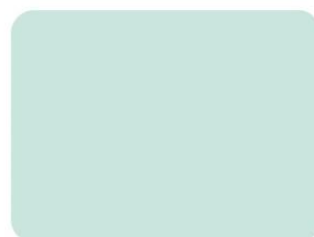
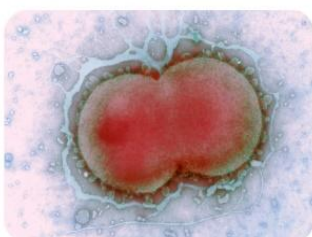
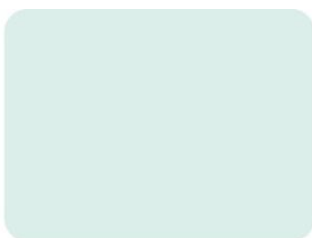




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UK Standards for Microbiology Investigations

Identification of *Streptococcus* species, *Enterococcus* species and morphologically similar organisms



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	12/01.10.26
Issue number discarded	4
Insert issue number	5
Anticipated next review date*	01.10.29
Section(s) involved	Amendment
Whole document	Document has been presented in a new template and restructured. Hyperlinks have been updated to direct the reader to the RCPATH website. Public Health England replaced by UK Health Security Agency. References to NICE accreditation removed and UK Health Security Agency logo updated. Devolved administrations links have been updated.
Introduction	Information regarding characteristics of individual species has been summarised into table 1. Information and references have been updated. Target organisms section removed and replaced with section 4.1: Target organisms of clinical significance.
Safety considerations	Standard references have been replaced with a link to the relevant page on the website.
Identification	Section has been restructured to reflect identification procedures. Table 1 added to summarise morphology of target organisms. Technical information section has been removed and information has been moved into the relevant sections.

Reporting	Section headings and information have been updated as appropriate.
Public health responsibilities in diagnostic laboratories	Section has been added.
Algorithm	Algorithm has been updated to better align with current laboratory practices.
References	Updated where appropriate.

*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 SMI document describes the identification of *Streptococcus*, *Enterococcus* and morphologically related organisms 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 identification systems yield inconclusive results or are unavailable. Molecular platforms may also be used 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 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](#).

This document focuses on clinically relevant species isolated from human infections. Organisms morphologically similar to streptococci, 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. Further identification may be necessary where clinically or epidemiologically indicated.

This identification document does not focus on screening, typing or antimicrobial susceptibility testing (AST). However, key information is included where necessary to provide context and ensure completeness. Refer to [UK SMI B 58: Detection of carriage of group B streptococci](#) for detection of Group B Streptococci. To support effective antimicrobial stewardship, laboratories should perform Antimicrobial Susceptibility Testing (AST) on all clinically significant isolates, particularly in cases of poor treatment response. Further information on AST methodologies, interpretation and reporting is available in the relevant sections in the UK SMI Bacteriology documents.

Please note that some streptococcal species have been reclassified, and the updated nomenclature of these species have been included in this document for reference.

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

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

4 Introduction

4.1 Target organisms of clinical significance

Streptococcus and *Enterococcus* species include several clinically significant organisms associated with a range of human and animal infections. Many exist as commensals of the upper respiratory tract, gastrointestinal tract or genitourinary tract, but possess a high potential for virulence under certain conditions.

The most clinically significant *Streptococcus* species include the following:

S. pyogenes also widely known as Group A Streptococcus (GAS) is one of the most virulent *Streptococcus* species capable of causing skin and soft tissue infections such as impetigo, mucosal membrane infections such as tonsillitis, vulvovaginitis and also capable of causing severe, invasive infection (2,3).

S. pneumoniae remains one of the most important causes of community-acquired pneumonia, meningitis, and invasive pneumococcal disease globally (4-6).

S. agalactiae also widely known as Group B Streptococcus (GBS) can produce many invasive and non-invasive diseases and is a major cause of newborn infections presenting as early-onset sepsis, pneumonia and meningitis, as well as late onset neonatal sepsis (3,7). It also causes skin and soft tissue infections, prosthetic joint infection, septic spontaneous abortion, and puerperal sepsis in adults.

S. dysgalactiae* subsp. *equisimilis causes infections that are clinically similar to those due to *S. pyogenes*, including pharyngitis, soft tissue infection and invasive disease, supported by the presence of virulence genes analogous to *emm* types found in *S. pyogenes* (8).

S. canis although primarily an animal-associated species, is a clinically relevant zoonotic pathogen capable of causing invasive disease in humans including bacteraemia, soft-tissue infections, cellulitis, necrotising fasciitis, and occasionally endocarditis.

The ***Streptococcus anginosus* group** comprises *S. anginosus*, *S. intermedius* and *S. constellatus*. Although variable in haemolysis, they are consistently associated with deep-seated purulent infections. Identification of these organisms from sterile sites is clinically significant because of their strong association with abscess formation across multiple organs (9).

Viridan streptococci, including *S. mitis*, *S. oralis*, *S. sanguinis*, *S. mutans*, and *S. salivarius*, are predominantly commensals of the oral, gastrointestinal and genitourinary tracts. Although often contaminants when recovered from blood cultures, certain species, particularly *S. sanguinis* and *S. oralis* are major contributors to native-valve infective endocarditis (10-12).

S. suis is an important zoonotic pathogen which is also isolated from human cases of meningitis and bacteraemia (3,11,13).

