IDENTIFICATION OF CORYNEBACTERIUM SPECIES

BSOP ID 2

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

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

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IDENTIFICATION OF CORYNEBACTERIUM SPECIES

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the identification to species level of Corynebacterium diphtheriae, Corynebacterium ulcerans and Corynebacterium pseudotuberculosis isolated from throat, skin and other sites. These organisms may be isolated from suspected cases of classical diphtheria, cutaneous diphtheria and very rarely from other clinical infections such as pharyngitis or chronic skin infections. The importance of toxin production by this species in the pathogenesis of disease is emphasised. The document also describes the identification of non-toxigenic species, Corynebacterium jeikeium, Corynebacterium striatum and clinically significant species (for Arcanobacterium haemolyticum, formerly known as Corynebacterium haemolyticum see BSOPID 3 - Identification of Listeria species, non-toxigenic Corynebacterium species and other non-sporing gram-positive rods.)

INTRODUCTION

This NSM covers four tests for the preliminary identification of the pathogenic Corynebacterium species and recommends that the organisms be sent to the Reference Laboratory for confirmation of identification and toxin testing if required.

Taxonomy

The potentially toxigenic corynebacteria comprise C. diphtheriae, C. pseudotuberculosis and C. ulcerans. C. diphtheriae consists of four biotypes: gravis, mitis, intermedius and belfanti.

Characteristics

Corynebacterium species are Gram-positive rods, often with clubbed ends, occurring singly or in pairs. Some cells may stain unevenly giving a beaded appearance. Agar containing blood and potassium tellurite, such as Hoyle’s tellurite medium, serves as a selective and differential medium.

C. diphtheriae grows as pinpoint grey/black colonies on Hoyle's tellurite agar in 16 - 18 hours and produces characteristic colonies after 48 hours. Isolates of potentially toxigenic Corynebacterium species will also grow on blood agar. Colonial morphology varies among the species. C. ulcerans and C. pseudotuberculosis colonies may be slightly β-haemolytic on blood agar. Although C. diphtheriae will grow on nutrient agar, the presence of serum or blood will improve the growth. C. diphtheriae, C. ulcerans and C. pseudotuberculosis are facultatively anaerobic, non-sporing, non-capsulated and non acid-fast. These organisms are non-motile and catalase-positive.

C. ulcerans and C. pseudotuberculosis are urease-positive which may be used to distinguish them presumptively from C. diphtheriae.

Strains of these species can all harbour the phage-borne diphtheria tox gene, which is required for the production of toxin. Toxigenic strains may cause diphtheria or diphtheria-like illness. Possible toxigenic strains of Corynebacterium species should be referred to the Reference Laboratory for detection of toxin production as soon as possible. A polymerase chain reaction (PCR) directed at the A subunit of the diphtheria toxin gene can also be used to detect the tox gene, the structural gene for diphtheria toxin, although it does not confirm toxin production. PCR is rapid and can be completed within four hours of receipt of the strain, although toxin production must always be verified by the phenotypic test for toxigenicity.

Non-toxigenic strains of corynebacteria eg non-toxigenic C. diphtheriae, C. ulcerans, C. jeikeium and C. striatum are also known to cause infections in humans including endocarditis, pulmonary infection, leukaemia and endocarditis. Both C. jeikeium and C. striatum are non-haemolytic, urease negative and catalase positive.
Principles of identification

Isolates from primary culture are identified by colonial appearance, Gram stain, and four preliminary tests, which permit the presumptive identification of the potentially toxigenic *Corynebacterium* species within four hours. Additional identification may be made using a commercial identification kit in conjunction with toxin testing. It is advisable that suspected toxigenic cultures are sent to the Streptococcus and Diphtheria Reference Unit (SDRU) for confirmation of identification and toxigenicity testing. Use of Albert’s stain is not recommended in this NSM, as metachromatic granules are not specific to *C. diphtheriae* or any of the potentially toxigenic corynebacteria.

The interpretation of the clinical significance of *Corynebacterium* isolated from microbiological samples can be problematic. *Corynebacterium* isolated as a predominant organism from a specimen from a normally sterile site, wound, abscess or purulent sputum, from more than one blood culture set or present at $\geq 10^5$ cfu/mL in a pure culture from urine should be considered for identification to species level. The clinical significance is strengthened when isolating *Corynebacterium* species from multiple samples or when they are seen in a Gram stained smear as the predominant organism or associated with a significant leucocyte response.

**TECHNICAL INFORMATION/LIMITATIONS**

N/A
1 SAFETY CONSIDERATIONS

C. diphtheriae, C. ulcerans and C. pseudotuberculosis are Hazard Group 2V organisms, and in some cases the nature of the work may dictate full Containment Level 3 conditions.

