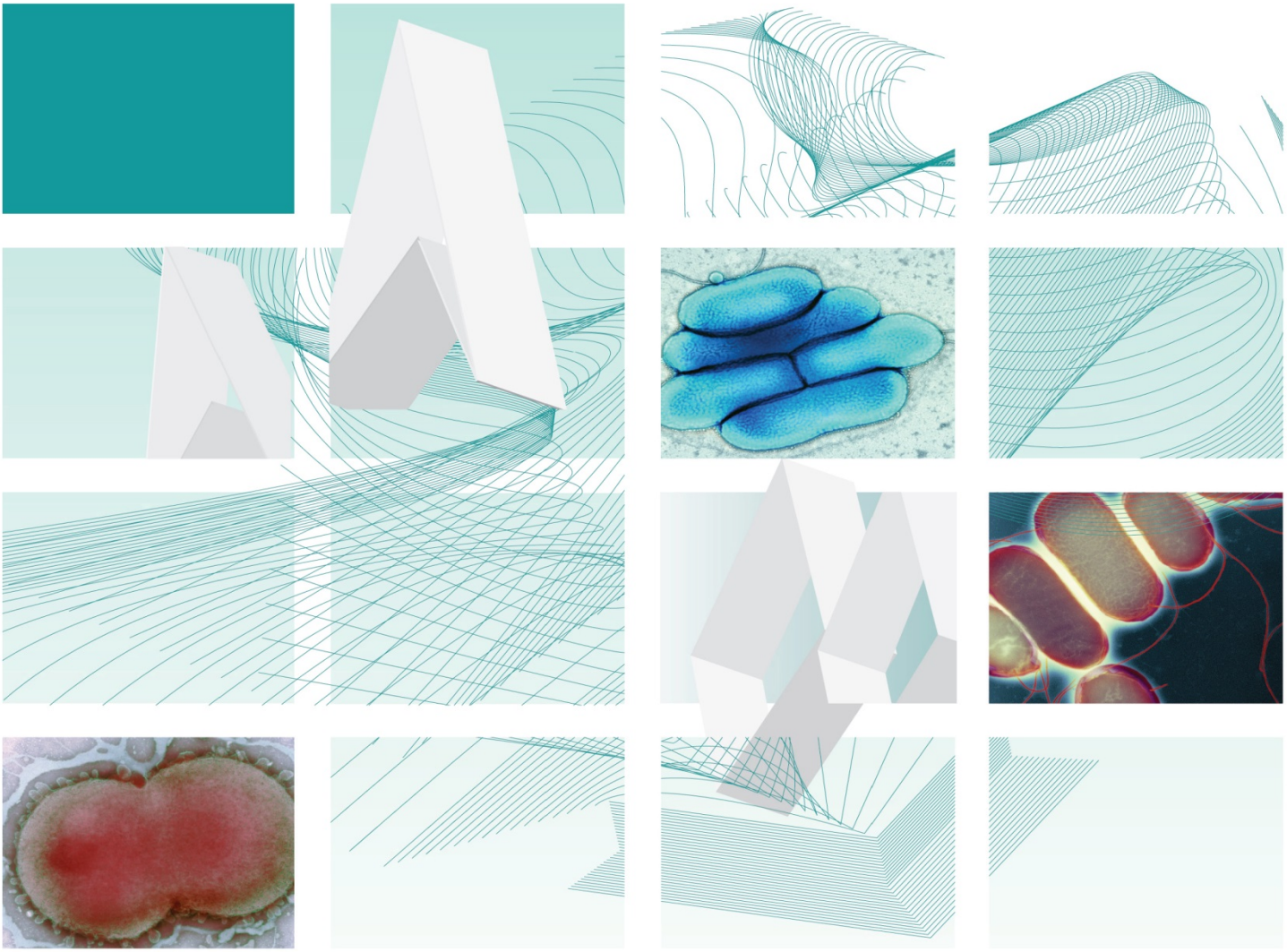
Public Health England

UK Standards for Microbiology Investigations

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



NICE accredited
www.nice.org.uk/accreditation

"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011.**"

This publication was created by Public Health England (PHE) in partnership with the NHS.