The ***Streptococcus bovis*/Streptococcus equinus complex (SBSEC)**, comprising *S. gallolyticus* (and its subspecies), *S. equinus*, *S. infantarius* subsp. *infantarius*, *S. infantarius* subsp. *coli*, *S. lutetiensis*, and *S. alactolyticus*, is clinically relevant due to its association with bacteraemia, endocarditis, and gastrointestinal pathology, including colorectal malignancy.

Enterococcus faecalis and ***Enterococcus faecium*** are the most clinically significant enterococcal species due to their prominent role in healthcare-associated infections, including bloodstream infection, intra-abdominal sepsis, and urinary tract infection. Intrinsic and acquired antimicrobial resistance, particularly in vancomycin-resistant enterococci (VRE), presents major therapeutic and infection prevention challenges (14).

Clinically and epidemiologically significant VRE are defined by the presence of acquired VanA or VanB glycopeptide resistance determinants, most commonly identified in *Enterococcus faecium* and *Enterococcus faecalis*. These plasmid- or transposon-mediated mechanisms confer moderate to high-level vancomycin resistance and are readily transmissible, underpinning their recognised importance for infection prevention and control (IPC).

In contrast, intrinsically vancomycin-resistant VanC-positive species, including *Enterococcus gallinarum*, *Enterococcus casseliflavus* and *Enterococcus flavescens*, possess chromosomally encoded, non-transferable resistance mechanisms associated with low-level vancomycin resistance. Owing to their limited transmissibility and differing epidemiological significance, these organisms are not universally classified as VRE.

For surveillance and reporting, laboratories should follow national guidance and classification used by UKHSA and relevant surveillance programmes, recognising that some systems include VanC enterococci within broader “glycopeptide-resistant enterococci” categories.

Irrespective of reporting classification, the IPC response should be informed by species identification and resistance mechanism. IPC interventions are typically targeted at VanA or VanB-producing enterococci; however, local definitions and risk assessments may vary. Where VanC enterococci are included within local VRE policies, additional species-specific IPC guidance should be applied to ensure that control measures are proportionate to the organism’s transmission risk and clinical impact.

Other Gram-positive cocci that may resemble streptococci include ***Abiotrophia*** and ***Granulicatella*** species which are part of the normal flora of the human urogenital and intestinal tracts, and have been isolated from blood, abscesses, oral ulcers, and urethral specimens. Recognition of these species is important for deep seated infections (notably endocarditis), to ensure appropriate antimicrobial therapy.

Additional genera, including ***Aerococcus***, ***Facklamia***, ***Gemella***, ***Lactococcus***, ***Leuconostoc***, and ***Pediococcus*** species, are generally environmental or commensal organisms with limited but recognised opportunistic pathogenic potential. Although infections are uncommon, these organisms have been implicated in sporadic cases of invasive disease, most notably bacteraemia and endocarditis, and, in the case of *Aerococcus* species, urinary tract infection. Their identification is mainly important to ensure correct antimicrobial therapy, as some of these genera exhibit intrinsic resistance patterns that differ from those of streptococci. *Leuconostoc* and *Pediococcus* species are intrinsically resistant to vancomycin. Accurate identification is required to prevent these organisms being misinterpreted as VRE.

4.2 Taxonomy and characteristics

The genus *Streptococcus* comprises many commensal and pathogenic species. With advances in microbial phenotyping and molecular identification, the genus *Streptococcus* has undergone a significant expansion and revision (14). There are now over 100 recognised species of *Streptococcus*, many of which are pathogens or commensals in humans and animals (2,15,16).

The classical differentiation of streptococci is based primarily on their haemolytic patterns on blood agar (Refer to Figure 1). Streptococci were historically separated into β -haemolytic, α -haemolytic, and non-haemolytic (γ -haemolytic) groups, with β -haemolysis most strongly associated with the traditional classification of “pyogenic” streptococci. These β -haemolytic species include *Streptococcus pyogenes*, *S. agalactiae*, *S. dysgalactiae* subsp. *equisimilis*, *S. canis*, and typically *S. constellatus* of the *Streptococcus anginosus* group (SAG), all of which are linked with acute, pus-forming infections of skin, soft tissue, and deeper sites. *S. anginosus* group are also notable for their variable haemolytic behaviour.

In contrast, the α -haemolytic and non-haemolytic streptococci comprise predominantly the viridans streptococci, *Streptococcus pneumoniae*, and members of the *Streptococcus bovis* / *Streptococcus equinus* complex (SBSEC). These organisms are classically classified as non-pyogenic streptococci, reflecting traditional microbiological grouping rather than clinical behaviour. While viridans streptococci and SBSEC are primarily commensals of the oral cavity and gastrointestinal tract and are not typically associated with classical pyogenic syndromes, they are nevertheless capable of causing purulent (suppurative) infection, particularly in invasive disease. *Streptococcus pneumoniae*, despite also being classically non-pyogenic, is a significant pathogen and a major cause of both invasive and non-invasive disease where suppurative complications may occur.

The genus name *Enterococcus*, was revived in 1984 when other bacteria were transferred to the genus. There are now more than 50 recognised species of the genus *Enterococcus* (2,11). Enterococci and other related streptococcus-like genera (see Section 4.1) are most commonly non-haemolytic or weakly α -haemolytic on blood agar and although not included in the pyogenic classification, all can cause invasive and suppurative disease, most notably endocarditis and deep-seated infections. Accurate laboratory identification is required, as these organisms may exhibit variable haemolysis and overlapping phenotypic features.

