IDENTIFICATION OF ANAEROBIC ACTINOMYCES SPECIES

BSOP ID 15

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Centre for Infections
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AMENDMENT PROCEDURE

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IDENTIFICATION OF ANAEROBIC ACTINOMYCES SPECIES

SCOPE OF DOCUMENT
This National Standard Method (NSM) describes the identification of anaerobic Actinomyces species. Colonies may be isolated on blood agar or egg containing media. For aerobic Actinomyces see BSOPID 10 – Identification of aerobic Actinomycetes species.

INTRODUCTION
Taxonomy
The nomenclature of the group comprising the Actinomyces species is complicated. Considerable morphological diversity is not only seen within genera but also among strains of the same taxon.

Characteristics
Actinomyces species require enriched culture (such as brain-heart infusion medium) and growth is enhanced by an atmosphere with 6 - 10% added carbon dioxide. The optimum growth temperature is 37°C. Colonies may appear after 3 - 7 days of incubation but detection may require 10 to 14 days incubation. Colonies are described often as ‘molar tooth’ colonies on agar and ‘breadcrumb’ colonies suspended in broth media. They appear as Gram-positive bacilli.

Nocardia species are morphologically indistinguishable from Actinomyces species on Gram - staining and also clinically resemble Actinomyces in that they produce chronic infections of the lung and CNS. Nocardia species are aerobic and some strains are partially acid fast.

Actinomyces species are frequently isolated from clinical specimens in mixed culture with Actinobacillus actinomycetemcomitans, Eikenella corrodens and species of Fusobacterium, Bacteroides, Capnocytophaga, Staphylococcus, Streptococcus and Enterococcus.

The pathogenic Actinomyces species do not exist freely in nature (eg in soil) but are commensals usually involved in mixed oral or cervicofacial, thoracic, pelvic, and abdominal infections. Certain species (A. viscosus and A. naeslundii) are involved in periodontal disease and dental caries. There is no person to person transmission.

Propionibacterium propionicum may produce actinomycosis-like disease. Actinobaculum species., Arcanobacterium species. and Varibaculum cambriense are closely related to Actinomyces species and may be involved in human infections.

Actinomyces israelii
Cells appear fine, filamentous, branching and beaded rods. Colonies are white, molar tooth or breadcrumb, pitting/adherent to agar, may be very gritty. Slow growing. Grows poorly or not at all in air and air and CO₂. Generally isolated from classic actinomycosis, canaliculitis, IUCDs and other soft tissue abscesses and bone infections.

Actinomyces gerencseriae
Formerly A. israelii serotype II. Cells, colonies, growth and sources similar to A. israelii but colonies are very white and not so gritty.

Actinomyces meyeri
Cells are small diphtheroids. Colonies <1 mm, white, convex, smooth, entire. No growth in air or air and CO₂. Isolated from pleural fluids, brain abscesses and other soft tissue abscesses.

Actinomyces georgiae
Similar to *Actinomyces odontolyticus* but non-pigmented (white). Grows poorly or not at all in air and air and CO₂. Part of oral flora. Uncommon in clinical specimens.

*Actinomyces turicensis*
Cells are small coccobacilli. Colonies <1 mm grey/translucent, shiny/smooth, covex, entire. Grows in air and CO₂ but poorly or not at all in air.

*Actinomyces radingae*
Cells grow similarly to *A. turicensis*.

**Principles of Identification**
The isolation of colonies anaerobically on blood agar, followed by identification via biochemical tests using the latest taxonomic tables and molecular methods.

**TECHNICAL INFORMATION/LIMITATIONS**
Commercial kits, although useful in providing basic biochemical information for these pathogens, can not be relied upon for accurate identification using their codes because their databases contain out of date information. This is particularly true as molecular techniques enable more species to be identified than was previously possible.
1 SAFETY CONSIDERATIONS

Refer to current guidance on the safe handling of all 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

2.1 ACTINOMYZES SPECIES REPORTED TO HAVE CAUSED HUMAN INFECTION

Actinomyces israelii (facultatively anaerobic)
Actinomyces naeslundii
Actinomyces funkei
Actinomyces europaeus
Actinomyces graevenitzii
Actinomyces urogenitalis
Actinomyces odontolyticus
Actinomyces viscosus
Actinomyces meyeri (rarely isolated)
Actinomyces gerencseriae
Actinomyces neuii (former CDC coryneform group 1)
Actinomyces radingae (former CDC coryneform group E)
Actinomyces turicensis (former CDC coryneform group E)

Other species may be associated with human disease

Actinomyces radicidentis
Actinomyces cardiffensis
Actinomyces oricola
Actinomyces nasicola

3 IDENTIFICATION

3.1 MICROSCOPIC APPEARANCE

Gram stain (BSOP TP 39 - Staining Procedures)

Branching, beaded, filamentous or diphtheroid-shaped or cocco-bacillary Gram-positive bacilli.

N.B. Propionibacterium species are pleomorphic bacilli that may appear to branch.

3.2 PRIMARY ISOLATION MEDIA

1) Fastidious anaerobe agar or equivalent without neomycin – many Actinomyces species may be inhibited by neomycin. Incubate anaerobically at 35-37 C for 5-10 days.

2) Actinomyces selective agar with metronidazole 10 mg/L and nalidixic acid 30 mg/L. Incubate anaerobically at 35-37 C for 5-10 days.

