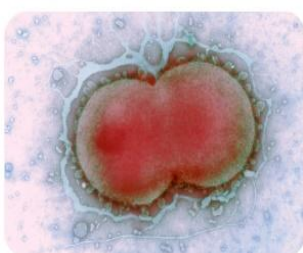
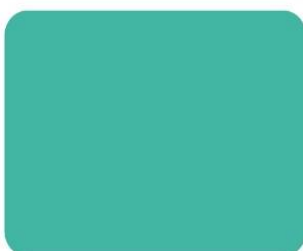
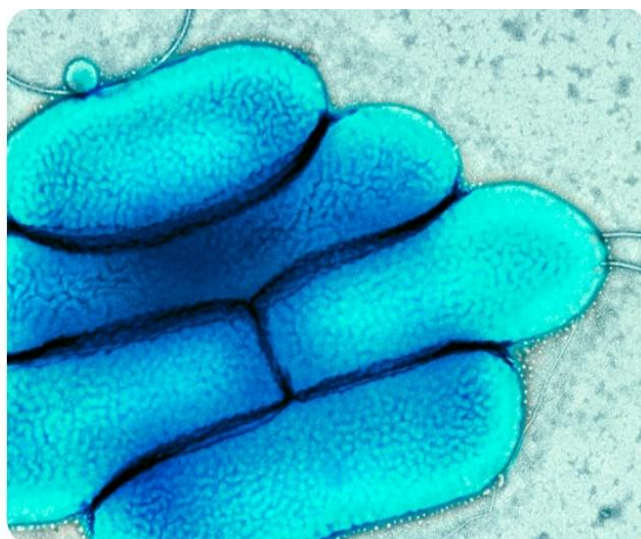
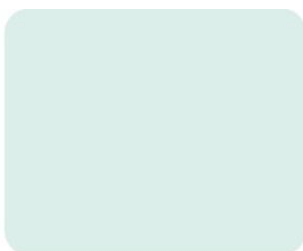




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UK Standards for Microbiology Investigations

Identification of *Staphylococcus* species, *Micrococcus* species and *Rothia* species



Acknowledgments

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Amendment table

The amendments since the previous version of this UK SMI document are listed in the amendments table below.

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

Amendment number/date	7/01.10.26
Issue number discarded	4
Insert issue number	5
Anticipated next review date*	01.10.29
Section(s) involved	Amendment
Whole document	Hyperlinks updated to direct reader to UK SMIs on the RCPATH website rather than GOV.UK. Subheadings have been revised and modified as required. All sections have been updated with current and relevant information and supported with recent literature where available. Some sections have been restructured as appropriate to align with current laboratory practices.
Scope of document	The scope has been updated to list the identification methods covered in the document. Links to other relevant UK SMIs that can be read in conjunction with this document have been added – UK SMI TP 39. Topics that are outside the scope of this document have been mentioned and links to relevant UK SMIs were provided if available – UK SMI B 26.
Introduction	The taxonomy of <i>Staphylococcus</i> species and related organisms has been updated. The information under the characteristics section on <i>Staphylococcus</i> species has been summarised in Table 1.
Technical information and limitations	The information under this section has either been moved to Section 8: Identification or removed if not relevant anymore.

Safety considerations	Standard references have been replaced with a link to the relevant page on the website.
Identification	Table 1 added to summarise the microscopic and colonial appearance of the target <i>Staphylococcus</i> species and related organisms. Guidance on latex agglutination, coagulase (slide and tube) and PYR testing has been added and clarified. Tube coagulase testing is described as a confirmatory method when additional phenotypic confirmation is required.
Reporting	The information has been updated as appropriate.
Referral to reference or specialist testing laboratories	The information has been updated and hyperlinks added where appropriate.
Public health responsibilities of diagnostic laboratories	New section added to the UK SMI document.
Algorithm	The structure and content of the algorithm have been updated to align with the current state of laboratory practices and knowledge.
References	References reviewed and updated.

*Reviews can be extended up to 5 years where appropriate

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 SMI) document describes the identification of *Staphylococcus* species, *Micrococcus* species and *Rothia* species from clinical material. In routine laboratory workflows, Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) usually serves as the primary method of identification. The test procedure for matrix assisted laser desorption ionization-time of flight mass spectrometry (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](#).

Further characterisation can be supported by phenotypic approaches and commercial biochemical systems as required. This also applies when MALDI-TOF MS or other automated systems yield inconclusive results, or when such systems are unavailable. Molecular platforms may also be employed when additional confirmation is necessary. Commercial identification systems and other *in vitro* diagnostic devices should be used in accordance with the manufacturer's instructions and relevant medical device regulations (1).

The focus of this UK SMI is on clinically relevant species isolated from human infections. Organisms morphologically similar to *Staphylococcus* species which may be found in clinical specimens are also included.

