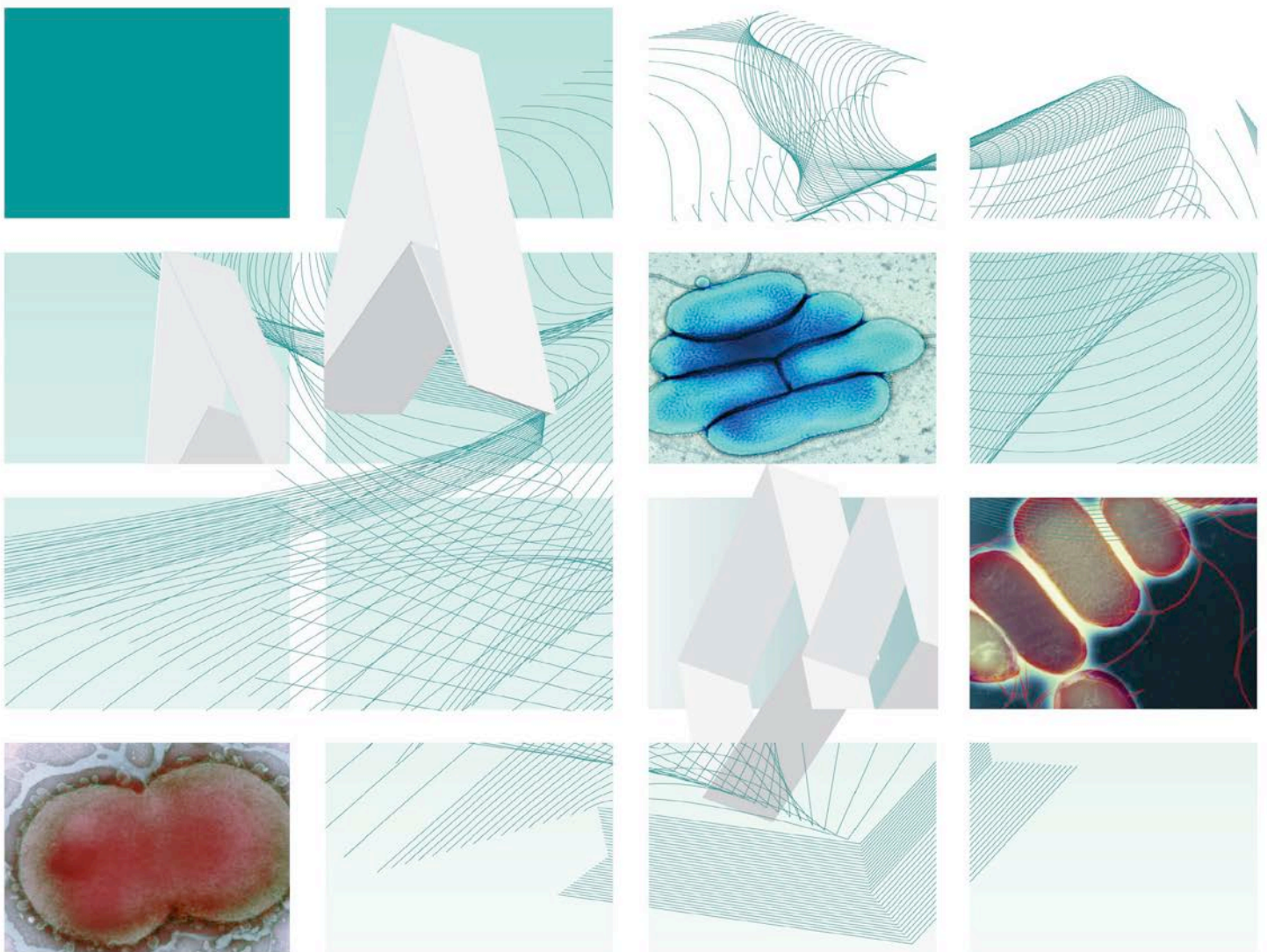




Protecting and improving the nation's health

UK Standards for Microbiology Investigations

Identification of Anaerobic *Actinomyces* species



Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

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Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	4/26.06.15
Issue no. discarded.	1.3
Insert Issue no.	2
Section(s) involved	Amendment
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Whole document.	Document presented in a new format. Title of the document amended. Reorganisation of some text. Edited for clarity. Test procedures updated. Updated contact details of Reference Laboratories.
Scope of document.	The scope has been edited for clarity. It has been updated to include webpage link for ID 10 document.
Introduction.	The taxonomy of <i>Actinomyces</i> species has been updated. More information has been added to the Characteristics section. The medically important species have been grouped and their characteristics described. Use of up-to-date references. Section on Principles of identification has been updated.
Technical Information/Limitations.	Addition of information regarding commercial identification systems, agar media and metronidazole susceptibility has been described and referenced.
Safety considerations.	Reference added.

Target Organisms.	The section on the Target organisms has been updated and presented clearly. References have been updated.
Identification.	<p>Amendments and updates have been done on 3.1, 3.2, 3.3, 3.4 and 3.6 have been updated to reflect standards in practice.</p> <p>Information added to table (in 3.3) to explain the different species and their colonial morphology.</p> <p>Section 3.4.3 and 3.4.4 have been updated to include MALDI-TOF MS and NAATs with references.</p> <p>Subsection 3.5 has been updated to include the Rapid Molecular Methods.</p>
Identification Flowchart.	Information has been provided as to how to identify these organisms being that there is constantly considerable morphological diversity among genera.
Reporting.	Subsections 5.1, 5.2 and 5.5 have been updated.
Referral.	The contact details of the reference laboratories have been updated.
References.	Some references updated.

UK Standards for Microbiology Investigations[#]: scope and purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests.
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>.

Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