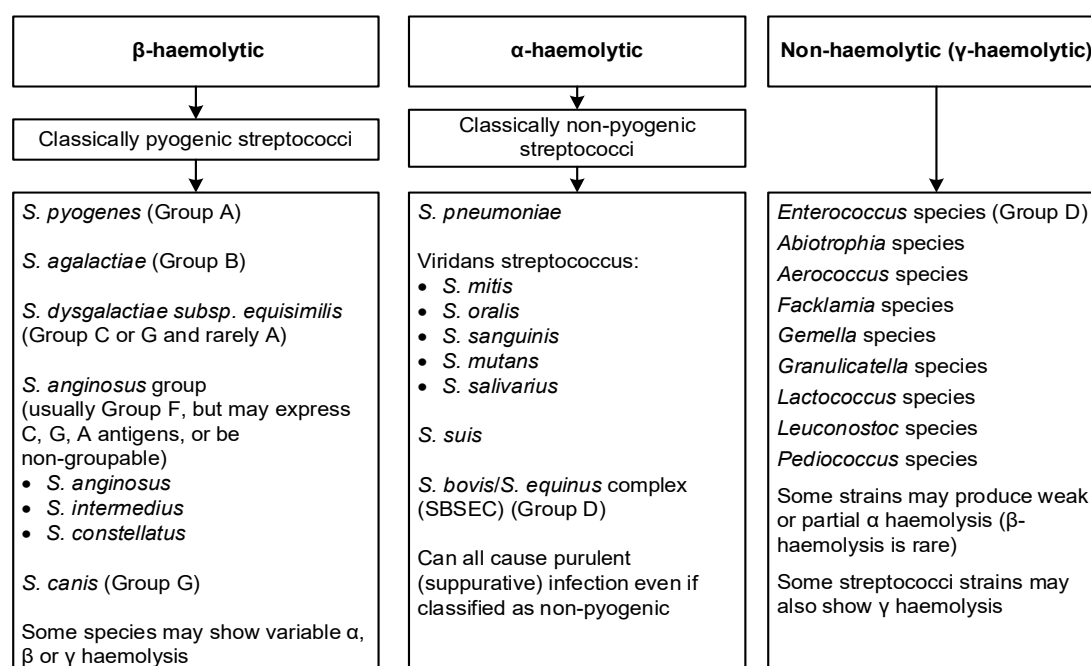


Figure 1: Classification of clinically relevant streptococcus species, *Enterococcus* species and other relevant organisms according to haemolysis patterns on blood agar, Lancefield grouping, and their clinical grouping into pyogenic and non-pyogenic organisms.

5 Safety considerations

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

Hazard group 2 organisms. The processing of diagnostic specimens 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 in line with local procedures.

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

6 Identification

Isolates from primary culture are assessed by their colonial morphology, haemolysis pattern, Lancefield grouping, and relevant physiological characteristics. Gram stain morphology, for example, Gram-positive cocci in chains, can further support identification, particularly from direct specimens or positive blood cultures. Catalase negativity in streptococci and enterococci also aids distinction from other Gram-positive cocci.

The sequence of testing, methods employed, and level of identification reported may vary depending on specimen type, site of infection, clinical context, and local laboratory practice when supported by appropriate validation and risk assessment (17). Identification pathways can therefore be applied flexibly.

In routine workflows, MALDI-TOF MS is usually the primary method for species-level identification. However, it should not be used in isolation, if there is a possibility of reduced discriminatory capacity for certain closely related streptococcal species (see section 6.4.1), or when results are inconclusive. In these circumstances or if MALDI-TOF MS is unavailable, further characterisation by phenotypic and biochemical tests should be used. Conversely, these traditional biochemical methods may also have limited discriminatory capacity for closely related or taxonomically complex species and should not be used in isolation where definitive identification is required (18).

Immunological and molecular rapid assays, including real-time Polymerase Chain Reaction (PCR) and other nucleic-acid amplification test (NAAT) platforms, may also be used when clinically appropriate.

Unusual or unexpected isolates should be referred to an appropriate specialist or reference laboratory.

The required level of organism identification depends on both the clinical significance of the isolate and the specimen type. For β -haemolytic streptococci, Lancefield grouping is commonly used for laboratory identification and in accordance with local laboratory policy, grouping alone may be sufficient for routine investigation of certain specimens, such as group A streptococci (GAS) from pharyngeal swabs or group B streptococci (GBS) from vaginal screening specimens. However, results should be interpreted with caution, as cross-reactivity between Lancefield antisera (as described in section 6.4.3) can occur and may lead to misidentification if not considered alongside colony morphology and clinical context. Reporting practices, including the use of Lancefield groups for β -haemolytic streptococci, are addressed separately in Section 8.

In contrast, species-level identification is required for isolates from blood cultures or other normally sterile sites, and in clinical scenarios where further differentiation has management or prognostic significance. For example, distinction between members of the *Streptococcus anginosus* group, such as *S. constellatus*, may be important in suspected endocarditis or deep-seated infection.

6.1 Microscopic appearance

Gram stain ([TP 39 – Staining Procedures](#))

Streptococci, *Enterococci* and related organisms appear as Gram-positive cocci. These are arranged in chains or pairs in direct Gram stains of specimens including blood cultures and cerebrospinal fluids.