In view of the constantly evolving taxonomy of this group of organisms, phenotypic methods alone may not adequately identify organisms to species level. This UK SMI adopts a simplified approach based on grouping organisms with similar phenotypic attribute. Further identification may be necessary where clinically or epidemiologically indicated.

This is an identification document and therefore does not focus on screening, typing or antimicrobial susceptibility testing. However, relevant information is included where necessary to provide context. For details on methicillin-resistant *Staphylococcus aureus* (MRSA) screening, refer to [UK SMI B 29 - Investigation of Specimens for Screening MRSA](#).

For the identification of catalase negative Gram-positive cocci, see [UK SMI ID 4 - Identification of *Streptococcus* species, *Enterococcus* species and morphologically similar organisms](#).

For further information on specific organisms and their associated clinical syndromes, please refer to the relevant UK SMI in the [Syndromic](#) and [Bacteriology](#) categories.

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

4 Introduction

4.1 Target organisms of clinical significance

Staphylococci are widespread in nature and include several species that colonise the skin and mucous membranes of human and animals. *Staphylococcus aureus* is the most clinically significant species within the genus.

The *Staphylococcus aureus* complex comprises several closely related species: *S. aureus*, *S. argenteus*, *S. schweitzeri* and *S. aureus* subsp. *anaerobius*. *S. aureus* subsp. *anaerobius* is a zoonotic pathogen primarily associated with sheep, and *S. schweitzeri* is mainly found in African wildlife (2-4). *S. aureus* and *S. argenteus* are relevant to human clinical disease and due to the close genetic and clinical relatedness, these organisms may have similar pathogenic potential and infection control implications (4).

Although a common coloniser of the anterior nares, skin and other mucosal surfaces, *S. aureus* can cause a wide range of infections including skin and soft tissue infections as well as bacteraemia, pneumonia and endocarditis. *S. aureus* is also responsible for toxin-mediated disease such as toxic shock syndrome and food poisoning. This pathogen produces several virulence factors such as protein A, capsular polysaccharides, and various toxins. Some strains of *S. aureus* produce Toxic Shock Syndrome Toxin-1 (TSST-1), Panton-Valentine Leukocidin (PVL), or other exotoxins, which contribute to their pathogenicity and ability to cause severe systemic illness. In addition, it is also a major cause of healthcare-associated infections (HCAs), particularly surgical site infections, catheter-related bloodstream infections (CRBSI) and ventilator-associated pneumonia.

Coagulase negative staphylococci (CoNS) are normal skin, nasal, and ear canal commensals that were once considered non-pathogenic. Although they lack many of the virulence factors of *S. aureus*, they are now increasingly recognised as important opportunistic pathogens. Their clinical significance has increased with the greater use of intravascular devices and the rising numbers of immunocompromised patients. CoNS are a major cause of nosocomial infections, including CRBSI, prosthetic valve infective endocarditis and other device-related infections, particularly in patients with prosthetic joint or cardiac implants.

There are more than 30 species of CoNS, representing a taxonomically diverse group of organisms with differing phenotypic characteristics and clinical importance (5).

Staphylococcus lugdunensis exhibits pathogenicity more similar to *Staphylococcus aureus* than to other CoNS species and has been isolated from a range of infections,

including skin, superficial and deep soft-tissue and wound infections, and in some cases from more invasive disease such as infective endocarditis (5-10).

S. haemolyticus and *S. capitis* have also been implicated with growing frequency in invasive and HCAs. *S. haemolyticus* has emerged as an important cause of healthcare-associated bloodstream infections and is frequently characterised by multidrug resistance, particularly in intensive care settings (5,11-13).

S. capitis has been implicated in outbreaks in neonatal intensive care units (5,14).

S. epidermidis and *S. saprophyticus* are the CoNS species most often associated with infections, but there is increasing recognition that other CoNS species are emerging as important pathogens. *S. saprophyticus* commonly causes uncomplicated urinary tract infections in young, sexually active women, while *S. epidermidis*, a normal skin commensal, is an important opportunistic pathogen in device-related and HCAs (5).

With the increasing use of advanced identification methods, particularly MALDI-TOF MS and molecular techniques, other less common CoNS species such as *S. cohnii*, *S. hominis*, *S. sciuri*, *S. schleiferi* subspecies *schleiferi*, *S. simulans*, *S. saccharolyticus* (previously known as *Peptococcus saccharolyticus*) and *S. warneri* have also been identified in clinical specimens (5,15). Their clinical significance should be interpreted in the context of patient factors, specimen type and clinical presentation.

Micrococcus species are generally considered harmless saprophytes that inhabit or contaminate the skin, mucosa, and the oropharynx; however, they can be opportunistic pathogens in certain immunocompromised patients. *Micrococcus* species that are associated with infections are *Micrococcus luteus* and *Micrococcus lylae* (16-18).

Amongst the *Rothia* species, *Rothia dentocariosa* and *Rothia mucilaginosa* are the species most commonly associated with human infections (19,20).

