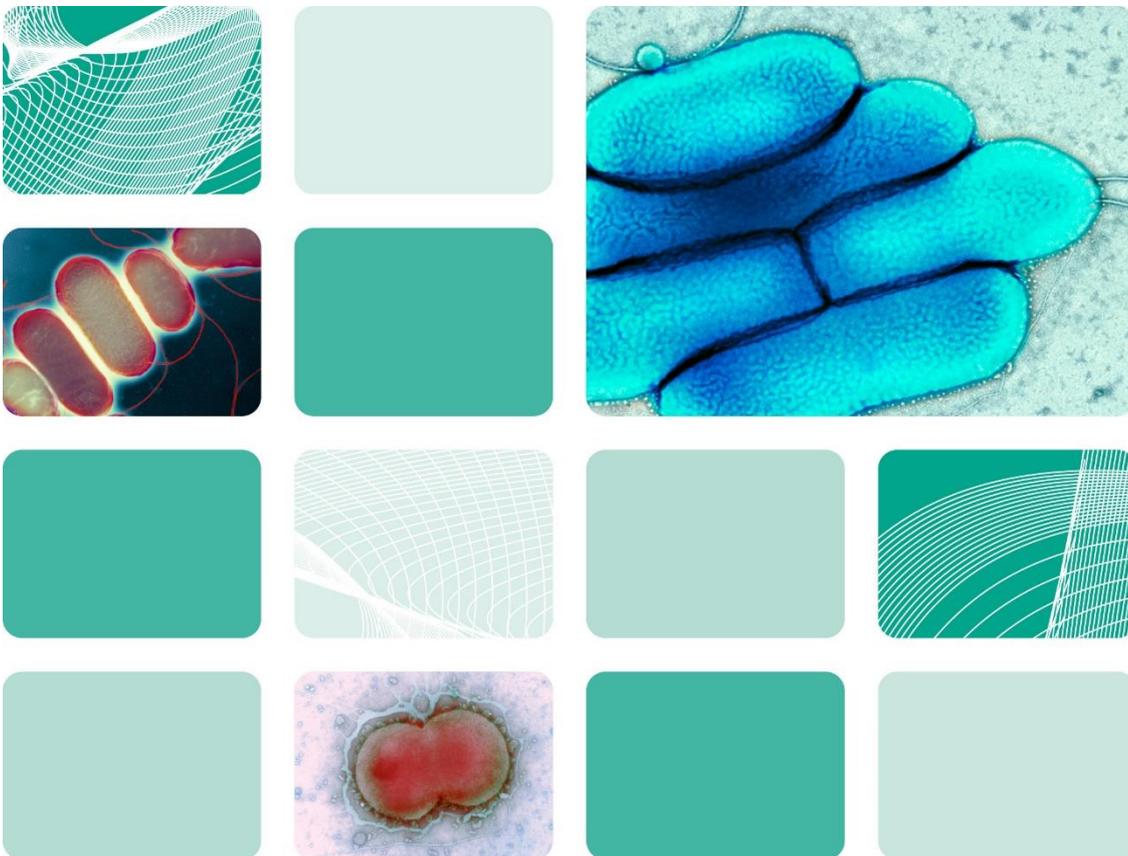




UK Health
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UK Standards for Microbiology Investigations

Identification of *Actinomyces* species



National Institute for Health and Care Excellence (NICE) has renewed accreditation of the process used by the UK Health Security Agency to produce UK Standards for Microbiology Investigations (UK SMIs). The renewed accreditation is valid until 30 June 2026 and applies to guidance produced using the processes described in 'UK Standards for Microbiology Investigations Development Process' (2021). The original accreditation term began on 1 July 2011.

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on [the UK SMI website](#). UK SMIs are developed, reviewed and revised by various working groups which are overseen by a [steering committee](#).

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.

UK SMIs are produced in association with:



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Amendment table

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

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	6/08.02.24
Issue number discarded	3
Insert issue number	3.1
Anticipated next review date	08.02.27
Section involved	Amendment
Referral to reference or specialist testing laboratories	Reference to the UK Anaerobe Reference Unit has been provided.