6.2 Primary isolation media

Most streptococci grow on a range of media.

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

Fastidious anaerobe agar (FAA) / Fastidious Anaerobe Neo Agar (FANEO) may help when β -haemolysis is better defined on anaerobic media or when recovering more fastidious or slow-growing streptococci and related organisms.

Media such as Cystine Lactose Electrolyte Deficient (CLED) agar can also prevent *Proteus* species and other motile Enterobacterales from swarming and allow discrete colonial growth of streptococci and enterococci, which is easier to distinguish in mixed cultures. This also applies to chromogenic media when used according to the manufacturer's instructions.

However, for initial assessment and identification, blood agar is preferred as it supports good recovery and clear haemolysis.

Some commercial biochemical identification kits and MALDI-TOF MS platforms specify that streptococcal or enterococcal colonies grown on blood agar are validated for use with their systems. Where other media are used, local validation is required.

Sheep blood generally gives the most distinct haemolysis, but the performance of blood source should be assessed through local media verification and routine quality control in accordance with [UK SMI Q 1: Evaluations, validations and verifications of diagnostic tests](#).

6.3 Colonial appearance

See Table 1 below.

This is not an exhaustive list of possible species.

Table 1. Presumptive identification of *Streptococcus* species, *Enterococcus* species and morphologically similar organisms from blood agar

Haemolysis	Colony morphology	Colony size	Genera / Organisms	Notes
Beta (β) haemolysis	Colonies are grey-white, domed shaped, with smooth or moist surface clear margins. Some may be mucoid.	Large	<i>S. pyogenes</i>	<i>S. pyogenes</i> may be mucoid;
	Colonies are spherical to ovoid, flat, grey-white, may be translucent.	Large	<i>S. agalactiae</i>	Some strains have yellow/orange pigment; colonies appear larger after 18–24h. Minority non-haemolytic. <i>S. agalactiae</i> may show yellow/orange pigment and appear larger after 18–24h; β-haemolysis stronger anaerobically (11,19).
	Colonies often resemble <i>S. pyogenes</i> .	Large	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> and <i>S. canis</i>	<i>S. dysgalactiae</i> colonies may closely resemble <i>S. pyogenes</i> (20).
Variable haemolysis	White or translucent colonies, convex, entire margins.	Small	<i>S. anginosus</i> Group	Haemolysis varies e.g., <i>S. constellatus</i> is often β or non-haemolytic; <i>S. intermedius</i> is mostly α or non-haemolytic.
Alpha (α) haemolysis	Grey, mucoid; domed early, later central depression (autolysis).	Small - medium	<i>S. pneumoniae</i>	α-haemolytic aerobically.
	Smooth greyish colonies; variable texture.	Small	Viridans streptococci	Includes <i>S. mitis</i> , <i>S. oralis</i> , <i>S. sanguinis</i> , <i>S. mutans</i> , <i>S. salivarius</i> ; generally, α-haemolytic. <i>S. mutans</i> may be sticky/hard; <i>S. mitis</i> group resembles <i>S. pneumoniae</i> but lacks capsule.

Haemolysis	Colony morphology	Colony size	Genera / Organisms	Notes
	Grey-white, smooth, translucent, moist.	Small - medium	<i>S. suis</i>	Usually α on sheep blood; may appear β on horse blood.
	Greyish colonies.	Small	SBSEC (<i>S. bovis</i> / <i>equinus</i> complex)	Includes <i>S. equinus</i> , <i>S. gallolyticus</i> , <i>S. infantarius</i> , <i>S. lutetiensis</i> , <i>S. alactolyticus</i> .
Gamma (γ) / non-haemolytic	Cream/grey/white smooth colonies; typically, γ -haemolytic.	Large	<i>Enterococci</i> species	Usually γ ; sometimes weak α . Colony morphology is similar across species, not distinguishable on appearance alone. Some species are motile (<i>E. casseliflavus</i> and <i>E. gallinarum</i>).
Other catalase-negative Gram-positive cocci	Colony morphology may overlap with <i>streptococci</i> and <i>enterococci</i> depending on species.	Varies	<i>Abiotrophia</i> , <i>Granulicatella</i> , <i>Aerococcus</i> , <i>Facklamia</i> , <i>Gemella</i> , <i>Lactococcus</i> , <i>Leuconostoc</i> , <i>Pediococcus</i>	See Table 2 for differentiation and detailed characteristics.

Footnotes:

- a** Large colony size refers to colonies of >0.5 mm after 24 h incubation, whereas small colony size is <0.5 mm. Colonies measuring **approximately 0.5 mm** are considered medium-sized.

6.4 Test procedure

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 is widely used in routine diagnostic laboratories for the identification of clinically relevant Gram-positive cocci, including streptococci, enterococci, and related organisms (21,22). Where MALDI-TOF MS is employed, laboratories should follow local policies that define score thresholds for acceptance of identifications and specify criteria for when additional testing is required.

In routine practice, MALDI-TOF MS may provide reliable species-level identification for a range of clinically significant streptococci and streptococcal-like organisms where identification scores are high, and the result is concordant with colonial morphology, haemolysis pattern and Gram stain appearance. 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, biochemical, or grouping methods may be required.

