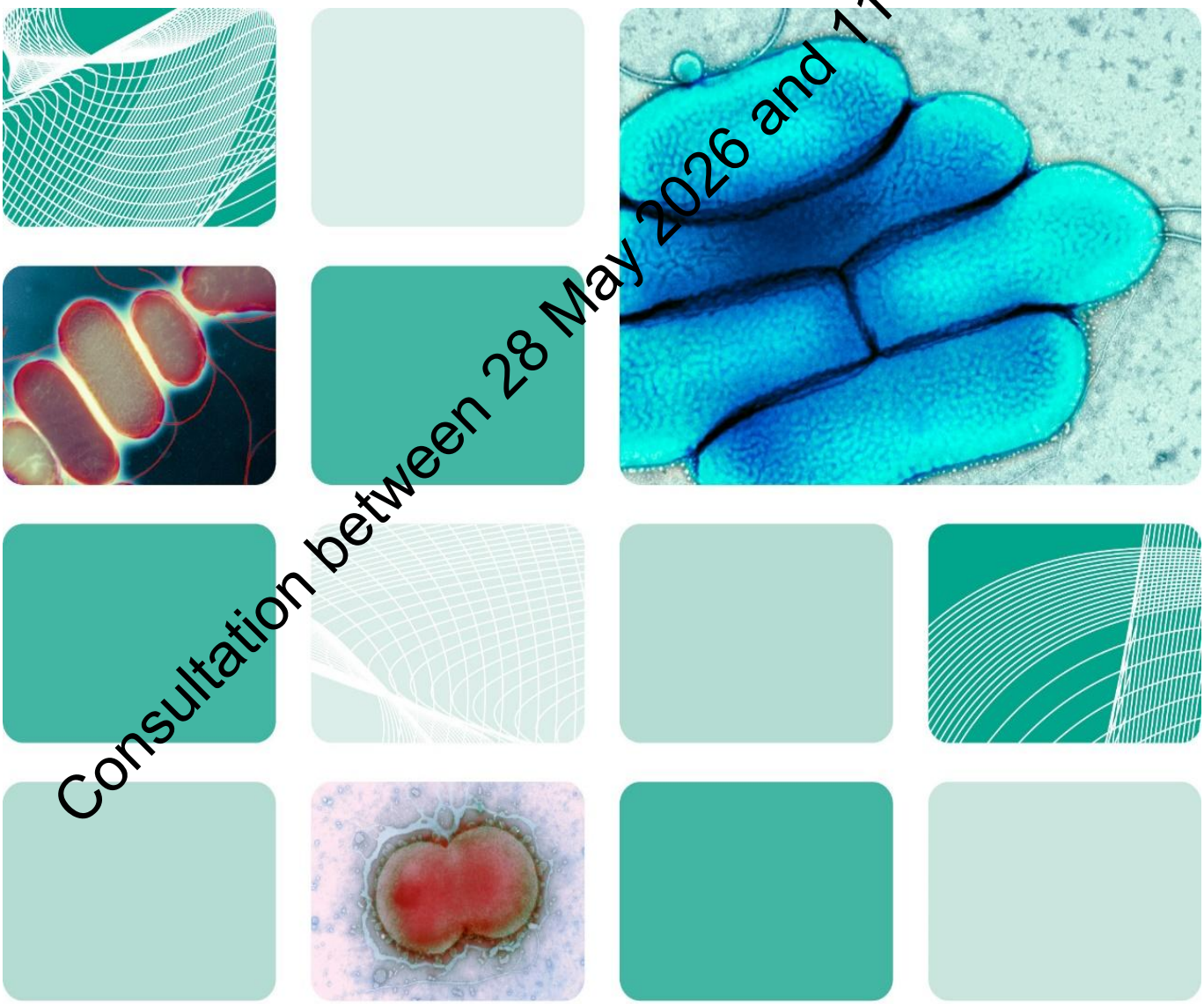
UK Health Security Agency

UK Standards for Microbiology Investigations

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



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 the UK SMI 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 editors for editing the medical and clinical content.