Suspected isolates of potentially toxigenic corynebacteria should always be handled in a microbiological safety cabinet. For the urease test a urea slope is considered safer than a liquid medium.

C. diphtheriae and C. ulcerans cause severe and sometimes fatal diseases. Laboratory acquired infections have been reported\(^9\). The organism infects primarily by the respiratory route. Vaccination against diphtheria is available; guidance is given in DH Green Book at [http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/GreenBook/fs/en](http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/GreenBook/fs/en). Diphtheria antitoxin for the treatment of clinical cases is distributed by the HPA Cfl Immunisation Department and should be given without waiting for bacteriological confirmation.

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

*Corynebacterium* species which are potentially toxigenic\(^2\)

*Corynebacterium diphtheriae* var *belfanti*
*Corynebacterium diphtheriae* var *gravis*
*Corynebacterium diphtheriae* var *intermedius*
*Corynebacterium diphtheriae* var *mitis*
*Corynebacterium pseudotuberculosis*
*Corynebacterium ulcerans*

*Corynebacterium* species which are non-toxigenic\(^5\)

*Corynebacterium diphtheriae*
*Corynebacterium pseudotuberculosis*
*Corynebacterium ulcerans*
*Corynebacterium jeikium*
*Corynebacterium striatum*

Other *Corynebacterium* species have been known to cause human infection\(^2,5\).
3 IDENTIFICATION

3.1 MICROSCOPIC APPEARANCE

Gram stain (see BSOPTP 39 - Staining Procedures)

Gram-positive rods, pleomorphic, slightly curved with tapered or clubbed ends. Cells may occur singly or in pairs, often in a “V” formation (forming “chinese letters”). Cells usually stain weakly and unevenly giving a beaded appearance.

3.2 PRIMARY ISOLATION MEDIA

Blood agar - skin swabs incubated in 5 -10% CO₂ at 35 - 37°C for 40 - 48 h and throat swabs incubated anaerobically at 35 - 37°C for 16 - 24 h.

Hoyle’s tellurite agar incubated in air at 35 - 37°C for 16 - 48 h.

β-haemolytic streptococci may also be present, particularly in throat swabs.

3.3 COLONIAL APPEARANCE

Appearance varies among species: for more information refer to Section 3.4 Test procedures.

3.4 TEST PROCEDURES

All potentially toxigenic corynebacteria are catalase positive (see BSOPTP 8 - Catalase Test) and pyrazinamidase* negative. For non-toxigenic corynebacterium the catalase test results are varied Rapid (4 h) tests should be performed for urease, pyrazinamidase, catalase and nitrate reductions.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Culture media</th>
<th>Biochemical tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hoyle’s tellurite agar</td>
<td>Blood agar</td>
</tr>
<tr>
<td><strong>C. diphtheriae biotype var gravis</strong>[^19,20]</td>
<td>dull, grey/black, opaque colonies, 1.5-2.0 mm in diameter, matt surface, friable, tending to break into small segments when touched with a straight wire</td>
<td>non-haemolytic</td>
</tr>
<tr>
<td><strong>C. diphtheriae biotype var mitis</strong>[^19,20]</td>
<td>grey/black, opaque colonies, 1.5 - 2.0 mm in diameter, entire edge and glossy smooth surface; size variation is common</td>
<td>colonies exhibit a small zone of β-haemolysis</td>
</tr>
<tr>
<td><strong>C. diphtheriae biotype var intermedius</strong>[^19,20]</td>
<td>small, grey/black, shiny surface, discrete, translucent colonies, 0.5-1.0 mm in diameter</td>
<td>colonies exhibit a small zone of β-haemolysis</td>
</tr>
<tr>
<td><strong>C. diphtheriae biotype var belfanti</strong>[^19,20]</td>
<td>grey/black, opaque colonies, 1.5-2.0 mm in diameter, entire edge and glossy smooth surface; size variation is common</td>
<td>colonies exhibit a small zone of β-haemolysis</td>
</tr>
<tr>
<td><strong>C. ulcerans</strong>[^19,20]</td>
<td>grey/black, very dry opaque colonies</td>
<td>colonies exhibit a small zone of β-haemolysis</td>
</tr>
</tbody>
</table>

[^19,20]: References or notes for specific strain characteristics.
**3.5 FURTHER IDENTIFICATION**

It is important that a preliminary identification of possible colonies of *C. diphtheriae* or other potentially toxigenic *Corynebacterium* species is made as rapidly as possible with the use of 4h tests.

All isolates which undergo the four 4 h preliminary tests should be subcultured to a blood agar plate for purity and to a blood agar slope (preferably) or Loeffler (for possible referral to the Streptococcus and Diphtheria Reference Unit (SDRU)) at the time that the tests are set up.

