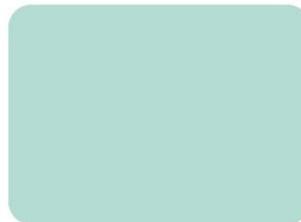
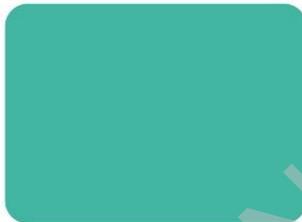
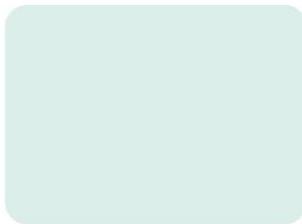




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## UK Standards for Microbiology Investigations

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



## Acknowledgments

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

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UK SMIs are produced in association with:



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## Amendment table

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

<b>Amendment number/date</b>	x/dd.mm.yy
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<b>Anticipated next review date*</b>	dd.mm.yy
<b>Section(s) involved</b>	<b>Amendment</b>

\*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 *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) serves as the primary method of identification, with further characterisation supported by phenotypic approaches and commercial biochemical systems as required. Molecular platforms may also be employed when additional confirmation is necessary.

Some identification methods, including certain biochemical tests, molecular assays or other supplementary techniques, are not performed routinely. They are performed only when additional confirmation is required, when MALDI-TOF MS or other automated systems yield inconclusive results, or when those systems are unavailable.

The focus is 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 (1). Further identification may be necessary where clinically or epidemiologically indicated.

This identification document does not focus on screening, typing or antimicrobial susceptibility testing. 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 \(\*Streptococcus agalactiae\*\)](#) for detection of Group B Streptococci.

Please note that some of the *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 capable of causing a wide range of infections in both humans and animals. Many exist as commensals of the upper respiratory tract, gastrointestinal tract or genitourinary tract, but possess a high potential for virulence under certain conditions. Refer to Section 6.3, Table 1 for all species relevant to this document.

The most clinically significant *Streptococcus* species include ***Streptococcus pyogenes***, ***Streptococcus pneumoniae***, ***Streptococcus agalactiae***.

***S. pyogenes*** (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, vulvo-vaginitis 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 (4,5).

***S. agalactiae*** can produce many invasive and non-invasive diseases and is a major cause of newborn infections presenting as early-onset sepsis infections, typically pneumonia and meningitis, which may lead to early or late onset neonatal sepsis (3). It also causes septic spontaneous abortion and puerperal sepsis.

***S. dysgalactiae* subsp. *equisimilis*** causes infections clinically similar to those due to GAS, including pharyngitis, soft tissue infection and invasive disease, supported by the presence of virulence genes analogous to *emm* types found in *S. pyogenes* (6).

***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** - *S. anginosus*, *S. intermedius* and *S. constellatus* - although variable in haemolysis, are consistently associated with deep-seated purulent infections. Identification of these organisms from sterile sites is clinically significant due to their strong association with abscess formation across multiple organs (7).

**The viridans 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 (1,8,9).

***S. suis*** is an important zoonotic pathogen which is also isolated from human cases of meningitis and bacteraemia (1,3,10).

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

Within the enterococci, ***Enterococcus faecalis*** and ***Enterococcus faecium*** are particularly important due to their role in healthcare-associated infections such as bloodstream infection, intra-abdominal sepsis and urinary tract infection. Their intrinsic and acquired antimicrobial resistance, particularly in vancomycin-resistant enterococci (VRE), pose major therapeutic and infection control challenges (11).

Other enterococcal species, including ***E. gallinarum***, ***E. casseliflavus***, and ***E. flavescens***, are less commonly implicated in disease but may be relevant in opportunistic infections of immunocompromised hosts. In addition, these species carry the intrinsic VanC phenotype therefore correct identification to species level is essential, as VanC enterococci exhibit low-level vancomycin resistance but are not classified as VRE, preventing them from being erroneously reported as such.

Only enterococci carrying acquired VanA or VanB resistance mechanisms require infection prevention and control (IPC) intervention, as these represent true VRE of clinical and epidemiological significance.

Other Gram-positive cocci that may resemble streptococci include **Abiotrophia** and **Granulicatella** 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 samples.

Recognition of these species is important for deep seated infections (notably endocarditis) to ensure the most appropriate antimicrobial therapy.