A recognised limitation of MALDI-TOF MS is its reduced ability to differentiate certain closely related streptococcal species. Although ongoing database updates have improved performance, challenges remain in reliably distinguishing specific taxa, including:

- differentiation between β -haemolytic streptococci associated with Groups A, C and G,
- differentiation of *Streptococcus dysgalactiae* from *Streptococcus canis*
- differentiation within the SBSEC, including species relating to *Streptococcus gallolyticus*,
- distinction of *Streptococcus pneumoniae* from closely related members of the *S. mitis* group

For these organisms, MALDI-TOF MS results should be interpreted with caution and supported by additional testing where clinically appropriate, such as Lancefield grouping for β -haemolytic streptococci or supplementary phenotypic tests for pneumococci. MALDI-TOF MS should therefore not be used in isolation where species-level discrimination carries clinical, public health, or infection prevention significance.

MALDI-TOF MS is an effective identification technique for Enterococci species including *E. faecalis* and *E. faecium* compared to other automated methods (23). This method has also been used for the identification of aerococci to species level. However, the accuracy of MALDI-TOF MS in identification of bacterial species that are uncommon in clinical specimens, such as aerococci, needs to be further evaluated (22,24).

Refer to [UK SMI TP 40 – Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry \(MALDI-TOF MS\) test procedure](#) for more information on the technical limitations.

6.4.2 Biochemical tests

A wide range of biochemical tests are available for the characterisation of streptococci; some common ones are listed in Table 2. Commercially available test identification kits designed specifically for streptococci have been developed to include carbohydrate fermentation and other traditional biochemical tests, however these kits may not identify more recently recognised species (11).

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

Streptococci and morphologically similar organisms are usually catalase negative.

Enterococcus species are catalase negative, but some strains reveal pseudocatalase activity when cultivated on blood-containing agar media.

6.4.3 Streptococcal grouping kits

Commercial streptococcal grouping kits based on latex agglutination are available for routine use. A positive reaction is indicated by the clumping of the latex particles.

Laboratories should follow manufacturer's instructions and rapid tests and kits should be validated and be shown to be fit for purpose before use (11).

It should be noted that expression of Lancefield antigens is not species-specific. For example:

- Some isolates of *S. dysgalactiae subsp. equisimilis* (SDSE) may express Lancefield Group A antigen.
- *Streptococcus equi subsp. zooepidemicus* expresses Lancefield Group C antigen.
- Members of the *Streptococcus anginosus* group may express Lancefield Groups A, C, F or G antigens.
- *Vagococcus fluvialis* may give a weak reaction with Lancefield group D antiserum and may be confused with some enterococci.
- *Streptococcus pseudoporcinus*, has been reported to cross react with commercial group B streptococcal reagents when using commercial kits.

These limitations highlight the importance of correlating Lancefield grouping results with phenotypic characteristics and, where appropriate, supplementary identification methods.

Lancefield antigen groupings associated with streptococci, enterococci and morphologically similar Gram-positive cocci are outlined in Table 2.

6.4.4 Commercial identification systems (phenotypic panels)

Some commercial kits may give unreliable results with the identification of alpha haemolytic streptococci. Some commercial identification systems have limited ability to differentiate *S. pneumoniae* from closely related members of the *S. mitis* group because of their close genetic relatedness.(25).

Commercial agglutination tests for pneumococcal antigen detection are available, but these should be used with caution, as cross-reactions may occur with the *S. oralis* and *S. mitis* groups.

Species belonging to the *S. mitis* and *S. sanguinis* groups, often regarded as a single group, are difficult to differentiate and may give discordant results due to the low quality of some identification systems used.

In some commercial identification systems, *Helcococcus kunzii* may be misidentified as *A. viridans* but can be differentiated based on colony size and haemolysis (11,26,27). *A. sanguinicola* may also be misidentified as *A. viridans*. This makes the reports of infections caused by *A. viridans* problematic when identification is based on these methods. Reports of infection attributed to *A. viridans* should be interpreted cautiously when identification is based solely on these systems.

In some commercial identification systems, “viridans” streptococci can be misidentified as *Gemella* species (11,27-29).

Table 2: Presumptive phenotypic identification of *Streptococcus* species, *Enterococcus* species and morphologically similar organisms based on haemolysis, Lancefield grouping and biochemical characteristics

Species	Lancefield group	Bile Aesculin hydrolysis ^a	Optochin sensitivity ^b	Bile solubility test ^c	Bacitracin sensitivity ^d	PYR ^e	CAMP
<i>S. pyogenes</i>	A	-	-	-	+	+	-
<i>S. agalactiae</i>	B	-	-	-	-	-	+
<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> .	C, G, A and L	-	-	-	-	-	-
<i>S. canis</i>	G	-	-	-	-	-	+
<i>S. anginosus</i> Group: <i>S. anginosus</i> , <i>S. constellatus</i> , <i>S. intermedius</i>	A, C, F and G	-	-	-	-	-	-
<i>S. pneumoniae</i>	None	-	+	+	-	-	-
<i>S. mitis</i>	None	-	-	-	-	-	-
<i>S. oralis</i>	None	-	-	-	-	-	-
<i>S. sanguinis</i>	H	-	-	-	-	-	-
<i>S. mutans</i>	None	-	-	-	-	-	-
<i>S. salivarius</i>	K	-	-	-	-	-	-
<i>S. suis</i>	R, S and T or ungroupable	-	-	-	-	-	-