The preliminary tests provide an indication of the likely presence or absence of *C. diphtheriae*, *C. ulcerans* or *C. pseudotuberculosis*. The results should be considered together with the clinical details.

Commercial identification kit

Additional biochemical/molecular tests\(^{19,20}\).

**3.6 STORAGE AND REFERRAL**

Refer the presumptive *C. diphtheriae*, *C. ulcerans* or *C. pseudotuberculosis* isolate on a Loeffler or blood agar slope immediately to the SDRU. The SDRU operates a 24 hours a day, 7 days a week diagnostic service.

**4 IDENTIFICATION FLOWCHART**

N/A

**5 REPORTING**

**5.1 PRESUMPTIVE IDENTIFICATION**

Presumptive identification may be made if appropriate growth characteristics, colonial appearance, Gram stain of the culture; catalase and 4 hour test results are demonstrated.

**5.2 CONFIRMATION OF IDENTIFICATION**

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### Identification of Corynebacterium species

<table>
<thead>
<tr>
<th><strong>C. pseudo-tuberculosis</strong>(^{19,20})</th>
<th>grey/black, very dry opaque colonies</th>
<th>small zone of β-haemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C. striatum</strong></td>
<td>grey/black, colonies</td>
<td>non-haemolytic white moist smooth colonies &gt; 2 mm after 24 h</td>
</tr>
<tr>
<td><strong>C. jeikeium</strong></td>
<td>grey/black, colonies</td>
<td>non-haemolytic grey/white low convex colonies</td>
</tr>
</tbody>
</table>

† see **BSOPTP 36 - Urease Test**

*If results of these 4h tests indicate *Corynebacterium* species immediately inform medical microbiologist and refer isolate to the Reference Laboratory. *C. xerosis* can be used as a positive control for this test.

If these preliminary tests do not indicate *Corynebacterium* species then consider further identification tests if clinically indicated.

Use commercial identification kit and refer isolate to the Laboratory of Healthcare Associated Infection (LHCAI) if clinically indicated.

**Note:** Fresh culture of control organism is advisable
Confirmation of identification and toxigenicity are undertaken only by the HPA Streptococcus and Diphtheria reference unit (SDRU).

5.3 **MEDICAL MICROBIOLOGIST**

Inform the medical microbiologist of presumptive and confirmed *C. diphtheriae*, *C. ulcerans* or *C. pseudotuberculosis* species. The medical microbiologist should also be informed if the request form bears relevant information eg

- Suspected case of contact with diphtheria or foreign travel
- Membranous/Pseudomembranous tonsillitis
- Ulcerating skin lesions acquired overseas. The medical microbiologist should be aware of possible factors from overseas protocols that could influence results
- Any of the above, with neurological or cardiological manifestations
- History of farming or veterinary work
- Any foreign travel, especially Russia and Former Soviet States, Africa, South America and South-East Asia

For presumptive and confirmed non-toxigenic *Corynebacterium* species the medical microbiologist should be informed when the request form bears relevant information eg

- Cases of suspected endocarditis associated with appropriate specimen.
- Infection of indwelling medical devices (prosthetic valves, pacemakers, peritoneal and vascular catheters, CSF shunts)
- History of substance abuse, alcoholism, immunodeficiency or other serious underlying disorder such as cancer, or patients receiving treatment for cancer, inducing neutropenia and/or mucositis.

Follow local protocols for reporting to the clinician.

5.4 **CCDC**

Refer to local Memorandum of Understanding

As diphtheria is a notifiable disease in the UK, for public health management of cases, contacts and outbreaks, all suspected cases should be notified to the local health protection unit immediately.

http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/GreenBook/fs/en

5.5 **CENTRE FOR INFECTIONS**

Refer to current guidelines on CfI and COSURV reporting

5.6 **INFECTION CONTROL TEAM**

Inform the infection control team of presumptive and confirmed isolates of *C. diphtheriae*
6 REFERRALS

6.1 REFERENCE LABORATORY

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

http://www.hpa.org.uk/cfi/reference_tests_index.htm

Potentially toxigenic corynebacteria (C. diphtheriae, C. ulcerans, C. pseudotuberculosis)

Streptococcus and Diphtheria Reference Unit
WHO Collaborating Centre for Streptococcal and Diphtheria Infections
Respiratory and Systemic Infection Laboratory
Health Protection Agency
Centre for Infections
61 Colindale Avenue
London
NW9 5HT


Service is provided 24 hours a day, 7 days a week.
Contact CfI main switchboard: Tel. +44 (0) 20 8200 440

The SDRU is a designated WHO collaborating centre for reference and research on diphtheria.

Other Corynebacterium species

Laboratory of Healthcare Associated Infection
Health Protection Agency
Centre for Infections
61 Colindale Avenue
London
NW9 5HT


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

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REFERENCES


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