Amendment number/date	5/30.01.24
Issue number discarded	2
Insert issue number	3
Anticipated next review date	30.01.27
Section(s) involved	Amendment
Title	The title has been changed from 'Identification of Anaerobic <i>Actinomyces</i> species' to 'Identification of <i>Actinomyces</i> species'
Whole document	<p>Hyperlinks updated to direct reader from UK SMIs webpages on GOV.UK to RCPATH website.</p> <p>Subheadings have been revised and modified where needed.</p> <p>All sections have been updated with current and relevant information and supported with recent literature where available.</p> <p>Some sections have been restructured as appropriate to align with current laboratory practices.</p>

Identification of *Actinomyces* species

<p>Scope of document</p>	<p>The scope has been updated to list the identification methods covered in the document.</p> <p>Links to other relevant UK SMIs that can be read in conjunction with this document have been added – UK SMI TP 40 and UK SMI ID 10.</p> <p>Topics that are outside the scope of this document have been mentioned and links to relevant UK SMIs were provided if available.</p> <p>The reader is also made aware of the reclassification and updated nomenclature of some <i>Actinomyces</i> species mentioned in the document.</p>
<p>Introduction</p>	<p>The taxonomy of <i>Actinomyces</i> species has been updated.</p> <p>The information under the characteristics section on <i>Neisseria</i> species has been summarised in Table 3.</p>
<p>Technical information and limitations</p>	<p>The information under this section has either been moved to Section 8: Identification or removed if not relevant anymore.</p>
<p>Safety considerations</p>	<p>Information about the hazard group classification of <i>Actinomyces</i> species has been added to this section.</p>
<p>Target organisms</p>	<p>The target organisms have been listed in Table 1.</p>
<p>Identification</p>	<p>All the sections have been updated with current and relevant information.</p> <p>This section has been restructured as appropriate to align with the current laboratory practices.</p>
<p>Referral to reference or specialist testing laboratories</p>	<p>Hyperlinks were updated where appropriate.</p>
<p>Algorithm</p>	<p>The structure and content of the algorithm has been updated to align with the current state of laboratory practices and knowledge.</p>
<p>References</p>	<p>References reviewed and updated.</p>

1 General information

[View general information](#) related to UK SMIs.

2 Scientific information

[View scientific information](#) related to UK SMIs.

3 Scope of document

This UK Standards for Microbiology Investigations (UK SMIs) document describes the identification of *Actinomyces* species and includes routine culture, Gram stain, and matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for identification. The test procedure for MALDI-TOF MS is covered in [UK SMI TP 40: Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry \(MALDI-TOF MS\) test procedure](#). It also includes conventional and molecular methods for alternative identification and confirmation.

This document also describes the differentiation of *Actinomyces* species from other related genera including the aerobic actinomycetes, *Nocardia* species. The identification of aerobic actinomycetes are covered in [UK SMI ID 10: Identification of aerobic actinomycetes](#).

This document does not provide information on antimicrobial susceptibility testing of *Actinomyces* species.

Some of the *Actinomyces* species have been reclassified, and the updated nomenclature of these species have been included in this document for reference.

UK SMIs should be used in conjunction with other relevant UK SMIs.

4 Introduction

4.1 Taxonomy and characteristics

Actinomyces species are Gram-positive, filamentous, microaerophilic to facultative anaerobes with high G-C DNA content (1). The genus *Actinomyces* is in the family Actinomycetaceae of the order Actinomycetales which belongs to the phylum Actinomycetota (Actinobacteria) - one of the largest and most diverse phyla among the bacteria (2-4).

There are currently more than 30 *Actinomyces* species validly published with the correct nomenclature and taxonomic status (1,3). Please refer to the most up to date nomenclature available as changes within the genus are commonplace and may occur following the publication of this UK SMI.

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The genus was revised in 2018 and a number of clinically significant species have been designated to alternative genera; refer to Section 7, Table 1. For simplicity the original nomenclature has been retained throughout this UK SMI.

Actinomyces species are opportunistic pathogens that form part of the usual microbiota of humans, they typically colonise the oral cavity, gastrointestinal tract, and female urogenital tract (1). Actinomycosis is a relatively rare and generally polymicrobial infection caused by *Actinomyces* species especially in immunocompromised individuals (5).