Species	Lancefield group	Bile Aesculin hydrolysis ^a	Optochin sensitivity ^b	Bile solubility test ^c	Bacitracin sensitivity ^d	PYR ^e	CAMP
<i>S. bovis</i> / <i>S. equinus</i> complex (SBSEC): <i>S. equinus</i> , <i>S. gallolyticus</i> , <i>S. infantarius</i> , <i>S. lutetiensis</i> , and <i>S. alactolyticus</i>	D	+	-	-	-	-	-
<i>Enterococcus</i> species: <i>E. faecalis</i> ^f , <i>E. faecium</i> , <i>E. gallinarum</i> , <i>E. casseliflavus</i> , and <i>E. flavescens</i>	D	+	-	-	-	+	-
<i>Abiotrophia</i> and <i>Granulicatella</i> : <i>A. defectivus</i> , <i>G. adiacens</i> , <i>G. balaenopterae</i> , <i>G. elegans</i>	None	-	-	-	-	-	-
<i>Aerococcus</i> species	None	Variable (often -)	-	-	-	-	-
<i>Facklamia</i> species ^g (<i>Facklamia hominis</i>)	None	-	-	-	-	+	-
<i>Gemella</i> species	None	-	-	-	-	-	-

Species	Lancefield group	Bile Aesculin hydrolysis ^a	Optochin sensitivity ^b	Bile solubility test ^c	Bacitracin sensitivity ^d	PYR ^e	CAMP
<i>Lactococcus</i> species	None	-	-	-	-	-	-
<i>Leuconostoc</i> species	None	-	-	-	-	-	-
<i>Pediococcus</i> species	None	-	-	-	-	-	-

Footnotes:

Unless otherwise stated, '+' indicates a positive reaction and '-' indicates a negative reaction. For optochin and bacitracin tests, '+' indicates sensitivity and '-' indicates resistance.

- a** *Enterococcus* species., *Lactococcus* species., and Lancefield Group D streptococci hydrolyse aesculin in the presence of 40% bile; other streptococci do not.
Most strains of *A. viridans* and *A. sanguinicola* give positive bile aesculin reaction while *A. urinae* is bile aesculin negative.
Some strains of *Leuconostoc* species can hydrolyse aesculin. In addition, some viridans group streptococci may show variable bile aesculin hydrolysis (for example, *Streptococcus salivarius*). Refer to [UK SMI TP 2 – Aesculin Hydrolysis Test](#)
- b** Occasional strains of *S. oralis*, *S. mitis* and *S. pseudopneumoniae* are optochin sensitive.
Some *S. pneumoniae* may be resistant to optochin. If there is a clinical suspicion of pneumococcal infection, confirm by additional tests such as automated identification systems, bile solubility testing, pneumococcal latex agglutination, or PCR-based detection of pneumococcal targets.
Refer to [UK SMI TP 25 – Optochin Test](#)
- c** *S. pneumoniae* is soluble in 10% bile salts, *S. pseudopneumoniae* is partially soluble and other α -haemolytic streptococci are insoluble; bile solubility test is used to confirm optochin sensitivity. Refer to [UK SMI TP 5 – Bile Solubility Test](#)
- d** This test uses lower-strength bacitracin disc than those used for the presumptive identification of *Haemophilus* species.
- e** PYR is more specific for the identification of *S. pyogenes* (GAS) compared to bacitracin sensitivity. This test is positive for *S. pyogenes* (GAS) and is negative for most other Lancefield group streptococci, although some human strains of group C and G may be positive.

- f** Identification of *E. faecalis* may involve tellurite resistance testing.
- g** *Facklamia* species are differentiated from each other by hydrolysis of hippurate.

6.5 Further identification and additional approaches

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

6.5.1 Nucleic Acid Amplification Tests (NAAT)

NAATs including multiplex PCR-based syndromic panels, are increasingly used in clinical microbiology for the rapid detection of *Streptococcus* and *Enterococcus* species. These assays are commonly applied to respiratory, meningitis and bloodstream infections and support early diagnosis alongside culture-based methods (30). For *Streptococcus* species, there are various NAATs for the different groups and their target genes and depending on clinical details, the appropriate NAAT should be performed.

Several NAATs have been developed for the identification of *S. agalactiae*, these can be either performed following culture enrichment or directly on clinical specimens (31,32). Real time PCR have also been developed for the identification of *S. pneumoniae* from culture isolates and serum specimens (3,33).

NAATs can provide simultaneous species identification and detection of clinically relevant antimicrobial resistance genes in enterococci, including glycopeptide (vancomycin) and linezolid resistance determinants (34-36).

7 Storage

If required, subculture the pure isolate on a nutrient agar slope for referral to the reference laboratory.

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

Flexible terminology for *S. pyogenes* and *S. agalactiae* is used across clinical guidelines, diagnostic pathways and infection control policies. Therefore, it is recommended that regardless of the methodology used to identify these organisms, i.e. Lancefield group agglutinations alone and/or MALDI-TOF MS or other biochemical commercial systems, and considering the limitations of each method, these should be reported using their full species name and by their corresponding Lancefield group designations. For example:

Streptococcus pyogenes [Group A Streptococcus],

Streptococcus agalactiae [Group B Streptococcus]

The specific format used i.e., use of brackets as shown above or the addition of standard comments may be determined by the capabilities and configurations of laboratory information systems.