4.2 Taxonomy and characteristics

The genus *Staphylococcus* is in the bacterial family Staphylococcaceae, which includes five lesser-known genera, *Gemella*, *Jeotgalicoccus*, *Macrococcus*, *Nosocomiicoccus* and *Salinicoccus*. There are currently over 50 recognised species of staphylococci and more than 20 subspecies (5).

The genus *Micrococcus* belongs to the bacterial family Micrococcaceae which currently contains 17 species. These have been isolated from human skin, animal and dairy products as well as environment (water, dust and soil). Some of these species have been re-classified to other genera. Former members of the genus *Micrococcus*, now assigned to other genera, include *Arthrobacter agilis*, *Nesterenkonia halobia*, *Kocuria kristinae*, *K. rosea*, *K. varians*, *Kytococcus sedentarius*, and *Dermacoccus nishinomiyaensis*. The *Micrococcus* species that are associated with infections are *Micrococcus luteus* and *Micrococcus lylae* (16-18).

The genus *Rothia* is in the same family as the genus *Micrococcus* and currently contains 8 species. *Rothia dentocariosa* and *Rothia mucilaginosa* are the most commonly known species to cause infections in humans. *Rothia* species are increasingly identified in clinical specimens following the routine implementation of MALDI-TOF MS and are part of the normal oral and upper respiratory tract flora, but may act as opportunistic pathogens, particularly in immunocompromised individuals (20,21).

Staphylococcus species are Gram-positive cocci in clusters, facultative anaerobes, and catalase positive. *Micrococcus* species are Gram-positive cocci in tetrads, strictly aerobic, and catalase positive, while *Rothia* species are pleomorphic Gram-positive organisms (cocci to short bacilli), facultatively anaerobic, with variable catalase reactions. Despite these differences, all are part of the normal human microbiota and can act as opportunistic pathogens in certain clinical settings (18,20).

Coagulase production is an important characteristic used in the differentiation of *Staphylococcus* species. Latex agglutination and coagulase tests may support the presumptive identification and confirmation of *S. aureus*. See Section 6.4.2 and [UK SMI TP 10 – Coagulase test](#).

In addition, pyrrolidonyl arylamidase (PYR) activity may be useful in the identification of certain CoNS, particularly *S. lugdunensis*. Refer to Section 6: Identification for further information.

5 Safety considerations

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

None of the organisms included in this document are classified higher than Hazard Group 2 by the Advisory Committee on Dangerous Pathogens (ACDP) (22).

The processing of diagnostic samples should be carried out at Containment Level 2.

Appropriate Personal Protective Equipment (PPE) and techniques designed to minimise exposure of the laboratory workers should be worn and adhered to at all times.

When the presence of organisms requiring enhanced precautions cannot be ruled out, any procedure must be conducted in a microbiological safety cabinet.

Laboratory acquired infections should be reported to the Health and Safety Executive (HSE).

The above guidance should be supplemented with local Control of Substances Hazardous to Health Regulations (COSHH) and risk assessments. Compliance with postal and transport regulations is essential.

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

6 Identification

Isolates from primary culture are assessed by their colonial morphology, microscopic appearance as Gram positive cocci, and physiological characteristics. Direct Gram staining of certain sterile samples or liquid enrichment media, such as positive blood cultures, may indicate the presence of staphylococci when Gram-positive cocci in clusters are seen on microscopy.

In routine workflows, MALDI-TOF MS is usually the primary method for species-level identification. It enables accurate differentiation of *S. aureus* and clinically significant CoNS such as *S. lugdenensis* from other commensal or opportunistic CoNS. Where MALDI-TOF MS is unavailable, preliminary identification may be supported by Gram staining and biochemical tests, including catalase testing (to differentiate staphylococci from streptococci), followed by latex agglutination and coagulase testing to distinguish *S. aureus* from CoNS. Refer to the algorithm for the identification of *Staphylococcus*, *Micrococcus*, and *Rothia* species outlined later in this document.

Additional characterisation may be achieved using phenotypic or biochemical tests performed from non-selective media when required. Antimicrobial susceptibility patterns may also be used as adjunctive indicators where locally validated; however, these findings are not definitive or reliable for identification and should be interpreted with caution alongside confirmatory methods.

Species-level identification is essential for organisms such as *S. aureus* and other clinically significant CoNS (e.g. *S. lugdenensis*) from sterile site samples. However, the required level of identification of other CoNS depends on local epidemiology, laboratory practice, specimen type and clinical context. When the finding is likely to be clinically insignificant, then reporting at genus level without susceptibilities may be appropriate.

Non-routine or supplementary methods, including specific biochemical reactions (e.g., DNase or thermostable nuclease), molecular assays, or other specialist techniques, may be used when results are inconclusive, confirmation is required, or automated systems are unavailable. Ambiguous, unusual, or unexpected isolates should be referred to an appropriate specialist or reference laboratory.