[#]Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

Quality assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

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The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Scope of document

This SMI describes the identification of anaerobic *Actinomyces* species.

For aerobic *Actinomycetes*, see [ID 10 – Identification of aerobic *Actinomycetes*](#).

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

The genus *Actinomyces* currently contains 46 species and two subspecies that have been characterised by phenotypic and molecular methods¹. Twenty four of these species are associated with humans and five have been recently assigned to other genera. The genus comprises 17 species that are known to be the cause of a wide spectrum of clinical diseases in animals.

Considerable morphological diversity is not only seen within genera but also among strains of the same taxon².

Characteristics

Actinomyces species are facultatively anaerobic, Gram positive, irregularly staining bacilli that are non-acid-fast, non-sporing and non-motile. They may show rudimentary branching. They are catalase negative except *Actinomyces viscosus*, *Actinomyces neuii* subsp *neuii*, *Actinomyces neuii* subsp *anitratu*s and *Actinomyces radidentis*².

They require enriched medium and growth is enhanced with added carbon dioxide. Anaerobic conditions are favoured but some species can be cultured aerobically or in air plus 5% CO₂. The optimum growth temperature is 35-37°C. They are fermentative and produce acetic, formic, lactic and succinic acid but not propionic acid as end-products of glucose metabolism³.

Colonies may appear after 3-7 days of incubation but detection may require 10-14 days incubation. Colonies are described often as 'molar tooth' colonies on agar and 'breadcrumb' colonies suspended in broth media.

On selective medium containing mupirocin and metronidazole after incubation for 5-10 days anaerobically, there is a report of significant isolation of *Actinomyces* species than on non-selective media from both dental and intrauterine contraceptive devices (IUCD)⁴.

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.

Actinomyces species (*A. israelii*, *A. gerencseriae*) cause the chronic disease, Actinomycosis. However, some *Actinomyces* species are opportunistic pathogens and are associated with other mixed anaerobic infections and in infections in severely compromised patients⁵.

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 infections³.