Issued by the Standards Unit, National Infection Service, PHE

Identification | ID 4 | Issue number: 4 | Issue date: 22 September 21 | Page: 1 of 31

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of PHE working in partnership with the National Health Service (NHS), Public Health Wales and with the professional 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 (see the steering committee page on GOV.UK).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

PHE publications gateway number: GOV-8348

UK Standards for Microbiology Investigations are produced in association with:

Contents

Amendment table

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

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	11 / 22 September 2021
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	22 September 2024
Section(s) involved	**Amendment**
Whole document	Document presented in a new template. Reorganisation of some text.
Section 4.1	Taxonomy of *Streptococcus*, *Enterococcus* and related species updated.
Section 4.1	Species Lancefield grouping added in table 1 and removed from main text.
Section 4.1	Table ‘summary of test results’ removed due to duplication with section 4.1 and appendix 1
Section 8.5	Information on whole genome sequencing added
Appendix 1	Appendix 1 amended
References	References updated

*Reviews can be extended up to 5 years subject to resources available.

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* and *Enterococcus* species isolated from clinical material to genus or species level by phenotypic and molecular methods. 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 UK SMI includes both biochemical tests and automated methods for the identification of microorganisms. Some biochemical tests may not be done routinely in laboratory except in cases where confirmation by an alternative technique is required or automated methods are not available.

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

4 Introduction

4.1 Taxonomy and characteristics

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 genus *Streptococcus* comprises a large number of commensal and pathogenic species. With the help of recent rapid development of methods for microbial phenotyping and molecular identification, the genus *Streptococcus* has undergone a significant expansion and revision(3). There are now over 100 recognised species of *Streptococcus*, many of which are pathogens or commensals in humans and animals(2,4,5).

Streptococci are Gram positive and catalase negative bacteria that are facultatively anaerobic but some requiring CO_2 for growth. Cells are usually coccus-shaped (spherical or ovoid) and arranged in chains and/or pairs. Cells are non-motile, and less than 2 µm in diameter. Endospores are not formed. Carbohydrates are metabolised fermentatively; lactic acid is the major metabolite. Streptococci produce the enzyme leucine aminopeptidase (LAP), which has also been called leucine arylamidase.

Growth temperature varies among species, but optimum temperature is usually about 37°C(1,2,6).

Streptococci growth on solid media can be enhanced by the addition of blood, serum or glucose. On blood agar, the species exhibit various degrees of haemolysis, which can be used as an early step in identifying clinical isolates. Haemolysis produced by colonies on blood agar and Lancefield serological grouping are important factors in presumptive identification, however there are many overlapping characteristics therefore genetic analysis is a more definitive method for identification(1,2).

Haemolysis on blood agar:

- α-haemolysis – incomplete or partial lysis of the red blood cells around the colony causing a green or brown colour surrounding the colony
- β-haemolysis – complete lysis of the red blood cells surrounding a colony causing a clearing of the blood from the medium
- non-haemolytic (previously called γ-haemolysis) – no colour change or clearing of the medium
- α-prime (α) or “wide zone” α-haemolysis – with an obvious outer ring of clearing around the zone of discoloured (green) erythrocytes. This type of haemolysis can be confused with β-haemolysis[1,7]

Streptococci which are non-haemolytic have no effect on blood agar.

Lancefield grouping

β-haemolytic streptococci are further characterised via Lancefield serotyping, which describes group-specific carbohydrates antigen present on the bacterial cell wall. There are 20 described serotypes, named Lancefield groups A to V (excluding I and J) those that are clinically significant are A, B, C, D, F and G (group D composed of alpha haemolytic organisms). Lancefield serotyping is useful in identification of *Streptococcus*; however, it should be used in conjunction with other tests to get accurate identification(7,8). Lancefield grouping can be used to validate the results of other identification methods.