6.1 Microscopic appearance

Gram stain ([UK SMI TP 39 - Staining Procedures](#))

Staphylococci are Gram positive cocci, which appear in pairs, tetrads or irregular clusters in Gram stains from liquid culture medium or directly from sterile samples.

Micrococcus species are also Gram-positive cocci and typically appear in tetrads or irregular clusters.

Rothia species are Gram positive and may appear as cocci, cocco-bacilli or short bacilli, and can be pleomorphic in Gram-stained preparations.

6.2 Primary isolation media

Staphylococci and other related organisms may be recovered using a range of media and under different atmospheric conditions. Blood agar is generally preferred for initial assessment because it supports good recovery and typical colonial morphology.

Additional media such as Staph/Strep selective agar, Cystine Lactose Electrolyte Deficient agar (CLED) or Mannitol Salt Agar (MSA) may also assist with the isolation of staphylococci by limiting overgrowth from other organisms including swarming organisms such as *Proteus* species and by enabling differentiation based on characteristic reactions.

Chromogenic media can aid direct isolation and presumptive identification of *S. aureus* and may incorporate selective agents such as oxacillin or ceftiofur for the detection of presumptive MRSA. Chromogenic media can allow isolation and presumptive identification in a single step and may reduce the need for further confirmatory testing, provided their performance is verified locally and shown to be sufficiently sensitive and specific.

As with other organisms, laboratories should ensure that media used for identification are validated for compatibility with downstream methods such as MALDI-TOF MS, other commercial platforms and biochemical tests. Performance should be verified as part of routine quality control in accordance with local quality assurance procedures.

6.3 Colonial appearance

Colony morphology, including size, is variable and influenced by growth conditions, limiting its diagnostic utility. While some species such as *S. aureus* tend to produce larger colonies and others such as *S. epidermidis* and *S. haemolyticus* may appear smaller, these features are not consistent and should be interpreted alongside other findings. Small-colony variants may occur and should also be interpreted in context.

See Table 1 below for examples of colonial appearance.

This is not an exhaustive list of possible species.

Table 1. Presumptive identification of selected *Staphylococcus* species and morphologically similar organisms from blood agar

Colony morphology	Organisms	Notes
Golden to cream, smooth, glistening, and convex	<i>S. aureus</i>	Most strains are facultatively anaerobic and will grow under both aerobic and anaerobic conditions, although routine growth and colony morphology are best assessed under aerobic /CO₂-enriched conditions (5,23). Beta haemolysis is frequently observed (5). Small-colony variants (SCVs) may occur. These slow-growing variants for colonies that are substantially smaller than the parental strain, are associated with chronic infections (e.g. cystic fibrosis), and may require prolonged incubation for recovery (24). <i>S. aureus</i> subspecies <i>anaerobius</i> is rarely isolated from human clinical specimens and is a strict anaerobe (2).
Creamy white	<i>S. argenteus</i>	Facultatively anaerobic and beta haemolytic (5). Small-colony variants (SCVs) may also be observed (see <i>S. aureus</i> section) (24). Difference to <i>S. aureus</i> in pigment is evident after growing on chocolate agar for 48 hr at 37 °C (2).
Pale yellow / orange, smooth, glossy, and convex	<i>S. lugdunensis</i>	Facultatively anaerobic and may exhibit beta haemolysis or appear non-haemolytic depending on strain (5).
White-cream and smooth	<i>S. intermedius</i> group	Includes <i>S. intermedius</i> , <i>S. pseudintermedius</i> , <i>S. delphini</i>
White to creamy/grey-white and smooth	<i>S. epidermidis</i>	Non-haemolytic (5).
Golden-yellow, smooth, and opaque	<i>S. haemolyticus</i>	Usually haemolytic but non-haemolysis occurs occasionally (5).
White/yellow, glossy, smooth, and convex	<i>S. saprophyticus</i>	—
White or tan, smooth, and opaque	<i>S. capitis</i> , <i>S. hominis</i> <i>S. warneri</i>	<i>S. warneri</i> may appear with orange rim around the colony.
Grey/White, smooth, and translucent	<i>S. simulans</i>	—

Colony morphology	Organisms	Notes
Yellow to cream white, smooth and convex Pink/red	<i>M. luteus</i> , <i>M. lylae</i> , <i>M. flavus</i> <i>M. roseus</i>	—
White to grey, dry surface, rough with irregular edges	<i>Rothia</i> species	Microcolonies on agar surface are composed of filamentous branched elements which rapidly fragment into bacillary or coccoid forms, resembling <i>Actinomyces</i> or <i>Nocardia</i> species (25). Exhibit good growth under aerobic or microaerophilic conditions, but poor or no growth anaerobically.

6.4 Test procedures

Test procedures may vary between laboratories, including the order in which methods are applied and the selection of tests used. These decisions are determined by local laboratory practice and supported by appropriate validation and verification. Therefore, the following section describes available tests without implying a fixed workflow.