The pathogenic genera within the anaerobic *Actinomyces* species are³:

Actinomyces israelii

Cells appear fine, filamentous, beaded and branching rods. Colonies are white- to -cream, molar tooth or breadcrumb, pitting/adherent to agar, may be very gritty. Older colonies may become pink. Slow growing. It grows poorly or not at all in air or air and CO₂. It is also generally isolated from classic actinomycosis, canaliculitis, Intrauterine Contraceptive Devices (IUCDs) and other soft tissue abscesses and bone infections.

Actinomyces gerencseriae

Previously known as *A. israelii* serovar II. Cells, colonies, growth and sources are similar to *A. israelii* but colonies are bright white and soft. They are equally slow growers.

Actinomyces meyeri

Cells are short fine rods. Colonies are small, white, convex, smooth and have entire edges. Slow growth in air or air and CO₂. Isolated from pleural fluids, brain abscesses and other soft tissue abscesses.

Actinomyces georgiae

Cells appear like diphtheroids. Colonies are similar to *Actinomyces neuii* and they appear as white or cream, smooth, convex and have entire edges. They may grow more rapidly than *A. israelii* and may grow well in air or in air plus CO₂. They are part of oral flora, uncommon in clinical specimens.

Actinomyces turicensis

Cells are small cocco-bacilli. Colonies are grey, semi -translucent, smooth, low convex and have entire edges. They grow in air and CO₂, but poorly or not at all in air. They may be mistaken for non-haemolytic streptococci, lactobacilli or other commensal organisms. It has also been isolated frequently from pilonidal sinuses, perianal and decubital abscesses, ear, nose and throat infections, post-operative wounds and IUCDs. *A. turicensis* has been described as a potential pathogen mostly in genital infections, followed by urinary tract infections and skin-related infections.

Actinomyces radingae

Cells grow similarly to *A. turicensis*. It is primarily isolated from IUCDs and various soft tissue infections.

Actinomyces viscosus

Cells are filamentous, branching rods. There are two types of colonies: large and smooth colonies with V, Y and T configurations or small and rough colonies with short branching filaments. They grow in the presence of carbon dioxide. They are catalase positive. Isolated from human dental calculus and root surface caries and there is no person to person transmission.

Actinomyces naeslundii sensu stricto

Formerly known as *Actinomyces naeslundii* genospecies 1⁶. Cells are medium rods and have clubbed ends and some branching. On plate, colonies appear as white, cream or pinkish, smooth, convex and have entire edges. Occasional rough forms may sometimes occur.

A. naeslundii is involved in periodontal disease and dental caries and there is no person to person transmission. They can also be isolated from IUCDs, Actinomycosis and bone infections.

***Actinomyces johnsonii*⁶**

Formerly known as *Actinomyces naeslundii* genospecies 3(WVA963). They are branching rods. Cells are similar to *Actinomyces naeslundii*. Facultatively anaerobic.

***Actinomyces oris*⁶**

Formerly known as *Actinomyces naeslundii* genospecies 2. They are branching rods. Cells are similar to *Actinomyces naeslundii*. Facultatively anaerobic.

They are commonly isolated in the human oral cavity and play a major role in the formation of oral biofilm or dental plaque.

Actinomyces funkei

Cells are fine, filamentous, beaded, branching bacilli. They appear as grey, semi-translucent, opaque centre, low convex and have entire edges. They have been isolated from IUCDs, bacteraemia and subacute endocarditis.

Actinomyces europaeus

Cells are short (length, 0.5 - 1.5µm) rods that are sometimes arranged in clusters. Cells are non-motile and do not form spores. Colonies are circular and smooth with a translucent whitish/greyish appearance and are not more than 0.5 mm in diameter after 48hr of incubation in a 5% CO₂, enriched atmosphere at 37°C. Facultatively anaerobic. They are negative for catalase, nitrate reduction and urea tests. Aesculin hydrolysis is variable. Acid is produced from glucose, maltose, galactose, and D-fructose.

They have been isolated from IUCDs and various soft-tissue infections.