Additional genera - **Aerococcus**, **Facklamia**, **Gemella**, **Lactococcus**, **Leuconostoc**, and **Pediococcus** - are generally environmental or commensal organisms with limited but recognised opportunistic potential, including sporadic cases of endocarditis, bacteraemia, urinary tract infection, or wound 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* 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 of a large number of commensal and pathogenic species. With the help of the recent rapid development of methods for microbial phenotyping and molecular identification, the genus *Streptococcus* has undergone a significant expansion and revision (12). There are now over 100 recognised species of *Streptococcus*, many of which are pathogens or commensals in humans and animals (2,13,14).

The genus name *Enterococcus*, previously called *Streptococcus faecalis* and *Streptococcus faecium*, was revived in 1984 when other bacteria were transferred to the genus. There are now more than 50 recognised species of the genus *Enterococcus*. *Enterococcus faecalis* and *Enterococcus faecium* are the most common enterococci isolated from human infections (1,2).

The classical differentiation of streptococci is based primarily on their haemolytic patterns on blood agar (Refer to figure 1). Streptococci were historically separated into  $\beta$ -haemolytic,  $\alpha$ -haemolytic, and non-haemolytic ( $\gamma$ -haemolytic) groups, with  $\beta$ -haemolysis most strongly associated with the traditional “pyogenic” streptococci. These  $\beta$ -haemolytic species include *Streptococcus pyogenes*, *S. agalactiae*, *S. dysgalactiae* subsp. *equisimilis*, *S. canis*, and typically *S. constellatus*, all of which are linked with acute, pus-forming infections of skin, soft tissue, and deeper sites.

In contrast, the  $\alpha$ -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 not generally associated with pyogenic disease but instead are important as commensals of the oral cavity and gastrointestinal tract, with pathogenic roles in endocarditis, bacteraemia, dental disease, and certain gastrointestinal conditions.

*Streptococcus anginosus* group (SAG) - *S. anginosus*, *S. intermedius*, and *S. constellatus* – are notable for their variable haemolytic behaviour but consistent association with purulent, abscess-forming infections across multiple anatomical sites.

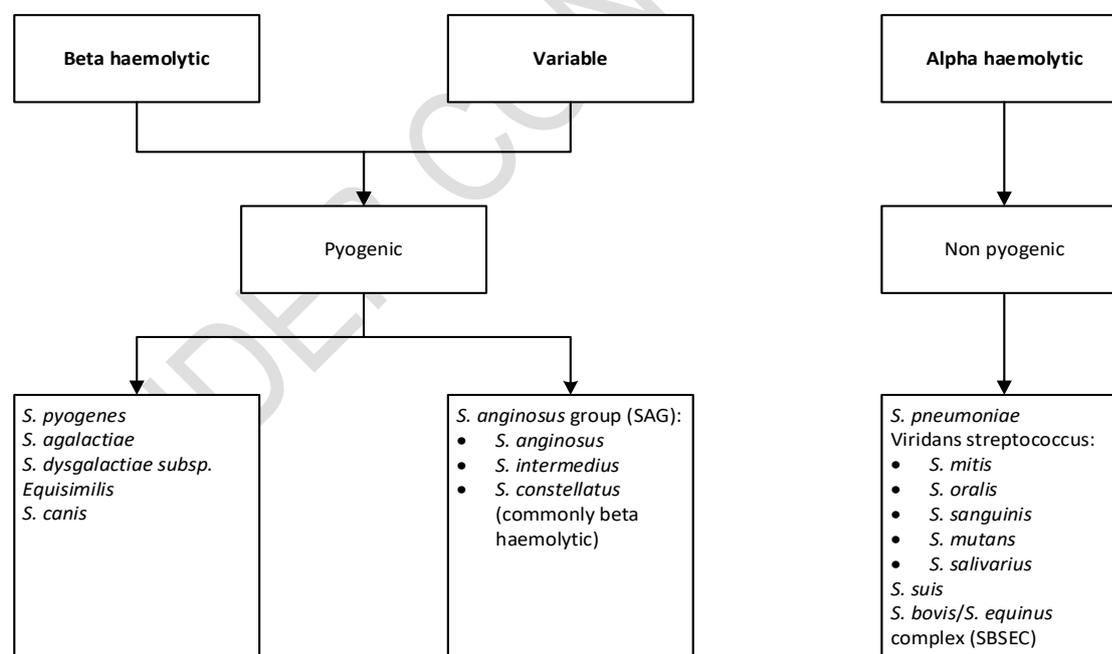


Figure 1: Classification of clinically relevant streptococcal species according to haemolysis patterns on blood agar and their clinical grouping into pyogenic and non-pyogenic organisms.