6.4.1 Matrix-Assisted Laser Desorption/Ionisation - Time Of Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS has largely replaced conventional biochemical methods for the identification of *Staphylococcus* species and is now used routinely in many laboratories. It enables rapid and accurate species-level identification and reliable discrimination between *S. aureus* and CoNS provided that identification scores meet locally defined acceptance criteria and are consistent with colony

morphology. Where identification scores fall below these thresholds, or where the organism identified falls outside the validated scope of the method, further characterisation using additional phenotypic or traditional biochemical methods may be required (15,26).

MALDI-TOF MS has also proved valuable in the description of newly recognised staphylococcal species and has improved species-level resolution among clinically relevant CoNS. Ongoing expansion and refinement of MALDI-TOF MS reference databases have substantially improved species-level resolution, leading to increased recognition of opportunistic species within the genera *Staphylococcus*, *Micrococcus* and *Rothia* (5,15).

However, differentiation of species within the *S. aureus* complex, for example *S. argenteus* and *S. schweizeri* from *S. aureus*, is dependent on the version and completeness of the MALDI-TOF MS reference database. As databases are updated, increasing recognition of these two organisms is expected (27).

Given these limitations, identification of *S. argenteus* or *S. schweizeri*, or results where differentiation is uncertain, should be interpreted with caution. Due to their close relatedness and similar clinical significance, these isolates may be investigated in line with *S. aureus*, including antimicrobial susceptibility testing and consideration of methicillin resistance where appropriate. Refer to [UK SMI B 29: Investigation of specimens for screening for MRSA](#). MALDI-TOF MS identification should be performed in accordance with manufacturer's instructions and supported by appropriate local validation and reporting policies.

6.4.2 Biochemical tests

A wide range of biochemical tests are available for the characterisation of staphylococci; some common ones are listed in Table 2.

Biochemical identification should be performed in accordance with the manufacturer's instructions for the identification system in use, including suitability of colony source and requirement for subculture where specified.

Catalase test (see [UK SMI TP 8 - Catalase test](#))

Staphylococcus, *Micrococcus* and *Rothia* species are catalase positive.

Notes:

- *S. aureus* subspecies *anaerobius* and *S. saccharolyticus* are catalase negative (5).
- Rare catalase negative strains of *S. aureus* have also been reported (28,29).
- *Rothia dentocariosa* are catalase positive. However, catalase negative strains have been reported and this will be more difficult to recognise with traditional tests, as they may mimic the rare *Bifidobacterium* strains that are able to grow aerobically, as well as *Actinomyces* and *Arcanobacterium* species, *Propionibacterium propionicum* and catalase negative *Listeria* strains (30).

Rothia mucilaginosa cells display variable catalase reactions ranging from negative to weakly positive or strongly positive.

Coagulase and latex agglutination tests (see [UK SMI TP 10 – Coagulase test](#))

Coagulase testing remains an important phenotypic method for differentiating coagulase-positive staphylococci from CoNS. Latex agglutination tests are commonly used for the rapid presumptive identification of *S. aureus* and detect clumping factor and/or protein A (a cell wall protein), whereas the tube coagulase test detects free coagulase and may be used to confirm positive or equivocal latex agglutination results when phenotypic confirmation is required. This may be particularly useful when MALDI-TOF MS is unavailable, inconclusive, or additional confirmation is needed. The slide coagulase test detects bound coagulase, also known as clumping factor. It may be used as a supplementary method but does not replace the tube coagulase test (5,15).

Notes:

- Rare coagulase-negative strains of *S. aureus* have been described (31).
- Several non-aureus staphylococci, including members of the *S. intermedius* group, may produce positive coagulase reactions (5).
- *S. lugdunensis* may express a clumping factor and therefore produce positive latex agglutination or slide coagulase reactions despite being tube coagulase negative (5,15).

- Coagulase and latex agglutination results should be interpreted alongside other identification findings

DNase test (see [UK SMI TP 12 – Deoxyribonuclease test](#))

The DNase test is useful for the presumptive identification of *Staphylococcus aureus*, as most CoNS are DNase negative.

Commercially available DNA containing agars are used to detect thermolabile nuclease activity. Addition of a weak acid (1N HCl) solution to an 18 – 24hr culture plate will demonstrate clearing around colonies of DNase positive species and if toluidine blue O solution is added, a bright rose-pink zone around colonies of DNase positive species can be seen (5,15).

PYR test

S. lugdunensis is typically PYR positive, and this characteristic may assist in differentiating it from other CoNS when additional characterisation is required.

6.4.3 Commercial identification systems (phenotypic panels)

Several commercial identification systems are available for the speciation of Staphylococci. Results should be interpreted in conjunction with the relevant test results shown in Table 2 and should be quality controlled following the manufacturer's instructions.