UK SMIs are produced in association with:

Displayed logos correct as of December 2024

Contents

Click on the links for the relevant sections.

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	x/dd.mm.yy
Issue number discarded	
Insert issue number	
Anticipated next review date*	dd.mm.yy
Section(s) involved	**Amendment**

*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. 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.

The focus 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 attributes (1). 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, key information is included where necessary to provide context and ensure completeness. For details on MRSA screening, refer to B 29 - Investigation of Specimens for Screening MRSA.

For the identification of catalase negative Gram positive cocci, see 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). *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 (3).

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 virulent 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 its pathogenicity and ability to cause severe systemic illness. In addition, it is also a major cause of healthcare associated infections (HCAIs), particularly in surgical wounds, catheter-related bloodstream infections and ventilator-associated pneumonia.

Coagulase negative staphylococci (CoNS) are normal commensals of the skin, anterior nares, and ear canals of humans and were considered as non-pathogenic and rarely reported to cause severe infections. However, because of the combination of increased use of intravascular devices and the increased number of hospitalised immunocompromised patients, CoNS have emerged as a major cause of nosocomial bloodstream infections. They are opportunistic pathogens which lack many of the virulence factors associated with *S. aureus*. There are more than 30 species of CoNS. The taxonomy of these coagulase negative staphylococci (CoNS) fall into clusters based on 16S rRNA sequences (4).

S. epidermidis and ***S. saprophyticus*** are the CoNS species most often associated with infection 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 healthcare-associated infections.

Other CoNS species such as ***S. lugdunensis, S. haemolyticus and S. capitis*** have been implicated with growing frequency in invasive and healthcare-associated

infections. *S. lugdunensis* has been isolated from a range of infections, including skin, superficial and deep soft-tissue and wound infections, and in some cases is associated with more invasive disease such as infective endocarditis (5-8). ***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 (9-11). *S. capitis* has been implicated in outbreaks in neonatal intensive care units (12).

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. Their clinical significance should be interpreted in the context of patient factors, specimen type and clinical presentation.

The ***Micrococcus* species** are generally considered harmless saprophytes that inhabit or contaminate the skin, mucosa, and also 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*.

Amongst the *Rothia* species, ***Rothia dentocariosa*** and ***Rothia mucilaginosa*** are the only two which have been known to cause infections in humans (13).

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 (14).

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) (15). 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*.

The genus *Rothia* is in the same family as the genus *Micrococcus* and currently contains 8 species. *Rothia dentocariosa* and *Rothia mucilaginosa* are the only two which have been known to cause infections in humans (13). *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.

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 (16-37).

Hazard group 2 organisms. 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.

The above guidance should be supplemented with local the 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 microscopic appearance as Gram positive cocci, colonial morphology and physiological characteristics. Direct microscopy and Gram staining on certain sterile samples or liquid enrichment media i.e., positive blood cultures, indicates the presence of staphylococci by Gram-positive cocci in clusters.

In routine workflows, MALDI-TOF MS is usually the primary method for species-level identification. It enables accurate differentiation of *Staphylococcus aureus* and clinically significant coagulase-negative staphylococci (CoNS) such as *S. lugdenensis* from other commensal or opportunistic CoNS. Additional characterisation may be achieved using phenotypic or biochemical tests performed from non-selective media when required.

Species-level identification is essential for organisms such as *Staphylococcus aureus* and other clinically significant CoNs (e.g. *S. lugdunensis*) 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 (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.

6.2 Primary isolation media

Most staphylococci grow on a range of media.

Staph/Strep agar can be useful in mixed primary culture, particularly where swarming organisms such as *Proteus* species are present.

Blood agar is generally preferred for initial assessment because it supports good recovery and typical colonial morphology. Additional media such as CLED or Mannitol Salt Agar (MSA) may also assist with the isolation of staphylococci by limiting overgrowth from other organisms and enabling differentiation based on characteristic reactions.