The main causative agent is *Actinomyces israelii*, but other *Actinomyces* species have also been reported including *Actinomyces odontolyticus*, *Actinomyces meyeri*, *Actinomyces gerencseriae* and *Actinomyces naeslundii* (6-9). The clinical presentation of the actinomycosis can vary depending on the severity and site of infection (7,10-14).

Species within this group have also been identified as clinically significant pathogens in breast abscesses and other non-classical actinomycosis infections, of particular note *Arachnia propionica*, associated with canaliculitis (15,16).

5 Technical information and limitations

Advancements in technology and gene sequencing have significantly contributed to the evolving taxonomy of *Actinomyces*, leading to numerous reclassification and changes in nomenclature of these species (3,4,17). Refer to Section 7, Table 1 for the reclassified *Actinomyces* species to date.

The complex taxonomy of *Actinomyces* species can lead to uncertainty and inconsistency in their identification, therefore it is important that clinicians stay up to date with the latest taxonomic revisions and resources and incorporate them into their interpretation of laboratory results. It is also important that the databases of identification methods such as MALDI-TOF MS and 16S rRNA gene sequencing reflect any changes in the taxonomy of *Actinomyces* (18,19).

6 Safety considerations

This section covers specific safety considerations (20-41) related to this UK SMI and should be read in conjunction with the general [safety considerations](#).

Actinomyces species and associated Gram-positive species are Hazard group 2 organisms. The processing of diagnostic samples should be carried out at Containment Level 2. Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

Identification of *Actinomyces* species

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet (29).

The above guidance should be supplemented with local COSHH and risk assessments. Compliance with postal and transport regulations is essential.

7 Target organisms

Table 1. *Actinomyces* species reported to have caused human infection (1,6,8,9,12-14,42-54).

Previous nomenclature	Current nomenclature
<i>Actinomyces israelii</i>	<i>Actinomyces israelii</i>
<i>Actinomyces graevenitzii</i>	<i>Actinomyces graevenitzii</i>
<i>Actinomyces gerencseriae</i>	<i>Actinomyces gerencseriae</i>
<i>Actinomyces naeslundii</i>	<i>Actinomyces naeslundii</i>
<i>Actinomyces odontolyticus</i>	<i>Schaalia odontolytica</i>
<i>Actinomyces viscosus</i>	<i>Actinomyces viscosus</i>
<i>Actinomyces funkei</i>	<i>Schaalia funkei</i>
<i>Actinomyces europaeus</i>	<i>Gleimia europaea</i>
<i>Actinomyces urogenitalis</i>	<i>Actinomyces urogenitalis</i>
<i>Actinomyces meyeri</i>	<i>Schaalia meyeri</i>
<i>Actinomyces neuii</i>	<i>Winkia neuii</i>
<i>Actinomyces neuii</i> subsp. <i>neuii</i>	<i>Winkia neuii</i> subsp. <i>neuii</i>
<i>Actinomyces neuii</i> subsp. <i>anitratus</i>	<i>Winkia neuii</i> subsp. <i>anitrata</i>
<i>Actinomyces radingae</i>	<i>Schaalia radingae</i>
<i>Actinomyces turicensis</i>	<i>Schaalia turicensis</i>
<i>Actinomyces radidentis</i>	<i>Actinomyces radidentis</i>
<i>Actinomyces cardiffensis</i>	<i>Schaalia cardiffensis</i>
<i>Actinomyces oricola</i>	<i>Actinomyces oricola</i>
<i>Actinomyces nasicola</i>	<i>Bowdeniella nasicola</i>
<i>Actinomyces massiliensis</i>	<i>Actinomyces massiliensis</i>
<i>Actinomyces johnsonii</i>	<i>Actinomyces johnsonii</i>

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Previous nomenclature	Current nomenclature
<i>Actinomyces dentalis</i>	<i>Actinomyces dentalis</i>
<i>Actinomyces hongkongensis</i>	<i>Pauljensenia hongkongensis</i>
<i>Actinomyces hominis</i>	<i>Gleimia hominis</i>
<i>Actinomyces oris</i>	<i>Actinomyces oris</i>
<i>Actinomyces timonensis</i>	<i>Actinomyces timonensis</i>
<i>Actinomyces georgiae</i>	<i>Schaalia georgiae</i>