Table 2: Phenotypic test characteristics of selected *Staphylococcus* species and morphologically similar organisms

Species	Tube coagulase test ^a	Latex Agglutination Test ^b	DNase test ^c	PYR ^d
<i>S. aureus</i> complex ^e	+	+	+	-
<i>S. lugdunensis</i>	-	+/-	-	+
<i>S. intermedius</i>	+	+	+	+
<i>S. epidermidis</i>	-	-	-	-
<i>S. haemolyticus</i>	-	-	-	+
<i>S. saprophyticus</i> - (<i>S. saprophyticus</i> subsp. <i>Saprophyticus</i> , and <i>S. saprophyticus</i> subsp. <i>bovis</i>)	-	-	-	-
<i>S. capitis</i>	-	-	-	-
<i>S. hominis</i>	-	-	-	-
<i>S. warneri</i>	-	-	-	-
<i>S. simulans</i>	-	-	-	+
<i>Micrococcus</i> species (<i>M. luteus</i> , <i>M. lylae</i> , <i>M. flavus</i> and <i>M. roseus</i>)	-	-	-	-
<i>Rothia</i> species (<i>R. dentocariosa</i> , <i>R. mucilaginoso</i>)	-	-	-	-

Footnotes:

- a** Positive or equivocal latex agglutination results should be confirmed with a tube coagulase test to avoid false positives.

Not all *S. aureus* are coagulase positive, and not all coagulase positive staphylococci are *S. aureus*. Therefore, to improve the identification of *S. aureus*, other tests in conjunction with coagulase test should be performed (31) .

Several species may produce positive tube coagulase tests, including *S. intermedius*, *S. pseudintermedius*, *S. argenteus*, *S. hyicus*, *S. schweitzeri*, *S. coagulans* and *S. delphini* (rare in humans) (5).

S. lugdunensis can show positive slide coagulase reactions due to strong clumping factor activity.

Occasional false-positive coagulase reactions may occur with non-staphylococcal organisms.

For more information on Coagulase test, see [UK SMI TP 10 - Coagulase test](#)

- b** Latex agglutination tests may be used as a rapid adjunct to the identification of presumptive *S. aureus* isolates. Results should be interpreted with caution, as both false-positive and false-negative reactions may occur.

Non-specific agglutination reactions may occasionally occur in latex agglutination tests, including with non-staphylococcal organisms such as yeasts. Speciation of presumptive staphylococci should be considered where clinically relevant to avoid missing clinically significant organisms such as *S. aureus*, *S. lugdunensis* or yeasts.

Positive or equivocal latex agglutination results should be confirmed using a tube coagulase test, and isolates producing atypical or discrepant results should be investigated further using additional identification methods, such as MALDI-TOF MS, where appropriate (5).

S. sciuri may occasionally produce false-positive latex reactions.

S. lugdunensis may yield weak, partial, or negative results in latex agglutination tests (5).

- c** DNase production helps support identification when used alongside coagulase/latex results.

Some non-aureus staphylococci may produce nuclease activity, giving misleading results.

- d** PYR is a useful test for identifying *S. lugdunensis* and may assist in differentiating it from other coagulase-negative staphylococci when MALDI-TOF MS is unavailable

Identification of *Staphylococcus* species, *Micrococcus* species and *Rothia* species

- e *S. aureus* complex includes *S. aureus* and *S. argenteus*, *S. schweitzeri*

6.5 Further identification

A range of molecular methods may be used for further characterisation or confirmation of *Staphylococcus* isolates. These include techniques such as 16S rRNA gene sequencing, whole genome sequencing, or other in-house molecular assays.

6.5.1 Nucleic Acid Amplification Tests (NAATs)

NAATs are usually considered to be a good method for bacterial detection as they are simple, sensitive and specific. However, there are limitations.

Multiplex NAAT assays have also been used for detection of genes encoding surface protein adhesins, toxins or antibiotic resistance in staphylococci and more recently, for species identification of coagulase positive staphylococci by targeting the thermonuclease (*nuc*) gene locus (5,32,33). The assay provides a valuable tool for the rapid and accurate characterization of staphylococci which is essential in modern hospital practice, as well as being valuable for surveys (34).

6.5.2 Whole Genome Sequencing (WGS)

WGS is useful in the detection of methicillin resistant *S. aureus* in an outbreak (35). It has also been used to highlight extensive differences in genome content between the closely related *Staphylococcus intermedius* group (*S. intermedius*, *S. pseudintermedius* and *S. delphini*) inhabiting distinct host niches as well as providing new avenues for research into pathogenesis and bacterial host-adaptation (36).

7 Storage

If required, store pure isolate on a nutrient agar slope for short-term preservation prior to referral to a reference or specialist testing laboratory.

Significant isolates should be stored on beads at –20 °C or –70 °C for long-term preservation.

For information regarding retention and storage of pathological records and specimens, please refer to the Royal College of Pathologists – Best practice recommendations (37).