Chromogenic media can aid direct isolation and presumptive identification of *Staphylococcus aureus* and may incorporate selective agents such as oxacillin or cefoxitin for the detection of MRSA. These media can reduce the need for additional confirmatory testing when used according to manufacturer instructions.

However, some non-*aureus* staphylococci may produce misleading colony colours on MRSA-selective chromogenic media. For example, *S. sciuri* may carry mecA and grow with similar pigmentation to MRSA, and species such as *S. intermedius* may also appear similar. Further testing is therefore required to confirm identity where results are unexpected or inconsistent.

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

6.3 Colonial appearance

See Table 1 below.

This is not an exhaustive list of possible species

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

Colony morphology	Colony size	Organisms	Notes
Golden to cream, smooth, glistening, and convex	Large	*S. aureus*	Strains may be identified by better growth anaerobically. Beta haemolysis activity is frequently observed. *S. aureus* subspecies *anaerobius* is rarely isolated from human clinical specimens.
Creamy white	Large	S. *argenteus*	Facultatively anaerobic. Small colony variants (SCVs) 1/10th normal size can occur. Beta haemolytic. Difference to *S. aureus* in pigment is evident after growing on chocolate agar for 48 hr at 37 °C.
Pale yellow / orange, smooth, glossy, and convex	Large	*S. lugdunensis*	Facultatively anaerobic and may exhibit β-haemolysis or appear non-haemolytic depending on strain
White-cream and smooth	Large	*S. intermedius group*	Includes *S. intermedius, S. pseudintermedius, S. delphini*
White to creamy/grey-white and smooth	Small	*S. epidermidis*	Non-haemolytic.
Golden-yellow, smooth, and opaque	Small	*S. haemolyticus,*	Usually haemolytic but non-haemolysis occurs occasionally
White/yellow, glossy, smooth, and convex	Large	*S. saprophyticus,*	
White or tan, smooth, and opaque	Small	*S. capitis, S. hominis S. warneri,*	*S. warneri* may appear with orange rim around the colony
Grey/White, smooth, and translucent	Large	*S. simulans*	
Yellow to cream white, smooth and convex Pink/red	Large	*M. luteus, M. lylae, M. flavus* *M. roseus*	
White to grey, dry surface, rough with irregular edges	Small	*Rothia* species	Microcolonies on agar surface are composed of filamentous branched elements which rapidly fragment into bacillary or coccoid forms, resembling *Actinomyces* or *Nocardia* species (38). 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 individual laboratory practice with appropriate local validation. The following section therefore describes 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 identification methods and is now routinely used for the identification of *Staphylococcus* species. It enables rapid and accurate species-level identification and reliable discrimination between *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) where identification scores are high, and the result is concordant with colonial morphology. Where identification scores fall below locally defined acceptance thresholds, or where the organism identified falls outside validated or established scope, further characterisation using additional phenotypic or traditional biochemical methods may be required.

MALDI-TOF MS has also proved valuable in the description of newly recognised staphylococcal species and in strain profiling, including the identification of distinct clonal lineages of *S. epidermidis* associated with either human or environmental origins (39).

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* species.

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

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 and MALDI-TOF MS identification must 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 must 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 TP 8 - Catalase test)

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

Notes:

- *S. aureus* subspecies *anaerobius* and *S. saccharolyticus* are catalase negative (40).
- *Rothia dentocariosa* are catalase positive. However, catalase negative strains have been reported and this will be more difficult to recognise with traditional tests, since 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 (41).

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

DNase test (see TP 12 – Deoxyribonuclease test)

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.

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 key test results indicated in Table 2 and should be quality controlled following manufacturers guidelines.