In contrast, Lancefield Groups C, G and F comprise multiple streptococcal species, reporting only the Lancefield group (e.g. “Group C *Streptococcus*” or “Group G *Streptococcus*”) lacks species-level specificity and may obscure clinically relevant differences in epidemiology, pathogenicity, and antimicrobial susceptibility. As modern identification methods such as MALDI-TOF MS or other commercial systems enable accurate species-level identification, it is preferable to report the organism by its full species name and the corresponding Lancefield group designation where agglutinations have been performed.

8.1 Designated infection specialist

The infection specialist should be informed of all presumed and confirmed identification of *Streptococcus* and *Enterococcus* species and morphologically similar organisms isolated from specimens obtained from normally sterile sites.

Due to the potential for invasive disease and the development of immunologically or toxin-mediated sequelae, any “new” putative isolates of *S. pyogenes* should be promptly escalated to the infection specialist in accordance with local protocols. Early identification helps support timely risk assessment, outbreak detection, and implementation of control measures to limit transmission, particularly in high-risk settings such as healthcare facilities, schools, or care homes.

Notification to public health authorities of invasive *S. pyogenes* is mandatory throughout the UK to support surveillance, contact tracing, and wider population health protection.

Similarly, “large colony” isolates expressing Lancefield Group C or G antigens should be escalated, as these may also be associated with severe disease and cluster events. Discussion with an infection specialist is recommended to decide whether further in-house testing and/or confirmation by a reference laboratory is required.

Other clinical conditions must be notified to the laboratory associated infection specialist. Typically, when the request includes relevant or additional information suggestive of invasive or severe streptococcal infection such as:

- toxin mediated phenomena (toxic shock syndrome or scarlet fever)
- (necrotising) fasciitis or myositis, puerperal sepsis
- endocarditis
- investigation of possible outbreaks or apparent cross-infection within a hospital or other institution

- unusual antimicrobial resistance patterns, including vancomycin or other glycopeptide resistant *Enterococcus* species and penicillin resistant *S. pneumoniae*

Follow local protocols for reporting *S. agalactiae* (GBS) isolated from pregnant or recently postpartum patients and from neonates.

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 (38).

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 in accordance with local policy, these should be issued as soon as actionable findings are available (e.g., significant microscopical 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)

Invasive Group A Streptococcal (iGAS) infection and scarlet fever are notifiable conditions across all UK nations, and suspected or confirmed cases must be reported in accordance with national legislation and local health protection arrangements. This involves notification to the relevant public health authority via local Health Protection Teams (HPTs) or equivalent services.

National notification requirements and reporting procedures are available at:

- England: [UKHSA guidance on notifiable diseases and reporting](#).
- Scotland: [Public Health Scotland guidance on notifiable diseases, health risk states and infections](#).

- Wales: [Public Health Wales guidance on notifiable diseases and reporting through the AWARe/Health Protection Team service.](#)
- Northern Ireland: [Public Health Agency guidance on notification of infectious diseases and reporting requirements.](#)

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

The hospital infection control team should be informed of Group A streptococcal disease identified, glycopeptide-resistant *Enterococcus* (VRE), and penicillin-resistant pneumococci isolated from in-patients in accordance with local protocols.

Enterococci with acquired glycopeptide resistance mechanisms (e.g., VanA or VanB) are of established IPC significance. In contrast, intrinsically VanC-positive species, including *Enterococcus gallinarum*, *Enterococcus casseliflavus* and *Enterococcus flavescens*, possess chromosomally encoded, low-level, non-transferable glycopeptide resistance. Approaches to reporting and IPC management of these organisms may vary according to national guidance and local policy, and IPC measures should be proportionate to species and resistance mechanism.

Consideration should be given to informing the relevant infection control staff of such isolates from patients currently in the community (including nursing homes and prisons) in accordance with local arrangements, notably if suspecting cross-transmission.

There is no nationally mandated IPC guidance for either VRE or penicillin-resistant pneumococci. Management of cases should follow local IPC policies with escalation to a microbiology clinician and referral to UKHSA where indicated.

Exceptional resistance phenotypes or isolates associated with outbreaks, unusual patterns, or clinical concern should be discussed with IPC teams. Infection prevention and control measures should follow local protocols and applicable national IPC guidance (40-42).

Exceptional isolates should be referred to UKHSA in line the ARMHAI (Antimicrobial Resistance and Healthcare Associated Infections) user manual.

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.

The following should be referred:

- All streptococcal isolates from invasive disease should be referred for surveillance and epidemiological purposes:
 - *S. pyogenes* (GAS) for *emm* typing
 - Whole genome sequencing (WGS) for *S. agalactiae* (GBS), group G streptococci and group C streptococci including *S. dysgalactiae* subsp. *equisimilis*, *S. canis*
- All *S. pneumoniae* isolates recovered from a normally sterile site should be referred for species confirmation and capsular serotyping.
- Isolates from superficial infections which are associated with an infection control or cluster investigation, particularly in high-risk settings such as maternity or neonatal units, may also be referred to support assessment of strain relatedness. Please inform the reference laboratory of the investigation details.
- *S. agalactiae* (GBS) and *S. pyogenes* (GAS) isolates demonstrating reduced susceptibility to penicillin should be referred for confirmation and WGS, particularly where unusual resistance patterns or emerging phenotypes are identified.

Note: Reduced penicillin susceptibility has been reported internationally, including in vulnerable populations (43). Confirmation and genomic analysis are required to characterise resistance mechanisms and support public health monitoring.