Actinomyces graevenitizii

Cells are medium length, thick, granular and branched. Colonies are white, pronounced molar tooth or smooth and convex. The rough and smooth forms occur together. They may also produce pink- to- red colonies, particularly in old cultures. Isolated from respiratory tract secretions and infants' saliva.

Actinomyces urogenitalis

Cells are cocco-bacilli. Colonies appear as cream to pink, with darker rings, smooth, convex and have entire edges. Old colonies may become red. Isolated from IUCDs and urogenital tracts.

Actinomyces odontolyticus

Cells are coryneform-like and may have some clubbed ends. Colonies are cream to red, smooth, convex and have entire edges. Old colonies may be dark brown. They are negative for nitrate and urease tests. They have been isolated from IUCDs, case of actinomycosis, bacteraemia and subacute endocarditis and periodontal diseases.

Actinomyces neuii

Formerly CDC coryneform group1. This has 2 subspecies namely; *Actinomyces neuii* subsp *neuii* and *Actinomyces neuii* subsp *anitratu*⁷.

Actinomyces neuii subsp neuii

Cells are predominantly diphtheroidal, but cocco-bacilli may occur; the rods are mainly arranged in clusters or V or Y forms and have no branching. Cells are non-motile and non-spore forming. Colonies are circular, smooth, convex, opaque, more white than

creamy in colour, and with entire edges. Colony sizes range from 0.5 - 1.5mm in diameter after 48hr of incubation in 5% CO₂, on sheep blood agar. Cells are facultatively anaerobic. For most strains, α-haemolysis is observed on sheep blood agar and for all strains on human blood agar. Cells are catalase positive but in recent years, a catalase negative strain was identified and so this reaction can no longer be considered a key reaction for the diagnosis of this species but must be interpreted in conjunction with other characteristics⁸. Nitrate is reduced to nitrite. They are negative for urea, aesculin and gelatin hydrolysis.

This organism is mainly isolated from abscesses in association with mixed anaerobic flora as well as from human blood cultures and from synovial and preoperative joint fluids.

***Actinomyces neuii* subsp *anitratu*s**

The morphological characteristics of *A. neuii* subsp *anitratu*s are similar to those of *A. neuii* subsp *neuii*, except that *A. neuii* subsp. *anitratu*s is non-haemolytic and does not reduce nitrate to nitrite.

They are isolated mainly from abscesses in association with mixed anaerobic flora as well as from human blood cultures.

Actinomyces radidentis

Cells are coccoid. Colonies are cream to pink, smooth, convex and have entire edges. Old colonies may become red. *Actinomyces radidentis* are catalase positive and give variable results on nitrate and urease tests. They have been isolated from infected root canals.

***Actinomyces cardiffensis*⁹**

Cells are pleomorphic, slender, straight-to-curved rods; beaded branching filaments occur. Cells stain Gram positive, are non-acid-fast and non-motile. Colonies are similar to *A. radidentis*. They are non-haemolytic, facultatively anaerobic and are negative for catalase and aesculin hydrolysis tests. Nitrate may or may not be reduced. They have been isolated from human clinical sources, including pleural fluid, brain, jaw, pericolic and ear abscesses, antrum, and IUCDs.

Actinomyces oricola

Cells are coryneform to filamentous, beaded, may display branching, Gram positive, rod-shaped cells. Cells are non-acid-fast, non-spore-forming and non-motile. On agar, colonies are tiny, white and breadcrumb-like and the agar appears pitted. They are catalase negative and facultatively anaerobic but grow best under anaerobic conditions. The end products of glucose metabolism are acetic, lactic and succinic acids. They are also negative for gelatin and starch hydrolysis and positive for aesculin hydrolysis. *Actinomyces oricola* have been isolated from human dental abscess.