Growth in air and in air plus 5-10% CO₂ is variable. Broth enrichment is rarely beneficial.

N.B: Some species may require longer incubation.
### 3.3 Colonial appearance

<table>
<thead>
<tr>
<th>Species</th>
<th>Colonies</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. israelii</em></td>
<td>White to cream, breadcrumb or molar tooth, gritty, pitting</td>
<td>Slow growing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old colonies may become pink</td>
</tr>
<tr>
<td><em>A. gerensceriae</em></td>
<td>Bright white, breadcrumb or molar tooth, pitting and softer than <em>A. israelii</em></td>
<td>Slow growing</td>
</tr>
<tr>
<td><em>A. naeslundii</em></td>
<td>White, cream or pinkish, smooth, convex, entire edged</td>
<td>Occasional rough forms occur</td>
</tr>
<tr>
<td><em>A. odontolyticus</em></td>
<td>Cream-to-red, smooth, convex, entire edged</td>
<td>Old colonies may be dark brown</td>
</tr>
<tr>
<td><em>A. meyeri</em></td>
<td>Small, white, smooth, convex, entire edged</td>
<td>Slow growing</td>
</tr>
<tr>
<td><em>A. deticolens</em> like</td>
<td>White to pink, heaped or molar tooth, pitting</td>
<td></td>
</tr>
<tr>
<td><em>A. georgiae</em></td>
<td>White or cream, smooth, convex, entire edged</td>
<td></td>
</tr>
<tr>
<td><em>A. neuii</em> sub sp. <em>neuii</em> and <em>anitratius</em></td>
<td>White or cream, smooth, convex, entire edged</td>
<td></td>
</tr>
<tr>
<td><em>A. radingae</em></td>
<td>Grey-to-white, semi-translucent, smooth, low convex, entire edge</td>
<td></td>
</tr>
<tr>
<td><em>A. turicensis</em></td>
<td>Grey, semi-translucent, smooth, low convex, entire edge</td>
<td></td>
</tr>
<tr>
<td><em>A. europaeus</em></td>
<td>Whitish, semi-translucent, smooth, low convex, entire edged</td>
<td></td>
</tr>
<tr>
<td><em>A. graevenitzii</em></td>
<td>White pronounced molar tooth or smooth, convex</td>
<td>Red fluorescence. Rough and smooth forms occur together. Old colonies may become dark brown</td>
</tr>
<tr>
<td><em>A. radicidentis</em></td>
<td>Cream-to-pink, smooth, convex, entire edged</td>
<td>Old colonies may become dark brown</td>
</tr>
<tr>
<td><em>A. urogenitalis</em></td>
<td>Cream-to-pink, with darker rings, smooth</td>
<td>Old colonies may become dark brown</td>
</tr>
<tr>
<td><em>A. funkei</em></td>
<td>Grey, semi-translucent, opaque centre (fried-egg), low convex, entire edge</td>
<td></td>
</tr>
<tr>
<td><em>A. cardiffensis</em></td>
<td>Cream-to-pink, smooth, convex, entire edged</td>
<td></td>
</tr>
<tr>
<td><em>A. nasicola</em></td>
<td>White or grey, smooth, convex, entire edged</td>
<td></td>
</tr>
<tr>
<td><em>A. oricola</em></td>
<td>White, breadcrumb, pitting</td>
<td></td>
</tr>
<tr>
<td><em>P. propionicum</em></td>
<td>Off-white to buff, breadcrumb, gritty, pitting, or smooth, convex, entire edged</td>
<td>Red fluorescence. Rough and smooth forms occur together</td>
</tr>
</tbody>
</table>
3.4 **TEST PROCEDURES**

**Preliminary tests**

*Actinomyces* species are inherently resistant to metronidazole and are spot-indole negative. **N.B. Propionibacterium acnes** (a common skin commensal) is indole positive.

Colonies of *A. graevenitzii* and *Propionibacterium propionicum* on blood-containing media fluoresce red under long-wave (366 nm) UV illumination.

**Commercial identification kit**

Results should be interpreted with caution and in conjunction with other test results. In order to achieve accurate results with biochemical tests it is advisable to use taxonomic keys and not rely on the identification given by the code.

3.5 **CONFIRMATION**

Amplified rDNA Restriction Analysis

16S rDNA PCR and sequence

**Other more specialized tests:**

Gas liquid chromatography

3.6 **STORAGE AND REFERRAL**

If required, save the pure isolate on a blood agar slope incubated anaerobically, in anaerobic broth culture or and transport swab for referral to the Reference Laboratory.

4 **IDENTIFICATION FLOW CHART**

N/A
5 RESULTS AND REPORTING

5.1 PRESUMPTIVE IDENTIFICATION
If appropriate growth characteristics, colonial appearance and Gram’s stain of the culture are demonstrated and the isolate is metronidazole non-susceptible.

5.2 CONFIRMATION OF IDENTIFICATION
Following commercial identification kit results and/or the Reference Laboratory report.

5.3 MEDICAL MICROBIOLOGIST
Inform the medical microbiologist of presumptive or confirmed anaerobes when the request card bears relevant information

5.4 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
N/A

6 REFERRALS

6.1 REFERENCE LABORATORY
Anaerobe Reference Unit
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
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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