## 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](#) (15-36).

All organisms included in this document are Hazard group 2 organisms therefore, the processing of diagnostic samples should be carried out at Containment Level 2.

Appropriate Personal Protective Equipment (PPE) should be worn and techniques designed to minimise exposure of the laboratory workers should be 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 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 staining (for example, Gram-positive cocci in chains) can further support identification, particularly from direct samples or positive blood cultures.

In routine workflows, MALDI-TOF MS is the primary method for species-level identification. When MALDI-TOF MS results are inconclusive, confirmation is required, or automated systems are unavailable, alternative characterisation may be achieved using phenotypic or biochemical tests performed from non-selective media. Because related genera can share similar features, identification should draw on a combination of methods.

Immunological and molecular rapid assays, including real-time PCR and other NAAT platforms, may also be used when clinically appropriate.

The required level of identification depends on the clinical significance of the isolate and specimen type. Lancefield grouping may be sufficient in many cases, while species-level reporting is essential for organisms such as *Streptococcus pyogenes* and *Streptococcus pneumoniae*. Some isolates, such as enterococci from urine, may be reported at genus level only.

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

### 6.1 Microscopical 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 samples 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  $\beta$ -haemolysis is better defined on anaerobic media or when recovering more fastidious or slow-growing streptococci and related organisms.

Selective agars such as CLED can also prevent *Proteus* species and other motile *Enterobacterales* from swarming and allows 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 manufacturer instructions.

However, for the purposes of 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 as part of each laboratory's media verification and routine quality control in accordance with [UK SMI Q 2: Quality assurance in the diagnostic infection sciences laboratory](#).

## 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	Organisms	Notes
Beta 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; <i>S. agalactiae</i> may show yellow/orange pigment and appear larger after 18–24h; $\beta$ -haemolysis stronger anaerobically (1,37).
	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.
	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> (38).
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 $\beta$ or non-haemolytic; <i>S. intermedius</i> is mostly $\alpha$ or non-haemolytic.
Alpha haemolysis	Grey, mucoid; domed early, later central depression (autolysis).	Small - medium	<i>S. pneumoniae</i>	$\alpha$ -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 $\alpha$ -haemolytic. <i>S. mutans</i> may be sticky/hard; <i>S. mitis</i> group resembles <i>S. pneumoniae</i> but lacks capsule.
	Grey-white, smooth, translucent, moist.	Small - medium	<i>S. suis</i>	Usually $\alpha$ on sheep blood; may appear $\beta$ on horse blood.

Haemolysis	Colony morphology	Colony size	Organisms	Notes
	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 (non-haemolytic)	Cream/grey/white smooth colonies; typically, $\gamma$ -haemolytic.	Large	<i>Enterococci</i> species	Usually $\gamma$ ; sometimes weak $\alpha$ . Colony morphology is similar across species, not distinguishable on appearance. 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.

## 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 been developed and validated to determine species and lineages of clinically relevant Gram-positive cocci including *Streptococcus*, *Aerococcus* and *Enterococcus* (39). Laboratories should follow local policies on whether MALDI-TOF MS is used primarily for identification, but any low identification scores should be retested with other biochemical tests or Lancefield grouping especially with *S. dysgalactiae*.

One of the significant limitations of MALDI-TOF MS is that it cannot readily distinguish between closely related streptococcal species. Although recent database updates have improved differentiation between Group A and Group G streptococci, challenges remain in reliably separating *Streptococcus dysgalactiae* from *S. canis*. This is in addition to reliance on the quality and comprehensiveness of reference database and the continuous streptococcal taxonomy changes.

MALDI TOF MS is an effective identification technique for *Enterococci* compared to other automated methods which are less efficient in detecting non-*faecalis* and non-*faecium* *Enterococcus* species (40). 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 samples, such as *Aerococci*, needs to be further evaluated (41).

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

### 6.4.2 Biochemical tests

A wide range of biochemical tests are available for the characterisation of *streptococci*; some common tests 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 (1).

#### **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 diagnosis. A positive reaction is visualised by the clumping of the particles.

Laboratories should follow manufacturer's instructions and rapid tests and kits should be validated and be shown to be fit for purpose prior to use (1).

*Vagococcus fluvialis* may give a weak reaction with Lancefield group D antiserum and may be confused with some enterococci.

*Streptococcus porcinus*, a swine pathogen, has been reported to cross react with commercial group B streptococcal reagents when using commercial kits.