Table 2: Phenotypic Test Characteristics of Selected *Staphylococcus* Species and morphologically similar organisms

Species	Coagulase test [a]	Latex Agglutination Test [b]	DNase test [c]	PYR [d]
S. aureus complex[e]	+	+	+	-
S. argenteus	-	+	+	-
S. lugdunensis	-	+/-	-	+
S. intermedius	+	+	+	+
S. epidermidis	-	-	-	-
S. haemolyticus	-	-	-	+
S. saprophyticus - (*S. saprophyticus subsp. Saprophyticus*, and *S. saprophyticus subsp. bovis*)	-	-	-	-
S. capitis	-	-	-	-
S. hominis	-	-	-	-
S. warneri	-	-	-	-
S. simulans	-	-	-	+
Micrococcus species (*M. luteus*, *M. lylae, M. flavus and M. roseus*)	-	-	-	-
Rothia species (*R. dentocariosa*, *R. mucilaginosa*)	-	-	-	-

Footnotes:

a Positive or suspect slide latex 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* (42). Therefore, to improve the identification of *S. aureus*, other tests in conjunction with coagulase test should be performed (43).

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)

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

Non-staphylococcal organisms that clot citrated plasma (can be mistaken for coagulase-positive staph) (44): *Enterococcus faecalis, Pseudomonas* spp., *Serratia marcescens,* Certain *Streptococcus* strains

Yeasts can be misidentified as coagulase negative staphylococci due to cross reactivity or non-specific latex agglutination reactions. Speciation of presumptive staphylococci should be considered where clinically relevant to avoid missing clinically significant organisms such as *S. aureus*, *S. lugdunensis* or yeasts.

For more information on Coagulase test, see TP 10 - Coagulase test

b Modern latex tests provide rapid identification and can detect atypical *S. aureus* and some MRSA strains (45).

Some *S. aureus* strains may be protein A deficient, so would require alternative testing e.g., MALDI-TOF MS.

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

Positive or suspect 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 (40).

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 key test for identifying *S. lugdunensis* when MALDI-TOF MS is unavailable.

e *S. aureus* complex includes *S. aureus* and *S. argenteus*.

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)

PCR is usually considered to be a good method for bacterial detection as it is simple, sensitive and specific. However, it does have limitations.

Multiplex PCR 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 (46,47). 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 (48).

6.5.2 Whole genome sequencing (WGS)

This is useful in the detection of methicillin resistant *S. aureus* in an outbreak (49). 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 (50).

7 Storage

If required, save pure isolates on a nutrient agar slope for referral to the Reference Laboratory.

Chromogenic media are affected by direct light and plates should be stored in the dark and not left in the light long before or after inoculation.

Any strain of *S. aureus* suspected of demonstrating unusual resistance e.g., vancomycin or linezolid, must be referred to the Staphylococcal Reference Service for further examination.

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 enhanced MRSA, MSSA and Gram-negative bacteraemia, and Clostridioides difficile infection surveillance.

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.

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 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 (32).

8.7 Infection prevention and control team

Inform the infection prevention and control team of isolates of methicillin resistant *Staphylococcus aureus* 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, or associated with a laboratory or clinical problem, or an anomaly that requires investigation should be sent to the appropriate reference laboratory. 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