Actinomyces nasicola

Cells are short coryneform -shaped rods; some branching and coccoid forms occur. Cells are non-acid-fast and non-spore-forming. After 48hr anaerobic incubation on fastidious anaerobe agar, colonies are pinpoint, white or grey, opaque, shiny, entire and convex. They are facultatively anaerobic; they can grow in air but grow better under anaerobic conditions. The organism is negative for catalase, nitrate reduction as well as aesculin, gelatin and starch hydrolysis tests. Acetic, lactic and succinic acids

are the end-products of glucose metabolism. It has been isolated from the antrum aspirate.

Actinomyces massiliensis

Cells are anaerobic straight rods. Optimal growth occurs at 37°C but they also grow in the presence of air and in 5% CO₂. After 48hr growth on sheep-blood agar, surface colonies are white, pinpoint, circular and shiny. The rods are 0.5–1.7µm in length and 0.35–0.74µm in diameter. They are negative for catalase and urease test. This organism has been isolated from human blood¹⁰.

Actinomyces dentalis

They are filamentous, beaded, branching rod-shaped cells. Cells were non-acid-fast, non-spore-forming and non-motile. On agar, colonies are tiny, white and breadcrumb-like and the agar appears pitted. The organism is catalase negative and grows under anaerobic conditions; however, it does not grow in air or in air plus 5 %CO₂. The end products of glucose metabolism are lactic acid (major) and acetic acid (minor); succinic acid is not detected. They are also negative for gelatin and starch hydrolysis, urease and nitrate tests. It can be isolated from pus of a human dental abscess.

Actinomyces hongkongensis

The cells are strictly anaerobic, straight, non-sporulating rods. They grow on sheep blood agar as non-haemolytic, pinpoint colonies after 24 hours of incubation at 37°C in anaerobic environment. They are non-motile and do not produce catalase. They are isolated from pus or from pelvic and abdominal abscesses¹¹.

Actinomyces hominis

Cells are acid-fast and non-spore-forming rods. Colonies are white–greyish, convex, not lipophilic and 1mm in diameter after 72hr incubation on sheep blood agar at 35°C in a 5% CO₂ enriched atmosphere. They are positive for catalase, nitrate reduction but not for urease and gelatin hydrolysis tests. It has been isolated from wound swab from a patient¹².

Actinomyces timonensis

Cells are anaerobic straight rods, catalase negative and oxidase negative. Growth occurred between 25 and 37°C. Optimal growth occurs at 37°C. They also grow under microaerophilic conditions and in 5% CO₂. Growth in the presence of air is weak after 48hr incubation. After 48hr growth on sheep blood agar, surface colonies are α-haemolytic, pin-point, circular, white, dry and embedded in the agar. The rod size, estimated by electron microscopy, is 1.0–3.2µm in length and 0.3–0.5µm in diameter. Isolated from a human clinical osteo-articular sample¹³.

Principles of identification

The isolation of colonies anaerobically on blood agar or egg containing media, followed by identification via biochemical tests using the recent taxonomic tables and molecular methods will greatly help in the identification of the most problematic *Actinomyces* species¹⁴.

Technical information/limitations

Commercial identification systems

Commercial kits, although useful in providing basic biochemical information for these pathogens, cannot be relied upon for accurate identification using their codes because the databases contain out of date information, are incomplete or due to occasional weak enzymatic and sugar fermentation reactions^{15,16}. This is particularly true as molecular techniques enable more species to be identified than was previously possible¹⁶.

Agar media

Deep fill plates are recommended for long incubations to prevent desiccation.

Susceptibility to metronidazole

Actinomyces species are inherently resistant to metronidazole.

1 Safety considerations¹⁷⁻³³

Refer to current guidance on the safe handling of all organisms documented in this SMI.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet²⁵.