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. There is also poor discrimination between the *S. pneumoniae* and the *S. mitis* group as they are genetically inseparable, and so *Streptococcus mitis/oralis* species can be erroneously identified as *S. pneumoniae* (42).

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 of the 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 (1,43,44). *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.

In some commercial identification systems, "viridans" streptococci can be misidentified as *Gemella* species (1,44-46).

**Table 2: Conventional 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 <sup>a</sup>	Optochin sensitivity <sup>b</sup>	Bile solubility test <sup>c</sup>	Bacitracin sensitivity <sup>d</sup>	PYR <sup>e</sup>	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	-	-	-	-	-	-

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

Species	Lancefield group	Bile Aesculin hydrolysis <sup>a</sup>	Optochin sensitivity <sup>b</sup>	Bile solubility test <sup>c</sup>	Bacitracin sensitivity <sup>d</sup>	PYR <sup>e</sup>	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> <sup>f</sup> , <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	V (often -)	-	-	-	-	-
<i>Facklamia</i> species <sup>g</sup> ( <i>Facklamia hominis</i> )	None	-	-	-	-	+	-
<i>Gemella</i> species	None	-	-	-	-	-	-

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

### Footnotes:

- a** *Enterococci*, Lancefield Group D streptococci and Lactococci hydrolyse aesculin in the presence of 40% bile, other *streptococci* do not. Most strains of *A. viridans* and *A. anguinicola* give positive bile aesculin reaction while *A. urinae* is bile aesculin negative. Some strains of *Leuconostoc* species can hydrolyse aesculin. 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. For more information, refer to [UK SMI TP 25 – Optochin Test](#).
- c** *S. pneumoniae* is soluble in 10% bile salts, *S. pseudopneumoniae* is partially soluble and other  $\alpha$ -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 low-strength bacitracin disc compared to in *Haemophilus* species presumptive identification.
- e** PYR is more specific for the identification of *S. pyogenes* compared to bacitracin sensitivity. This test is positive for Group A streptococci 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 other molecular assays in-house.

### 6.5.1 Nucleic Acid Amplification Tests (NAATs)

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. For *Streptococcus* species, there are various PCRs for the different groups and their target genes and depending on clinical details, the appropriate PCR 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 samples (47). Real time PCR have also been developed for the identification of *S. pneumoniae* from culture isolates and serum specimens (3,48).

PCR has also been used for simultaneous detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci (*Enterococcus faecium*, *E. faecalis*) (49).

## 6.6 Storage

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

## 7 Reporting

### 7.1 Designated infection specialist

Inform the infection specialist of all presumed and confirmed cultures of *Streptococcus* and *Enterococcus* species and morphologically similar organisms obtained from specimens from normally sterile sites.

Due to the potential for invasive disease, and for development of immunologically mediated or toxin-mediated sequelae, “new” putative isolates of *Streptococcus pyogenes* should be brought to the attention of the infection specialist in accordance with local protocols, along with “large colony” isolates which possess Lancefield Group C or G antigens.

Certain clinical conditions must be notified to the laboratory associated infection specialist. Typically, when the request bears 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 protocol for reporting to the patients’ clinicians when isolates of  $\beta$ -haemolytic streptococci of Lancefield Group B in the following cases:

- the patient is pregnant, immediately post-partum
- new-born

### 7.2 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

### 7.3 UK Health Security Agency

Refer to current guidelines on Second Generation Surveillance System (SGSS) reporting (50).

### 7.4 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.

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

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 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 and referred to UKHSA as per the ARMHAI user manual

## 8 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 GAS, GBS, group G streptococci and group C streptococci from invasive disease should be referred for surveillance.
- Isolated from superficial infections which are associated with an infection control or cluster investigation. Please inform the reference laboratory of investigation details.
- Organisms with unusual or unexpected antimicrobial resistance. Examples include Linezolid-resistant *Enterococcus*, reduced penicillin susceptibility in GAS.
- Isolates associated with laboratory anomalies, clinical problems, or findings requiring further investigation

Examples of characterisation methods performed following referral include:

- ***Streptococcus pyogenes* (gas) - emm typing:**
  - All invasive Group A *Streptococcus* (GAS) isolates, including those recovered from normally sterile sites, should be referred for emm typing.
  - Isolates associated with suspected outbreaks or clusters, particularly in high-risk settings such as maternity or neonatal units, may also be referred to support assessment of strain relatedness.
- **Enterococcus typing or genomic analysis:**
  - 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.
  - *Enterococcus* isolates demonstrating linezolid resistance should be referred for confirmation and specific typing, irrespective of vancomycin susceptibility.