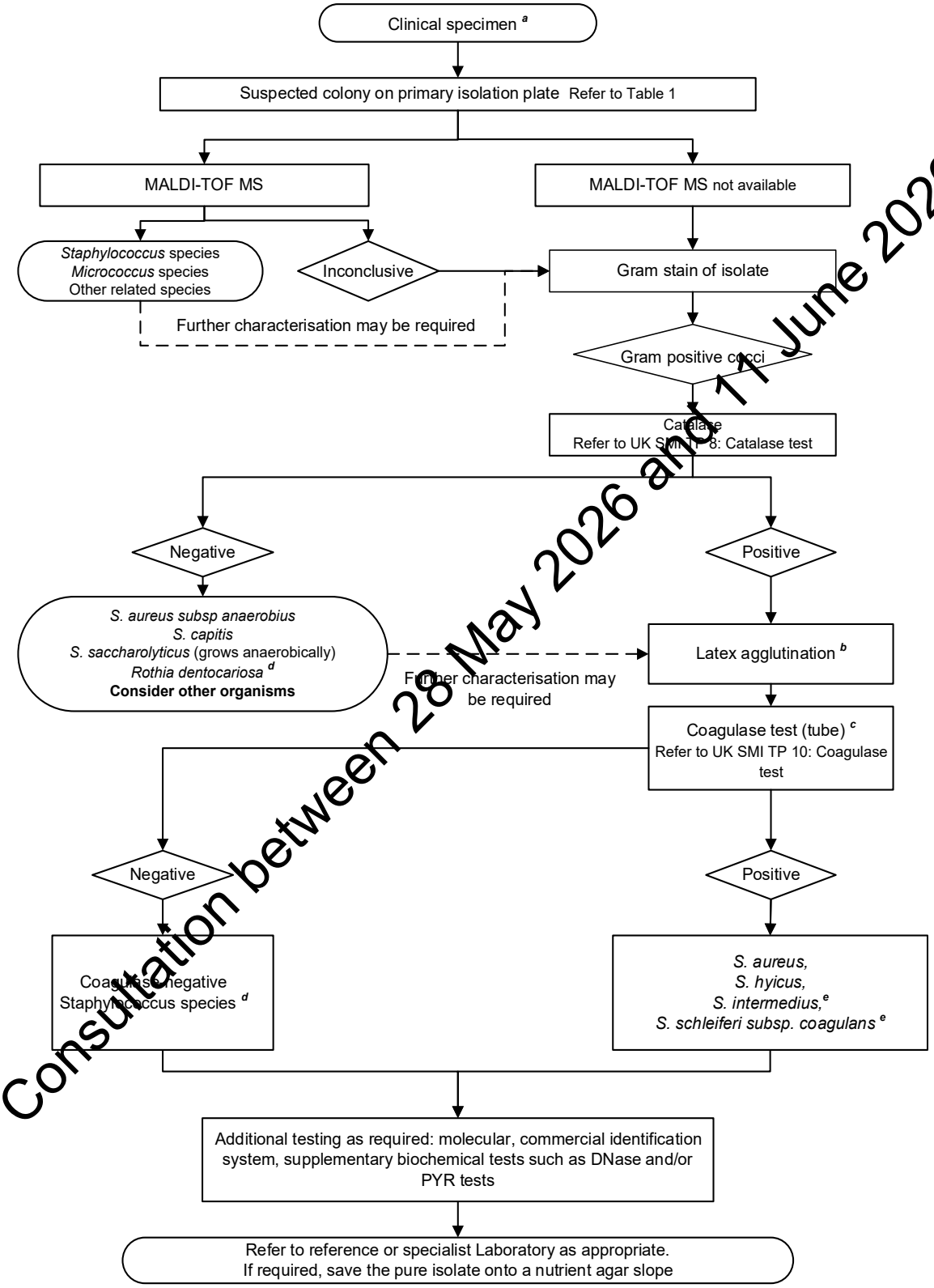
10 Public health responsibilities of diagnostic laboratories

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 samples may entail some costs that has 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 in liquid medium or body fluid, *Staphylococcus* species appear as Gram positive cocci in clusters

b Positive or suspect 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 reference standard for identification. 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 Strains may be positive to certain tests.

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.

1. Whiley RA, Hardie JM Genus, *Streptococcus*, *Enterococcus* and related species: Bergey's Manual of Systematics of Archaea and Bacteria. 1st ed.: Published by John Wiley & Sons, Inc., in association with Bergey's Manual Trust; 2015. **++**

2. Yan A and others. The Staphylococcus aureus complex: implications for the clinical microbiology laboratory. J Clin Microbiol 2025: volume 63, issue 7, pages e0127624. 10.1128/jcm.01276-24

3. Tong SYC and others. Novel staphylococcal species that form part of a Staphylococcus aureus-related complex: the non-pigmented Staphylococcus argenteus sp. nov. and the non-human primate-associated Staphylococcus schweitzeri sp. nov. Int J Syst Evol Microbiol 2015: volume 65, issue Pt 1, pages 15–22. 10.1099/ijs.0.062752-0

4. Takahashi T and others. Phylogenetic relationships of 38 taxa of the genus Staphylococcus based on 16S rRNA gene sequence analysis. IntJSystBacteriol 1999: volume 49 Pt 2, pages 725–8. **II**