Table:1 Lancefield group and species(1,6)

Species	**Lancefield group**
Streptococcus pyogenes	A
Streptococcus agalactiae	B
Streptococcus canis	G
Streptococcus dysgalactiae subspecies dysgalactiae	C
Streptococcus dysgalactiae subspecies equisimilis	C, G, A and L
Streptococcus equi subsp. zooepidemicus	C
Streptococcus equi subsp. equi	C
Streptococcus anginosus group	A, C, F and G

	or ungroupable
Streptococcus bovis group	D
Streptococcus suis	R, S and T or ungroupable
Enterococcus species	D

Other *Streptococcus* species such as *S. pneumoniae* and Viridans streptococci are classified as 'non-Lancefield streptococci'.

Streptococcus pyogenes

Streptococcus pyogenes or group A *Streptococcus* (GAS) are β-haemolytic and are one of the most virulent *Streptococcus* species, causing skin infections, pharyngitis, impetigo and other invasive diseases(2,6).

Cells are Gram positive cocci and arranged in chains. After 18 to 24hr of incubation at 35 to 37°C on blood agar colonies are approximately 0.5 to 1.0 mm in diameter, domed-shaped, with a smooth or moist surface, white to grey in colour and with clear margins. Some strains may produce mucoid colonies. The colony type largely depends on the growth conditions and production of hyaluronic acid. Growth is enhanced by the addition of broth with blood and serum. Haemolysis is best observed by growing the culture under anaerobic conditions because the haemolysins are more stable in the absence of oxygen(1,9).

S. pyogenes will not grow at 10°C, 45°C, in the presence of 6.5% NaCl, at pH 9.6 or in the presence of 40% bile(1).

GAS can be differentiated from other β-haemolytic streptococci (BHS) by bacitracin sensitivity and detection of enzyme pyrrolidonyl arylamidase (PYR) (also known as pyrrolidonyl aminopeptidase). Bacitracin susceptibility has been used by many laboratories as a presumptive screening test but it may give false positive results with Group C and Group G streptococci(8). PYR has been found to be 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. Enterococci are also PYR positive (1,10).

Streptococcus dysgalactiae subspecies equisimilis has been identified to possess Lancefield group A antigen. It has been found to cause serious human infections therefore it is essential to differentiate this organism from *S. pyogenes*(11).

Streptococcus agalactiae

Streptococcus agalactiae are facultatively anaerobic, oxidase-negative, catalase negative, Gram positive cocci occurring in chains. Cells are spherical or ovoid and 0.6 to 1.2 µm in diameter, flat, greyish-white and translucent to opaque. Some strains have yellow, orange or brick-red pigment. After 18 to 24hr incubation at 35 to 37°C colonies tend to be slightly larger than other streptococci (approximately 1 mm) and have a less distinct zone of β-haemolysis, although a very small proportion of strains are non-haemolytic. They will grow readily on blood agar and some strains can grow on media containing 40% bile(1).

S. agalactiae can produce many diseases, these can be invasive and non-invasive. *S. agalactiae* is a major cause of new born infections presenting as early-onset sepsis

and pneumonia and late-onset meningitis and sepsis(6). It also causes septic spontaneous abortion and puerperal sepsis.

Group A *Streptococcus* can be differentiated from *S. agalactiae* using CAMP (Christie, Atkinson, Munch, Peterson), bacitracin, PYR and SXT (sulfamethoxazole-trimethoprim) tests. The CAMP reaction refers to the synergistic lysis of erythrocytes by the haemolysin of *Staphylococcus aureus* and the extracellular CAMP factor of *S. agalactiae*(7).

Streptococcus porcinus is a swine pathogen that has been reported to cross react with commercial Group B *Streptococcus* reagents when using commercial kits. Both *Streptococcus* can be differentiated by the PYR test. S. *porcinus* is PYR positive and group B *Streptococcus* is PYR negative(12).

Streptococcus halichoeri is non haemolytic, originally identified in seals but now causes zoonotic infection in humans. *S. halichoeri* tests positive for Lancefield group B antigen but can be identified by MALDI-TOF MS and 16S rRNA gene sequencing(13).

