

NATIONAL STANDARD METHOD

IDENTIFICATION OF ANAEROBIC *ACTINOMYCES* SPECIES

BSOP ID 15

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections







IDENTIFICATION OF ANAEROBIC *ACTINOMYCES* SPECIES

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The reader is informed that all taxonomy in this document was correct at time of issue.

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AMENDMENT PROCEDURE

Controlled document reference	BSOP ID 15
Controlled document title	Identification Of Anaerobic <i>Actinomyces</i> 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

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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 *Actinomycetes* 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^{2,3}.

Characteristics⁴

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

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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 cocco-bacilli. Colonies <1 mm grey/translucent, shiny/smooth, convex, 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 tables⁵ 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^{6,7}. This is particularly true as molecular techniques enable more species to be identified than was previously possible⁷.

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1 SAFETY CONSIDERATIONS⁸⁻²⁰

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 *ACTINOMYCES* SPECIES REPORTED TO HAVE CAUSED HUMAN INFECTION^{21,22}

Actinomyces israelii (facultatively anaerobic)
Actinomyces naeslundii
Actinomyces funkei
Actinomyces europaeus
Actinomyces graevenitizii
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^{21,23}

Actinomyces radidentis
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.

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3.3 Colonial appearance⁴

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

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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 Analysis²⁴

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

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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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