5. Bowman TP and others. Staphylococcus lugdunensis in children: A retrospective analysis. Pediatric Investigation 2022: volume 6, issue 3, pages 163–70. https://doi.org/10.1002/ped4.12345

6. Morris TC and others. Endovascular stent-associated infection with Staphylococcus lugdunensis. BMJ Case Rep 2013: volume 2013. 10.1136/bcr-2012-008357

7. Peake CM and others. Staphylococcus lugdunensis Endocarditis in a Patient Being Treated for Pyelonephritis. Journal of Microbiology and Infectious Diseases 2018: volume 08, issue 01, pages 37–40. 10.5799/jmid.404245

8. Rose AM and others. Staphylococcus lugdunensis septic arthritis and epidural abscess in a patient with rheumatoid arthritis receiving anti-tumour necrosis factor therapy. Rheumatology 2014: volume 53, issue 12, pages 2231–. 10.1093/rheumatology/keu365

9. Manoharan M and others. Detection of Heteroresistant Vancomycin-Intermediate Staphylococcus haemolyticus Among Blood Isolates. Microbial Drug Resistance 2025: volume 31, issue 12, pages 422–30. 10.1177/10766294251389588

10. Dupin C and others. Emergence and establishment of Staphylococcus haemolyticus ST29 in two neonatal intensive care units in Western France. Journal of Hospital Infection 2025: volume 158, pages 38–46. https://doi.org/10.1016/j.jhin.2025.01.003

11. Lamberte LE and others. Staphylococcus haemolyticus is a reservoir of antibiotic resistance genes in the preterm infant gut. Gut Microbes 2025: volume 17, issue 1, pages 2519700. 10.1080/19490976.2025.2519700

12. Yuan J-M and others. Clinical outcomes of Staphylococcus capitis isolation from neonates, England, 2015–2021: a retrospective case–control study. Archives of Disease in Childhood - Fetal and Neonatal Edition 2024: volume 109, issue 2, pages 128. 10.1136/archdischild-2023-325887

13. Euzeby JP. List of prokaryotic names with standing in Nomenclature- Genus *Rothia*. 2013. **A, III**

14. Schleifer K-H BJA. Staphylococcaceae fam. Bergey's Manual of Systematic Bacteriology. 2nd ed.volume 3. New York: Springer, 2009.

15. Euzeby JP. List of Prokaryotic names with standing in Nomenclature- Genus *Micrococcus*. 2013. **A, III**

16. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive 2021. pages 1–39. **++**

17. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets 2000. **++**

18. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 2005. pages 1–44. **++**

19. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012: volume 61, pages 1–102.**+**

20. Department for Transport and others. Transport of infectious substances UN2814, UN2900 and UN3373 Guidance note number 17/2012 (revision 7). 2013. **++**

21. Department of Health. Health Protection Legislation (England) Guidance. pages 1–112. 2010. **++**

22. Gizzie N, Adukwu E. Evaluation of Liquid-Based Swab Transport Systems against the New Approved CLSI M40-A2 Standard. J Clin Microbiol 2016: volume 54, issue 4, pages 1152–6.**2+** 10.1128/JCM.03337-15

23. Health and Safety Executive. Managing risks and risk assessment at work (accessed 28/07/2021). https://www.hse.gov.uk/simple-health-safety/risk/index.htm. **++**

24. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 2009. **++**

25. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002 (as amended). Approved Code of Practice and guidance L5 (sixth edition). HSE Books. 2013. **++**

26. Health and Safety Executive. Risk assessment: A brief guide to controlling risks in the workplace. HSE. 2014. **++**

27. Health and Safety Executive, Advisory Committee on Dangerous Pathogens. Management and operation of microbiological containment laboratories. HSE. 2019. **++**

28. Health Services Advisory Committee. Safe working and the prevention of infection in clinical laboratories and similar facilities. Books. H 2003. **++**

29. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967. **++**

30. Home Office. Anti-terrorism, Crime and Security Act. 2001. **++**

31. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. 1998. pages 1–37. **++**