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^{3,9-13,34-41}

Actinomyces israelii, *Actinomyces graevenitizii*, *Actinomyces gerencseriae*, *Actinomyces naeslundii*, *Actinomyces odontolyticus*, *Actinomyces viscosus*, *Actinomyces funkei*, *Actinomyces europaeus*, *Actinomyces urogenitalis*, *Actinomyces meyeri* (rarely isolated), *Actinomyces neuii* (former CDC coryneform group 1) (subspecies: *Actinomyces neuii* subsp *neuii*, *Actinomyces neuii* subsp *anitratus*), *Actinomyces radingae* (former CDC coryneform group E), *Actinomyces turicensis* (former CDC coryneform group E), *Actinomyces radidentis*, *Actinomyces cardiffensis*, *Actinomyces oricola*, *Actinomyces nasicola*, *Actinomyces massiliensis*, *Actinomyces johnsonii*, *Actinomyces dentalis*, *Actinomyces hongkongensis*, *Actinomyces hominis*, *Actinomyces oris*, *Actinomyces timonensis*

3 Identification

3.1 Microscopic appearance

Gram stain ([TP 39 - Staining procedures](#))

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

Note: *Propionibacterium* species are pleomorphic bacilli that may appear to branch.

3.2 Primary isolation media

Fastidious anaerobe agar or equivalent without neomycin incubated anaerobically at 35-37°C for 5-10 days.

Note: Many *Actinomyces* species may be inhibited by neomycin.

Actinomyces selective agar with metronidazole 10mg/L and nalidixic acid 30mg/L (deep fill) incubated 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.

Note: Some species may require longer incubation.

OR

Blood agar with Mupirocin 128mg/L and metronidazole 2.5mg/L incubated anaerobically at 35-37°C for 5-10 days⁴.

Note: This media is not commercially available and so will have to be prepared in-house by laboratories wanting to use it.

3.3 Colonial appearance

Members of the genus demonstrate considerable variation in cell and colony morphologies and this may lead to difficulties in recognition of putative *Actinomyces* on primary isolation media.

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. georgiae</i>	White or cream, smooth, convex, entire edged	
<i>A. neuii</i> sub sp. <i>neuii</i> and <i>anitratus</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. graevenitzi</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 on the agar	

<i>A. viscosus</i>	The two types of colonies: large and smooth colonies with V, Y and T configurations or small and rough colonies with short branching filaments.	
<i>A. johnsonii</i>	Colonies are similar to <i>A. naeslundii</i>	
<i>A. oris</i>	Colonies are similar to <i>A. naeslundii</i>	
<i>A. massiliensis</i>	white, pinpoint, circular and shiny with entire edges	
<i>A. dentalis</i>	tiny, white and breadcrumb-like and pitting on the agar	
<i>A. hongkongensis</i>	non-haemolytic, pinpoint colonies	
<i>A. hominis</i>	white–greyish, convex, entire edges	
<i>A. timonensis</i>	α-haemolytic, pin-point, circular, white, dry and embedded in the agar	
<i>P. propionicum</i> *	Off white to buff, breadcrumb, gritty, pitting, or smooth, convex, entire edged	Red fluorescence, rough and smooth forms occur together
*Colonies of <i>A. graevenitzii</i> and <i>Propionibacterium propionicum</i> on blood containing media fluoresce red under long-wave (366 nm) UV illumination.		

3.4 Test procedures

3.4.1 Biochemical tests

Preliminary tests

Indole test (see [TP 19 – Indole test](#))

Actinomyces species are spot indole negative.

Note: *Propionibacterium acnes* (a common skin commensal) is indole positive.

Catalase test (see [TP 8 – Catalase test](#))

All *Actinomyces* species are catalase negative except *Actinomyces viscosus*, *Actinomyces neuii* subsp *neuii*, *Actinomyces neuii* subsp *anitratu*s and *Actinomyces radicidentis*.

3.4.2 Commercial identification systems

Laboratories should follow manufacturer's instructions and rapid tests and kits should be validated and be shown to be fit for purpose prior to use.

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.

3.4.3 Matrix-assisted laser desorption/ionisation - time of flight (MALDI-TOF) mass spectrometry

This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF as compared with other identification methods is that the results of the analysis are available within a few hours rather than several days. The speed and the simplicity of sample preparation and result acquisition associated with minimal consumable costs make this method well suited for routine and high throughput use. This approach potentially allows faster characterisation of organisms that are difficult to identify by conventional methods⁴².