8 Reporting

8.1 Designated infection specialist

Inform the infection specialist of preliminary and confirmed *Staphylococcus aureus* cultures when the test request contains relevant information, e.g.,

- toxin-mediated phenomena (e.g., Toxic Shock Syndrome, scalded skin syndrome, epidermal necrolysis, bullous impetigo, necrotising pneumonia, food poisoning)
- suspected outbreaks or instances of cross-infection

Certain clinical conditions must be notified to the laboratory associated infection specialist. Typically, these will include:

- osteomyelitis and septic arthritis
- infections involving indwelling medical devices, e.g., prosthetic valves, pacemakers, CSF shunts, peritoneal or vascular catheters
- isolates from normally sterile sites

Where laboratories are currently unable to identify and differentiate closely related species within the *S. aureus* complex, reporting as *S. aureus* is standard. However, in other settings where expanded MALTI-TOF MS databases or genomic approaches are available, reporting and downstream management may vary based on local laboratory policy.

In this case, laboratories should ensure that results are interpreted and communicated in a clinically meaningful way within the configuration of the local management system (e.g., Laboratory Information Management System-LIMS). This may include clearly indicating that the organism belongs to the *S. aureus* complex and highlighting its comparable pathogenic potential to *S. aureus*.

Given the similarity in clinical implications, laboratories may apply similar approaches to *S. aureus* in testing, escalation, and infection prevention and control measures (including those relating to methicillin-resistant isolates), in line with local and national policy. Practices should be agreed locally with infection specialists and public health teams to ensure consistency in clinical management and appropriate inclusion in surveillance systems, i.e. UKHSA's mandatory healthcare-associated infection (HCAI) surveillance programme ([HCAI Data Capture System](#))

Note: Public health guidance may differ throughout the UK in relation to *S. aureus* complex surveillance.

Further information is available in the UKHSA's [Bacteriology Reference Department User Manual](#).

Follow local protocols for reporting other organisms to clinicians. For information regarding communication of critical and unexpected pathology results, refer to Royal College of Pathologists best practice recommendations (38).

8.2 Interim report

Reporting interim or preliminary results may be conducted by competent staff as defined by local policy.

The provisional status of interim reports must be clearly indicated.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

8.3 Final report

Results should include appropriate interpretative comments and be communicated in accordance with local laboratory policy.

Any notifiable disease (i.e., Panton Valentine Leucocidin (PVL) positive isolates) should also be notified to the relevant body in accordance with statutory requirements.

8.4 Reporting time

If interim or preliminary results are required as per local policy, these should be issued as soon as actionable findings are available (significant microscopy results or initial culture growth), unless alternative arrangements have been agreed with the requestor.

Final reports should be issued as soon as all investigations are complete and authorised by competent staff.

8.5 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

8.6 UK Health Security Agency

Refer to current guidelines on Second Generation Surveillance System (SGSS) reporting (39).

8.7 Infection prevention and control team

Inform the infection prevention and control team of isolates of MRSA and any *S. aureus* bacteraemia (MSSA) in accordance with local protocols.

9 Referral to reference or specialist testing laboratories

In case of sending away isolates to reference or specialist testing laboratories 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 laboratories for sending isolates.

Organisms with unusual or unexpected resistance, associated with a laboratory or clinical problem, or an anomaly that requires investigation should be sent to the appropriate reference or specialist testing laboratory. Guidance on expected susceptibility profiles for staphylococci is available in the [EUCAST “Expected susceptible phenotypes” document](#) to support identification of unusual resistance.

Isolates requiring specialist AST testing, such as *Staphylococcus* species with suspected glycopeptide, daptomycin, or linezolid resistance, should always be sent to a specialist referral laboratory.

Whole genome sequencing (WGS) may be used for further characterisation of *Staphylococcus* isolates, particularly for outbreak detection and assessment of strain relatedness, including MRSA. WGS can also highlight differences among closely related species within the *Staphylococcus intermedius* group and support investigations into host adaptation and pathogenicity.

Referral for staphylococcal toxin gene detection may be indicated based on clinical presentation or local clinical pathways. Where PVL testing is available in-house, isolates requiring wider toxin profiling or confirmation via WGS should be referred to the appropriate reference laboratory. Specific toxin typing services vary regionally; consult local guidelines for referral criteria.

Contact the appropriate reference laboratory (refer to the links provided below) for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission.