32. Public Health England. Laboratory reporting to Public Health England: a guide for diagnostic laboratories. PHE. 2020. pages 1–31. **++**

33. Scottish Government. Public Health (Scotland) Act. 2008. **++**

34. The Royal College of Pathologists. The retention and storage of pathological records and specimens (5th edition). pages 1–59. 2015. **++**

35. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010. **++**

36. Tyrrell KL and others. Comparison of the Copan eSwab System with an Agar Swab Transport System for Maintenance of Fastidious Anaerobic Bacterium Viability. J Clin Microbiol 2016: volume 54, issue 5, pages 1364–7.**2+** 10.1128/JCM.03246-15

37. World Health Organization. Guidance on regulations for the transport of infectious substances 2019-2020. WHO. 2019. **++**

38. Georg LK, Brown JM. Rothia, Gen. Nov. an aerobic genus of the family actinomycetaceae. International Journal of Systematic Bacteriology 1967: volume 17, issue 1, pages 79–88.**B, III**

39. Dubois D and others. Identification of a variety of Staphylococcus species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. JClinMicrobiol 2010: volume 48, issue 3, pages 941–5.**B, III** JCM.00413-09 [pii];10.1128/JCM.00413-09 [doi]

40. Kloos W, Bannerman TL. Staphylococcus and Micrococcus. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of Clinical Microbiology. 7th ed. Washington DC: American Society for Microbiology; 1999. pages. 264–82. **B, IV**

41. von Graevenitz A. Rothia dentocariosa: taxonomy and differential diagnosis. ClinMicrobiolInfect 2004: volume 10, issue 5, pages 399–402.**B, IV** 10.1111/j.1469-0691.2004.00784.x [doi];CLM784 [pii]

42. Vandenesch F and others. Coagulase deficiency in clinical isolates of Staphylococcus aureus involves both transcriptional and post-transcriptional defects. JMedMicrobiol 1994: volume 40, issue 5, pages 344–9.**B, II**

43. Brown DF and others. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant Staphylococcus aureus (MRSA). JAntimicrobChemother 2005: volume 56, issue 6, pages 1000–18.**B, I** dki372 [pii];10.1093/jac/dki372 [doi]

44. Yrios JW. Comparison of rabbit and pig plasma in the tube coagulase test. JClinMicrobiol 1977: volume 5, issue 2, pages 221–4.**B, I**

45. Personne P and others. Comparative performances of six agglutination kits assessed by using typical and atypical strains of Staphylococcus aureus. JClinMicrobiol 1997: volume 35, issue 5, pages 1138–40.**B, II**

46. Stepan J and others. Molecular diagnostics of clinically important staphylococci. Folia Microbiol(Praha) 2004: volume 49, issue 4, pages 353–86.**B, II**

47. Hirotaki S and others. Rapid and accurate identification of human-associated staphylococci by use of multiplex PCR. JClinMicrobiol 2011: volume 49, issue 10, pages 3627–31.**B, III** JCM.00488-11 [pii];10.1128/JCM.00488-11 [doi]

48. Pichon B and others. Development of a real-time quadruplex PCR assay for simultaneous detection of nuc, Panton-Valentine leucocidin (PVL), mecA and homologue mecALGA251. JAntimicrobChemother 2012: volume 67, issue 10, pages 2338–41.**B, II** dks221 [pii];10.1093/jac/dks221 [doi]

49. Harris SR and others. Whole-genome sequencing for analysis of an outbreak of meticillin-resistant Staphylococcus aureus: a descriptive study. Lancet InfectDis 2013: volume 13, issue 2, pages 130–6.**B, IV** S1473-3099(12)70268-2 [pii];10.1016/S1473-3099(12)70268-2 [doi]

50. Ben Zakour NL and others. Comparative genomics of the Staphylococcus intermedius group of animal pathogens. Front Cell InfectMicrobiol 2012: volume 2, pages 44.**B, III** 10.3389/fcimb.2012.00044 [doi]