MALDI-TOF MS has shown to be a valuable tool for the identification of aero-tolerant *Actinomyces* species. The time taken for identification is less than 1hr. However, the addition or improvement of spectrometric patterns in the existing database may increase the confidence of species identification^{43,44}.

3.5 Further identification

Rapid molecular methods

Molecular methods have had an enormous impact on the taxonomy of anaerobic *Actinomyces* species. Analysis of gene sequences has increased understanding of the phylogenetic relationships of these species and related organisms and has resulted in the recognition of numerous new species and thus rendering previously published identification schemes obsolete. Molecular techniques have made identification of many species more rapid and precise than is possible with phenotypic techniques.

A variety of rapid typing methods have been developed for isolates from clinical samples; these include molecular techniques such as 16S rDNA Polymerase Chain reaction – Restriction Fragment Length Polymorphism (16S rDNA PCR-RFLP), and 16S rRNA gene sequencing. All of these approaches enable subtyping of unrelated strains, but do so with different accuracy, discriminatory power, and reproducibility.

However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

16S rDNA Polymerase chain reaction – restriction fragment length polymorphism (16S rDNA PCR-RFLP)

This has proved a useful typing technique for a number of groups of organisms, and can be used to identify species within some genera.

This has been used successfully in the differentiation between *Actinomyces* species and has helped facilitate rapid diagnosis and prompt initiation of the appropriate chemotherapy⁴⁵.

16S rRNA gene sequencing

A genotypic identification method, 16S rRNA gene sequencing is used for phylogenetic studies and has subsequently been found to be capable of re-classifying bacteria into completely new species, or even genera. It has also been used to describe new species that have never been successfully cultured.

The availability of gene sequencing has revolutionized the taxonomy of the *Actinomyces* species and has become an invaluable tool for the identification of clinical isolates. Partial or complete sequencing of the 16S rRNA ribosomal gene has

been successfully used to supplement phenotypic methods for the identification of aero-tolerant *Actinomyces* species^{6,9,43,46}.

However, DNA sequencing methods are expensive and labour-intensive, and, therefore, may not be available in many laboratories.

Amplified rDNA restriction analysis (ARDRA)

This has proved to be useful for discrimination of various bacterial species. It has been shown to be a simple, rapid, cost-effective and highly discriminatory method for identification of *Actinomyces* species of clinical origin⁴⁷. This may also be used to further clarify the taxonomy of the genus.

Other more specialized tests:

Gas liquid chromatography³.

3.6 Storage and referral

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

4 Identification flowchart

There is considerable morphological diversity seen among genera and also among strains of the same taxon, refer to the current journal articles for identification.

5 Reporting

5.1 Presumptive identification

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

5.2 Confirmation of identification

Confirmation of identification and toxigenicity are undertaken by Anaerobe Reference Laboratory, Public Health Wales Microbiology Cardiff.

5.3 Medical microbiologist

Inform the medical microbiologist of presumptive or confirmed anaerobes when the request bears relevant information.

5.4 CCDC

Refer to local Memorandum of Understanding.

5.5 Public Health England⁴⁸

Refer to current guidelines on CIDSC and COSURV reporting.

5.6 Infection prevention and control team

N/A

6 Referrals

6.1 Reference laboratory

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

Anaerobe Reference Unit
Public Health Wales Microbiology Cardiff
University Hospital of Wales
Heath Park
Cardiff CF14 4XW
Telephone +44 (0) 29 2074 2171 or 2378

<https://www.gov.uk/government/collections/anaerobe-reference-unit-arucardiff>

England and Wales

<https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services>

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

<http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm>

7 Notification to PHE^{48,49} or equivalent in the devolved administrations⁵⁰⁻⁵³

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAs) and Creutzfeldt–Jakob disease (CJD) under

'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](#)^{50,51}, [Wales](#)⁵² and [Northern Ireland](#)⁵³.

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