Public Health England

NHS

UK Standards for Microbiology Investigations

Identification of Anaerobic *Actinomyces* species

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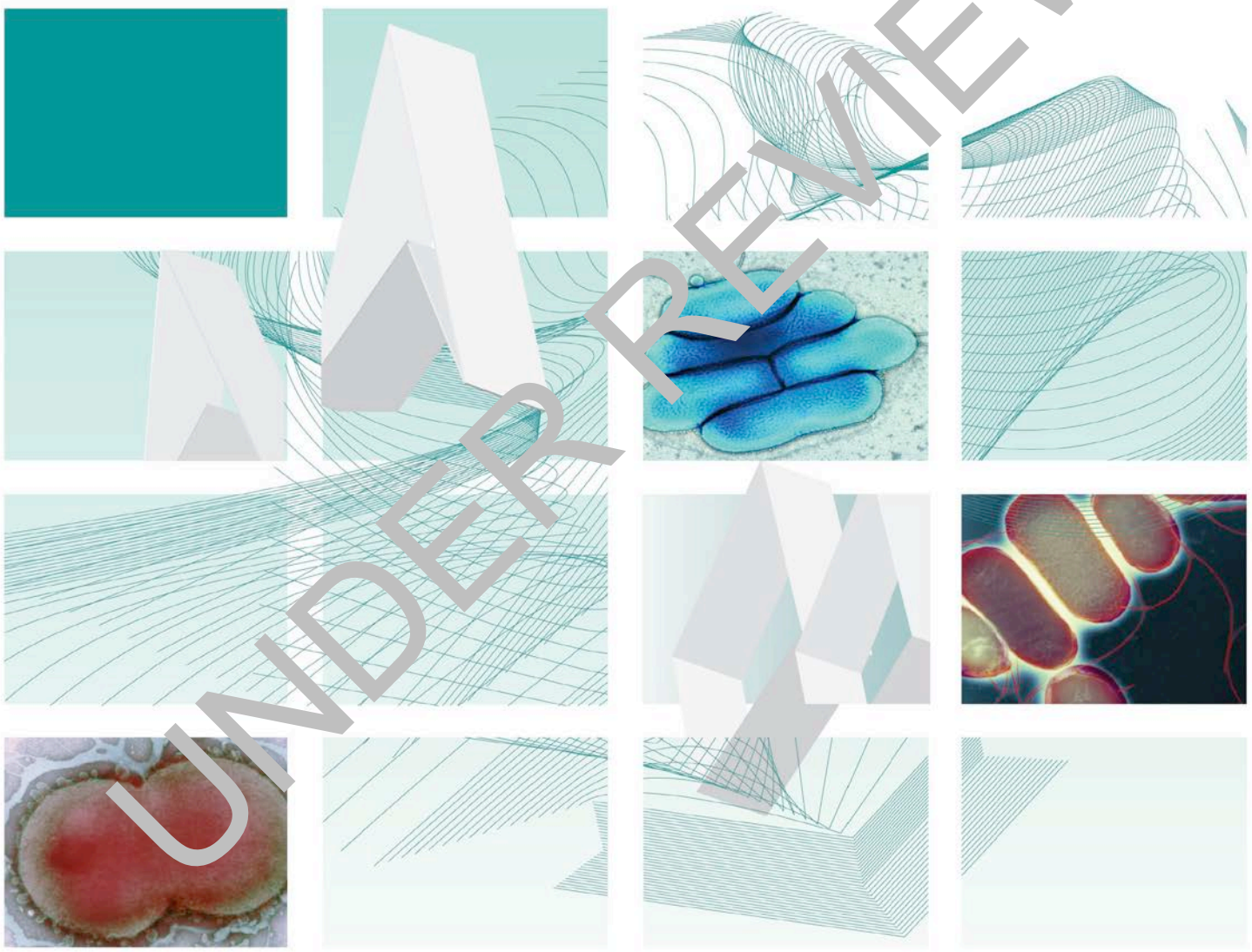

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Acknowledgments

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 http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

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.	3/11.03.14
Issue no. discarded.	1.2
Insert Issue no.	1.3
Section(s) involved	**Amendment**
Whole document.	Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety and notification references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	2/26.11.12
Issue no. discarded.	1.1
Insert Issue no.	1.2
Section(s) involved	**Amendment**
Whole document.	Minor formatting amendments.

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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 http://www.hpa.org.uk/SMI/Partnerships. 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.

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[#]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.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

The development of SMIs are subject to PHE Equality objectives http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

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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Suggested Citation for this Document

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Scope of Document

This UK SMI describes the identification of anaerobic *Actinomyces* species. Colonies may be isolated on blood agar or egg containing media.

For aerobic Actinomycetes, see ID 10 – Identification of Aerobic Actinomycetes species.

This SMI should be used in conjunction with other SMIs.

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[1,2].

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 10-14 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 CO_2. Generally isolated from classic actinomycosis, canaliculitis, IUCDs and other soft tissue abscesses and bone infections.

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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 <1mm, white, convex, smooth, entire. No growth in air or air and CO_2. 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 CO_2. Part of oral flora, uncommon in clinical specimens.

Actinomyces turicensis

Cells are small cocco-bacilli. Colonies <1mm grey/translucent, shiny/smooth, covex, entire. Grows in air and CO_2, 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[3].

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[4,5]. This is particularly true as molecular techniques enable more species to be identified than was previously possible[5].

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1 Safety Considerations[6-22]

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 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[23,24]

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[25,26]

Actinomyces radicidentis

Actinomyces cardiffensis

Actinomyces oricola

Actinomyces nasicola

3 Identification

3.1 Microscopic Appearance

Gram stain (TP 39 - Staining Procedures)

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

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

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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 10mg/L and nalidixic acid 30mg/L.

Incubate anaerobically at 35-37°C for 5-10 days. Growth in air and in air plus 5-10% CO_2 is variable. Broth enrichment is rarely beneficial.

Note: Some species may require longer incubation.

3.3 Colonial Appearance

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

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A. nasicola	White or grey, smooth, convex, entire edged	
A. oricola	White, breadcrumb, pitting	
P. propionicum	Off white to buff, breadcrumb, gritty, pitting, or smooth, convex, entire edged	Red fluorescence, rough and smooth forms occur together

3.4 Test Procedures

Preliminary tests

Actinomyces species are inherently resistant to metronidazole and are spot-indole negative.

Note: *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 (366nm) 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 Further Identification

Amplified rDNA Restriction Analysis[27]

16S rDNA PCR Sequencing.

Other more specialised 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 a dual transport swab for referral to the Reference Laboratory.

4 Identification Flowchart

N/A

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.

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5.3 Medical Microbiologist

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

5.4 CCDC

Refer to local Memorandum of Understanding.

5.5 Public Health England[28]

Refer to current guidelines on CDSC and COSURV reporting.

5.6 Infection Control Team

N/A

6 Referrals

6.1 Reference Laboratory

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

Anaerobe Reference Laboratory
Public Health Wales 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

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

England and Wales
http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370

Scotland

http://www.hpa.scot.nhs.uk/reflab/index.aspx

Northern Ireland

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

7 Notification to PHE[28,29] or Equivalent in the Devolved Administrations[30-33]

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.

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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 (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

Other arrangements exist in Scotland[30,31], Wales[32] and Northern Ireland[33].

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