[England](#)

[Wales](#)

[Scotland](#)

[Northern Ireland](#)

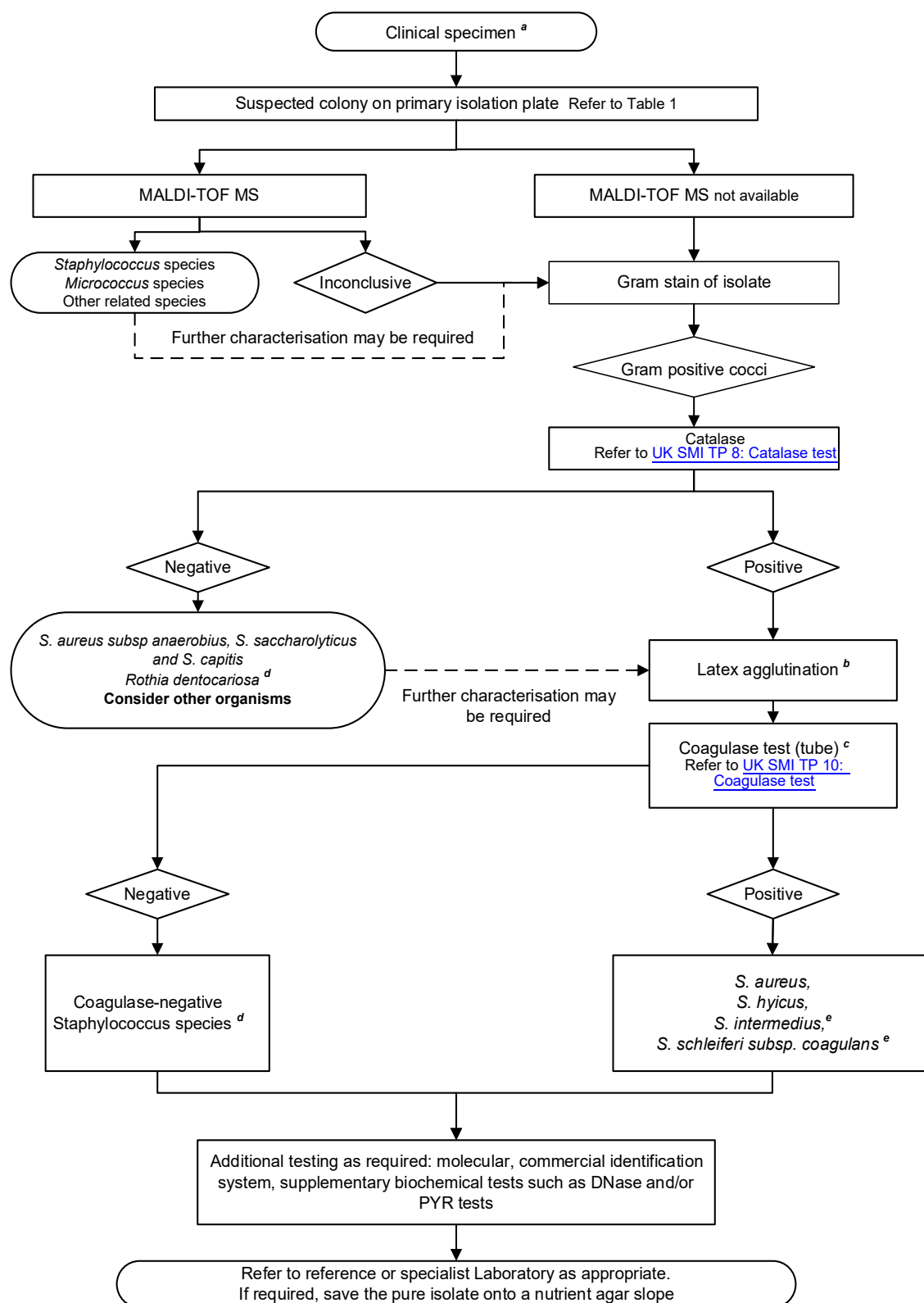
10 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required, primarily for public health purposes e.g., routine *cryptosporidium* detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of healthcare. It is recognised that additional testing and referral of specimen may entail some costs that have to be borne by the laboratory, but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

Algorithm: Identification of *Staphylococcus* Species, *Micrococcus* species and *Rothia* species



Footnotes:

Note: The algorithm is intended as a guide to best practice, but it is recognised that the sequence of testing and the methods used may vary according to specimen type, clinical context, and local laboratory practice.

- a** In certain sterile clinical specimens i.e. blood cultures or CSF, a Gram stain may be done directly from the specimen.
In liquid medium or body fluid, *Staphylococcus* species appear as Gram positive cocci in clusters
- b** Positive or equivocal slide latex results should be confirmed with a tube coagulase test to avoid false positives. *S. lugdunensis* may yield weak, partial, or negative results in latex agglutination tests
- c** Tube coagulase is the traditional confirmatory method for demonstrating free coagulase. Slide coagulase is rarely performed and, if used, is undertaken as an additional supplementary test and does not replace tube coagulase.
- d** *S. lugdunensis* may produce a clumping factor and give a false-positive slide or latex coagulase result but is tube coagulase negative.
- e** Several non-aureus staphylococci may be tube coagulase positive and should be differentiated from *S. aureus* using additional identification methods.

References

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

For suggested citation of UK SMIs, refer to [UK SMI Development](#).

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