant to human infections[3].

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 CO_2[3].

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[4].

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[5]. 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 produce[3].

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[6]. 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[7-18]

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[4]**

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

(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

Organism	Characteristics of growth on agar containing blood after anaerobic incubation at 35°C – 37°C for 40 – 48 h
C. perfringens	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
C. botulinum/ sporogenes	Large (3 mm), irregularly circular, smooth, greyish, translucent with a fibrillar edge that may spread. Most strains are β-haemolytic; produces lipase
C. difficile	Glossy, grey, circular colonies with a rough edge; fluoresce green-yellow under UV light. They are usually non-haemolytic, with a characteristic farmyard smell.
C. novyi	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
C. sordellii /bifermentans	Grey-white, convex, circular colonies with crenated edges, which may spread. They may be β-haemolytic; produce lecithinase; indole positive
C. septicum	Usually produce a thick swarming growth with a narrow zone of β-haemolysis
C. tetani	Fine swarming growth (may be difficult to see) which may appear β-haemolytic

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[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.

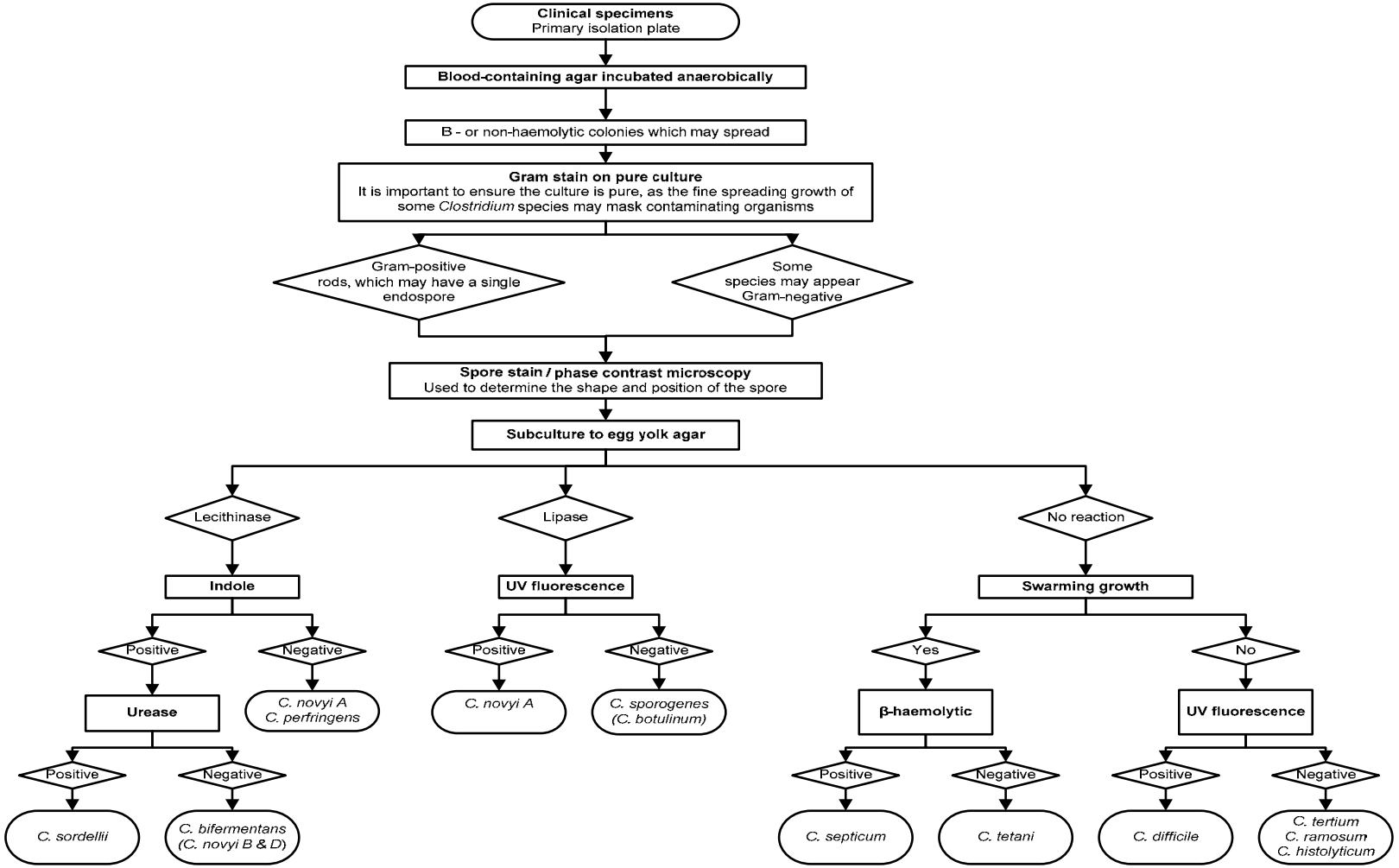
3.5 FURTHER TESTS

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



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) 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

5.3 CCDC

Refer to local Memorandum of Understanding.

5.5 CENTRE FOR INFECTIONS[20]

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.

The National Standard Methods are issued by Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency, London.

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