Table 2. Other organisms which may be misidentified as *Actinomyces* species (3,15,55-57)

Previous nomenclature	Current nomenclature
<i>(Pseudo)Propionibacterium propionicum</i>	<i>Arachnia propionica</i>
<i>Scardovia wiggisiae</i>	<i>Scardovia wiggisiae</i>
<i>Nocardia</i> species	<i>Nocardia</i> species

8 Identification

In clinical laboratories, the identification of *Actinomyces* species typically involves a combination of methods. Culture-based methods are primarily used for identification, with the integration of faster identification techniques such as MALDI-TOF MS, improving accuracy of identification. Conventional and molecular methods can provide an alternative means of identification and can be used for confirmation where appropriate.

8.1 Culture methods

Culture methods provide presumptive identification of *Actinomyces* species based on colony morphology, Gram stain and other phenotypic characteristics followed by identification via MALDI-TOF MS.

8.1.1 Bacterial growth media

Some *Actinomyces* species are fastidious and slow growing (for example, *Actinomyces israelii*, and *Actinomyces gerencseriae*) and require enriched medium, with growth enhanced by the addition of carbon dioxide. Anaerobic conditions are favoured but some species can be cultured aerobically or in air plus 5 - 10% CO₂ (1,58). The optimum growth temperature is 35 - 37°C (1,8,58). Colonies may appear after 3 - 7 days of incubation, but detection may require 10 - 14 days of incubation

Identification of *Actinomyces* species

(1,8). Refer to Section 8.2.1, Table 3 for the colony morphology of *Actinomyces* species.

Note: The majority of *Actinomyces* species are facultative anaerobes, except for *Actinomyces israelii*, *Actinomyces gerencseriae* and *Actinomyces meyeri* which are strict anaerobes (1,59).

8.1.1.1 Primary isolation media

Fastidious anaerobic agar or equivalent agar without neomycin incubated anaerobically at 35 - 37°C for 5 - 10 days (58).

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

8.1.1.2 Selective media

Actinomyces selective agar with metronidazole 10 mg/L and nalidixic acid 30 mg/L (deep fill) incubated anaerobically at 35 - 37°C for 5 - 10 days (58). Growth in air and in air plus 5 - 10% CO₂ is variable. Broth enrichment is rarely beneficial.

Note: Some species may require longer incubation.

8.1.2 Colonial appearance

Actinomyces species exhibit various appearances (1,8,60). Only a few species produce the classic breadcrumb/molar tooth colonies. The majority are white or grey in colour, with some producing pigmentation following prolonged incubation periods.

8.2 Microscopic appearance

Actinomyces colonies can be examined directly and/or following Gram staining to assess their colony morphology. Members of the genus demonstrate considerable variation in colony morphology, which can make their recognition and identification challenging (8,60,61). A combination of different laboratory techniques can aid in their accurate identification. Refer to Section 8.2.1, Table 3 for the colonial and microscopic appearance of *Actinomyces* species.

8.2.1 Gram stain

Refer to [UK SMI TP 39: Staining procedures](#).

Actinomyces appear as branching, beaded, filamentous, diphtheroid-shaped or coccobacillary Gram-positive bacilli (1).

Note: *Actinomyces* are easily decolourised during Gram staining. Excessive use of acetone and iodine/acetone during the decolourisation stage will remove the crystal violet from the cell wall, resulting in Gram-negative appearance.

Note: *Propionibacterium* and *Cutibacterium* species are pleomorphic bacilli that may appear to branch and *Nocardia* species are morphologically indistinguishable from *Actinomyces* species on Gram stain (55,56).

Identification of *Actinomyces* species

Table 3. Microscopic and colonial morphology of *Actinomyces* species (1,3,6,7,9,12-15,42-52,57)

The information here provides general characteristics of colony appearance, which can vary among different strains and culture conditions.