- **Whole genome sequencing (WGS):**

- Invasive Group B, C and G streptococcal isolates should be referred for WGS for surveillance and epidemiological analysis.
- *Streptococcus agalactiae* and *Streptococcus pyogenes* 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. Confirmation and genomic analysis are required to characterise resistance mechanisms and support public health monitoring.

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](#)

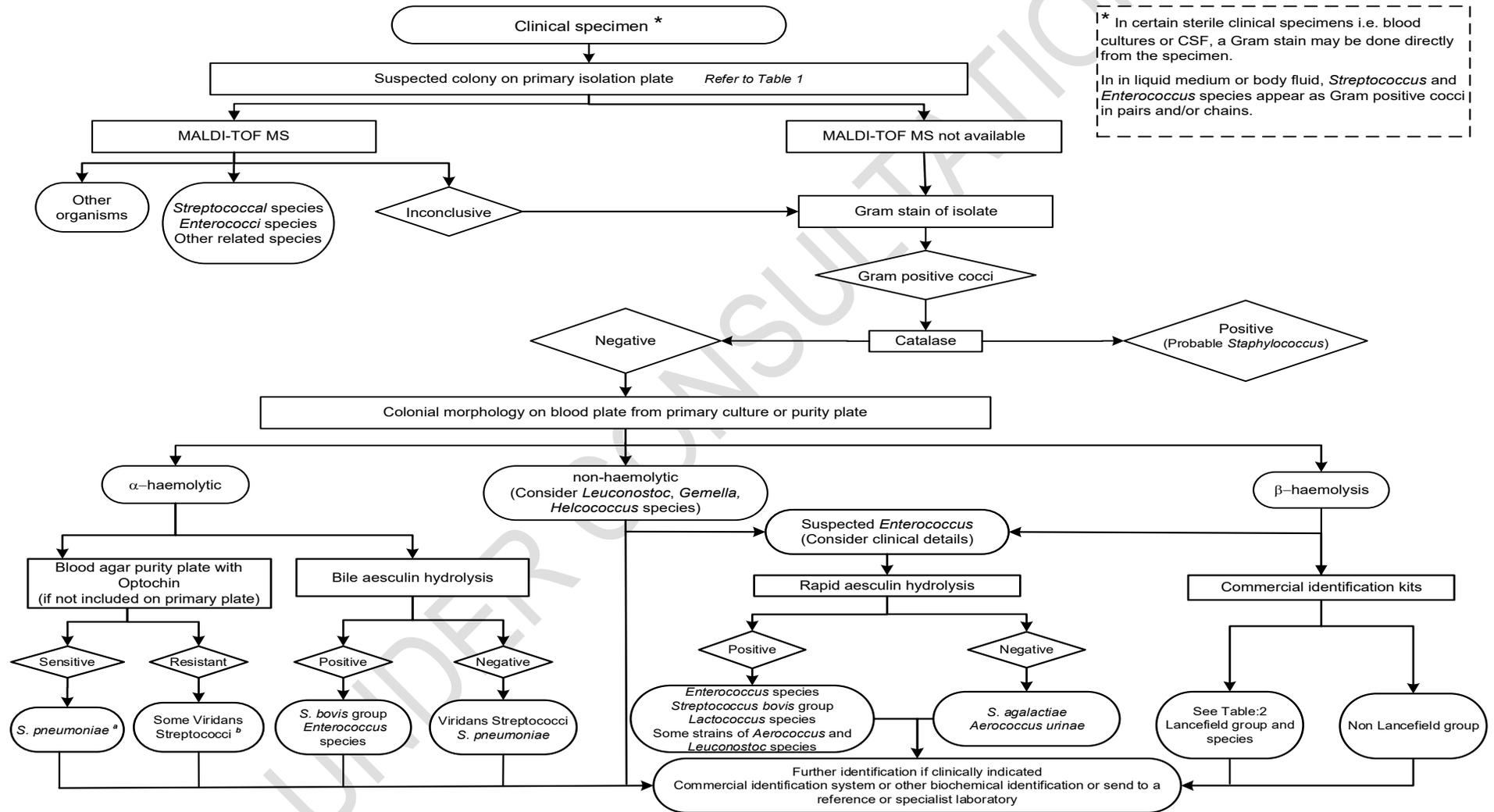
## 9 Public health responsibilities of diagnostic laboratories

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



### Footnotes:

The flowchart is for guidance only.

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 CO<sub>2</sub>.

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

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