- Typing or genomic analysis of vancomycin-resistant *Enterococcus* (VRE) isolates may be undertaken in the context of outbreaks involving sterile site infections, at the discretion of the national reference service.
- Organisms with unusual or unexpected antimicrobial resistance. Please refer to UKHSA's [Bacteriology Reference Department User Manual](#) for a full list.
- Isolates associated with laboratory anomalies, clinical concerns, or findings requiring further investigation

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 specimen submission.

[England](#)

[Wales](#)

[Scotland](#)

[Northern Ireland](#)

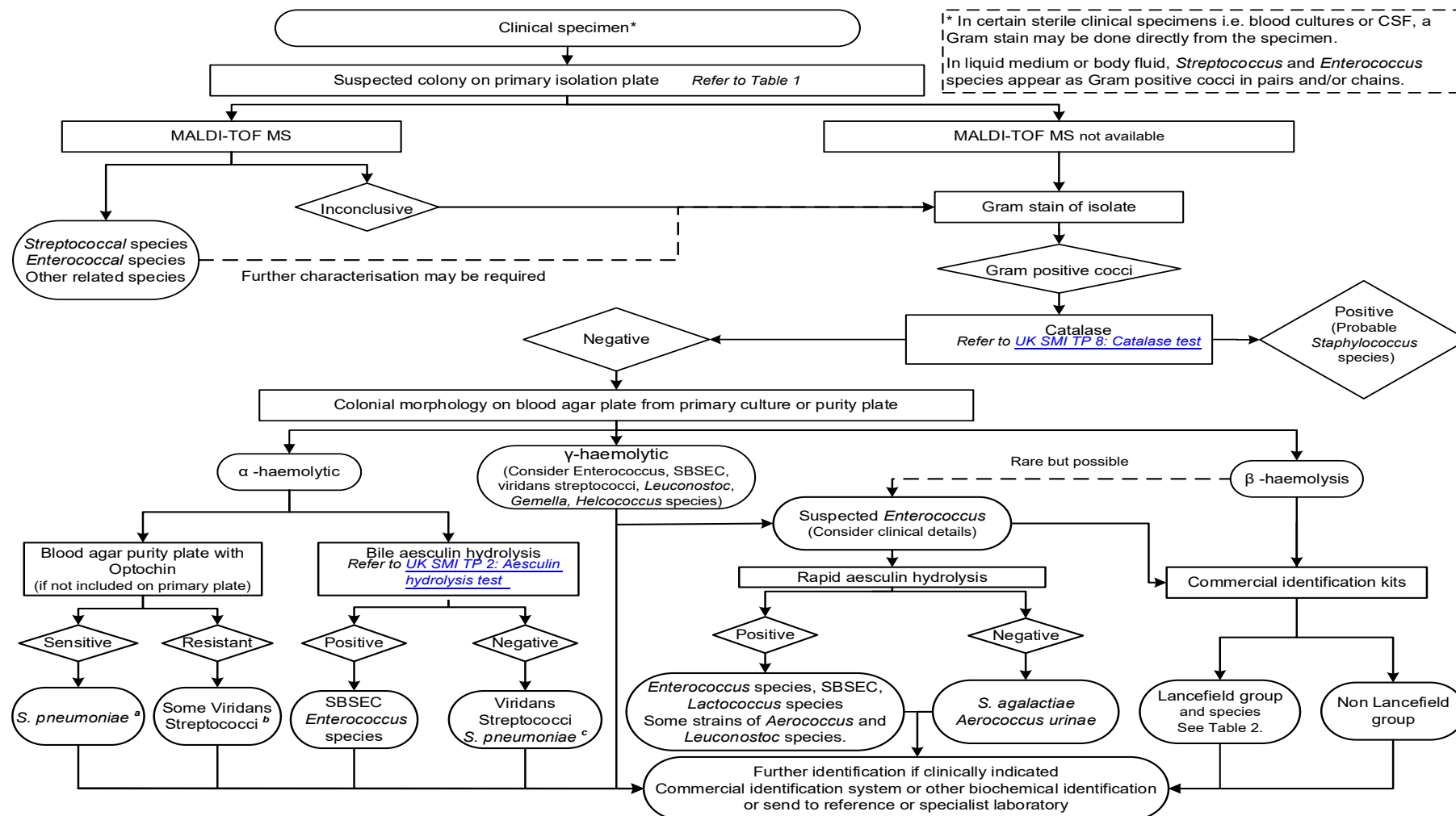
10 Public health responsibilities of diagnostic laboratories

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 *Streptococcus* species, *Enterococcus* species and morphologically similar organisms



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.

Note: Additional biochemical tests (e.g., PYR, CAMP, bacitracin sensitivity) may be performed where appropriate and are summarised in Table 2 for species identification when confirmation by an alternative technique is required or automated methods are not available.

- a** Some *S. pneumoniae* may be resistant to optochin: if there is a clinical suspicion of pneumococcal infection, confirm by additional tests as appropriate.
- b** Occasional strains of *S. oralis*, *S. mitis* and *S. pseudopneumoniae* may be optochin sensitive. *S. pseudopneumoniae* is optochin resistant when incubated in increased CO₂.
- c** Some viridans group streptococci may show variable bile aesculin hydrolysis (for example, *Streptococcus salivarius*).

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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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