Species	Colonies	Comments
<i>A. israelii</i>	White to cream, breadcrumb or molar tooth, gritty and pitting.	Slow growing.
<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 and convex, with entire edges.	Occasional rough forms occur. Acid production may affect viability in older cultures.
<i>A. odontolyticus</i>	Cream to red, smooth and convex, with entire edges.	Old colonies may be dark brown. Acid production may affect viability in older cultures.
<i>A. meyeri</i>	Small, white, smooth and convex, with entire edges.	Slow growing.
<i>A. georgiae</i>	White or cream, smooth and convex, with entire edges.	None.
<i>A. neuii</i> sub sp. <i>neuii</i> and <i>anitratus</i>	White or cream, smooth and convex, with entire edges.	None.
<i>A. radingae</i>	Grey to white, semi-translucent, smooth and low convex, with entire edges.	None.
<i>A. turicensis</i>	Grey, semi translucent, smooth and low convex, with entire edges.	None.
<i>A. europaeus</i>	Whitish, semi translucent, smooth and low convex, with entire edges.	None.
<i>A. graevenitzii</i> *	White pronounced molar tooth or smooth and convex.	Red fluorescence. Rough and smooth forms occur together. Old colonies may become dark brown.
<i>A. radidentis</i>	Cream to pink, smooth and convex, with entire edges.	Old colonies may become red.
<i>A. urogenitalis</i>	Cream to-pink, with darker rings and smooth.	Old colonies may become red. Acid production may affect viability in older cultures.
<i>A. funkei</i>	Grey, semi translucent, opaque centre (fried egg), low convex, with entire edges.	None.
<i>A. cardiffensis</i>	Cream to pink, smooth and convex, with entire edges.	None.

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Species	Colonies	Comments
<i>A. nasicola</i>	White or grey, smooth and convex, with entire edges.	None.
<i>A. oricola</i>	White, breadcrumb, pitting on the agar.	None.
<i>A. viscosus</i>	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.	None.
<i>A. johnsonii</i>	Colonies are similar to <i>A. naeslundii</i>	Acid production may affect viability in older cultures.
<i>A. oris</i>	Colonies are similar to <i>A. naeslundii</i>	Acid production may affect viability in older cultures.
<i>A. massiliensis</i>	White, pinpoint, circular and shiny with entire edges.	None.
<i>A. dentalis</i>	Tiny, white and breadcrumb-like and pitting on the agar	None.
<i>A. hongkongensis</i>	Non-haemolytic, pinpoint colonies.	None.
<i>A. hominis</i>	White–greyish and convex, with entire edges.	None.
<i>A. timonensis</i>	α-haemolytic, pinpoint, circular, white, dry and embedded in the agar	None.
<i>P. propionicum</i> *	Off white to buff, breadcrumb, gritty, pitting, or smooth and convex, with entire edges.	Red fluorescence, rough and smooth forms occur together
<i>Scardovia wiggisiae</i>	White to cream, breadcrumb, or molar tooth, gritty and pitting.	Very slow growing. May not identify by conventional methods.
<p>*Colonies of <i>A. graevenitzii</i> and <i>P. propionicum</i> on blood containing media fluoresce red under long-wave (366 nm) UV illumination.</p>		

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

MALDI-TOF MS is often used as the primary method for the identification of *Actinomyces* species in many diagnostic laboratories. Therefore it is important that this method is appropriately validated, manufacturer instructions carefully followed, available database updates installed and reviewed, and the use of an extraction step that can contribute to a more reliable species identification should be considered.

The changing taxonomy and reclassification of *Actinomyces* species poses a challenge for species-level identification (3,4,17). The reference database used by MALDI-TOF MS instruments need to be reviewed regularly and updated with the evolving nomenclature and classification of *Actinomyces* species to ensure accurate identification (5,18,19,61,62). Also, closely related *Actinomyces* species exhibit similar protein profiles, making it difficult to distinguish between them, which can lead to misidentification (18,62).

Another point for consideration is the influence colony age and morphology can have on the performance of MALDI-TOF MS. Older colonies or colonies with drier and 'chalkier' morphology can negatively impact the quality of the mass spectral output and subsequently reduce the accuracy of identification of *Actinomyces* species (61,63).

Therefore, confirmation of MALDI-TOF MS results may be required in certain cases, particularly if there are discrepancies in results, or if a species identification score is low or not obtained. In such cases, in-house confirmation using conventional or molecular methods should be performed or the isolates can be sent to a reference or specialist testing laboratory as appropriate.

8.4 Further identification

8.4.1 Biochemical tests and commercial identification systems

Biochemical tests including commercial identification kits provide basic biochemical information that can aid in the identification of *Actinomyces* species. However, relying solely on these tests is insufficient for accurate identification of *Actinomyces* species. Therefore, these tests are either not considered reliable for the identification of *Actinomyces* species or employed as part of a multi-step approach that combines alternative identification techniques to achieve more accurate results. They can also be used to confirm MALDI-TOF MS results as required.

In addition, biochemical test results should be interpreted with caution and in conjunction with other test results. To achieve accurate results with biochemical tests, it is advisable to use taxonomic keys and not rely on the identification given by the code. This is because the databases contain out of date information, are incomplete or

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due to variation in reaction strengths/occasional weak enzymatic and sugar fermentation reactions (64,65). This is particularly true as molecular techniques enable more species to be identified than was previously possible (65).

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

Refer to manufacturer's guidance or Manual of Clinical Microbiology for the biochemical properties of *Actinomyces* species and associated Gram-positive species (1).

8.4.1.1 Indole test

Refer to [UK SMI TP 19: Indole test](#).

Actinomyces species are spot indole negative (1).

Note: *Propionibacterium/Cutibacterium acnes* is indole positive (1,56).

8.4.1.2 Catalase test

Refer to [UK SMI TP 8: Catalase test](#).

All *Actinomyces* species are catalase negative except *Actinomyces viscosus*, *Actinomyces neuii* subsp. *neuii*, *Actinomyces neuii* subsp. *anitratius*, *Actinomyces radicidentis* and *Actinomyces hominis* (1,60).

8.4.2 Molecular methods

Molecular methods can serve as alternative identification methods to MALDI-TOF MS or be used for confirmation in cases where MALDI-TOF MS results need validation. The utilisation of 16S rRNA gene sequencing in particular has transformed the identification and taxonomy of *Actinomyces* species (4,17).

However, 16S rRNA gene sequencing also has limitations, these include the challenge of differentiating between some closely related *Actinomyces* species such as *Actinomyces naeslundii*, *Actinomyces viscosus* and *Actinomyces oris*, which can lead to misidentification (61). The databases may also contain erroneous sequences which can appear as the top match. Therefore it is important to corroborate results using a different identification method such as MALDI-TOF MS and to continue improving and expanding the reference databases to include a comprehensive range of *Actinomyces* species including novel species to reflect current classifications (18,61).

The implementation of 16S rRNA gene sequencing for *Actinomyces* identification can be challenging, as not all clinical laboratories have access to this sequencing technique. Therefore, *Actinomyces* isolates can be sent to a reference or specialist testing laboratory where required.

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8.4.2.1 Next Generation Sequencing

Next Generation Sequencing technologies that have been largely restricted to reference or specialist testing laboratories are gradually becoming more accessible and cost-effective. Clinical laboratories may implement them for routine identification and diagnostic purposes in the future.

9 Storage

If required, inoculate the pure isolate into anaerobic broth culture. Follow local regulations for the storage of *Actinomyces* species.

10 Reporting

10.1 Infection Specialist

Inform the infection specialist of presumptive or confirmed *Actinomyces* when the request contains relevant information.

10.2 Presumptive identification

Refer to Section 8: Identification for the identification of *Actinomyces* species.

10.3 Confirmation of identification

In certain cases, confirmation of identified isolates may be required.

Refer to Section 8: Identification for the identification and confirmation of *Actinomyces* species.

10.4 Health Protection Team (HPT)

N/A

10.5 UK Health Security Agency

N/A

10.6 Infection prevention and control team

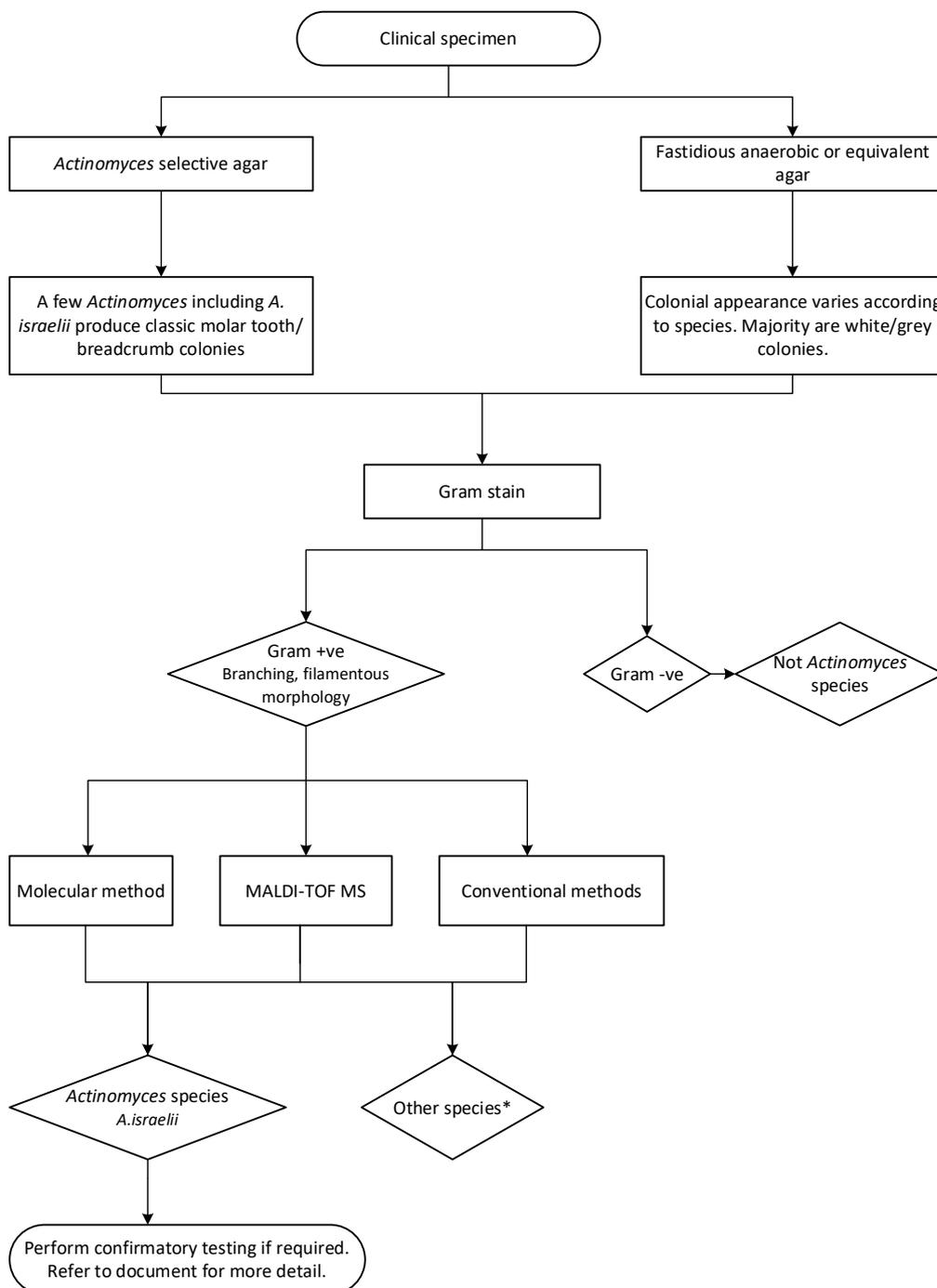
N/A

11 Referral to reference or specialist testing laboratories

In case of sending away isolates to a reference or specialist testing laboratory for processing, ensure that the specimen is placed in the appropriate package and transported accordingly. Follow local regulations and instructions provided by the reference or specialist testing laboratory for sending isolates.

For referral of isolates contact the [UK Anaerobe Reference Unit \(UKARU\)](#) or the appropriate specialist testing laboratory.

Algorithm: Identification of *Actinomyces* species



*Report as not *Actinomyces* species.

The flowchart is for guidance only.

References

An explanation of the reference assessment used is available in the [scientific information section on the UK SMI website](#).

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