Streptococcus dysgalactiae* subspecies *equisimilis

Streptococcus dysgalactiae subspecies equisimilis are large colony (≥0.5mm) β-haemolytic streptococci. Cells are Gram positive cocci, occurring in chains. Diseases caused by these organisms resembles infections caused by *S. pyogenes.* Other large colony strains are mainly found in animals and cause zoonoses, these can be alpha, beta or non-haemolytic. For example, *S. canis* causes infections in animals and occasionally in humans(14). Other closely related group C *Streptococcal* veterinary species are *Streptococcus equi subsp. zooepidemicus* and *Streptococcus equi subsp. equi,* both species have been reported to cause infections in humans(1,6). In addition, *Streptococcus dysgalactiae subspecies dysgalactiae* are alpha or non-haemolytic isolated from bovine but can rarely cause infections in humans(15,16).

***Streptococcus anginosus* group:**

***Streptococcus anginosus, Streptococcus anginosus subspecies whileyi, Streptococcus constellatus* subspecies *constellatus*, *Streptococcus constellatus* subspecies *pharyngis, Streptococcus constellatus* subspecies *viborgensis* and *Streptococcus intermedius* (formerly the "*Streptococcus milleri*" group)[17]**

The small colony forming (size ≤0.5 mm) *Streptococcus anginosus* group (SAG) consists of 3 distant species, *S. anginosus*, *S. constellatus* and *S. intermedius.* The species *S. constellatus* has been subdivided into the subspecies, *S. constellatus subsp. constellatus*, *S. constellatus subsp. pharyngis*, and *S. constellatus subsp. viborgensis*. These species are Gram positive, catalase negative, nonmotile, non-spore forming cocci, occurring in small chains or pairs of various lengths. Colonies on blood agar are 0.5 to 2.0mm in diameter, white or translucent, convex, and entire. They may exhibit variable haemolysis patterns: α, β or no haemolysis after 16 to 24hr at 35 to 37°C. For example, *S. constellatus* is generally β-haemolytic or non-haemolytic, while *S. intermedius* are mostly non-haemolytic or α-haemolytic on blood agar. Consideration of incubation conditions may be of some value for the presumptive identification of the *S. anginosus* group as growth is reduced under aerobic conditions and frequently enhanced by CO_2 addition. Organisms of this group normally colonise the upper respiratory tract, the digestive tract and the reproductive

tract. Streptococci in this group will grow on media containing bile although they are not salt tolerant(1,6,18).

Identification of an isolate from a clinical specimen as being a member of this group is potentially clinically significant, due to the propensity of this group to be associated with invasive pyogenic infections, such as superficial or deep soft-tissue infections and involve multiple organs(18).

Streptococcus anginosus and *Streptococcus constellatus subspecies constellatus* may cross react with the Lancefield group A and G antigen. *Streptococcus anginosus* and *Streptococcus constellatus subspecies pharyngis* may cross react with the Lancefield group C antigen.

Enterococcus species

Enterococci are Gram positive cocci that are facultatively anaerobic, but some species are CO_2 dependent. They are non-spore-forming. Haemolytic activity is variable and mostly species dependant. *Enterococcus* species can grow at 45°C, hydrolyse aesculin in the presence of 40% bile and most species grow in the presence of 6.5% NaCl. Some strains maybe motile such as *Enterococcus casseliflavus* and *Enterococcus gallinarum*. Cells are ovoid, can occur singly, usually in pairs, or in short chains of different lengths and are frequently elongated in the direction of the chain. Optimum growth temperature for most species is between 35 to 37°C however, some species are able to grow at 45°C and at 10°C. Enterococci are very resistant to drying. Colonies after 24hr of incubation on blood agar are 1 to 2mm in diameter, cream, grey or white in colour and are regular and circular with smooth surface. Enterococci are oxidase negative and ferment carbohydrates. Most species are catalase negative, however care should be taken when using blood containing agar because some strains can produce a pseudocatalase. This can be avoided by testing on MacConkey or other non-blood containing media. They are leucine aminopeptidase (LAPase) and PYR positive. Enterococci can grow and survive in harsh conditions, they can be found in the gastrointestinal tract of humans and animals, plants, soil, water, environment and fermented products. The most common species are *E. faecalis* (type species) and *E. faecium* due to its growing antibiotic resistance(1,7,19-21).

Most enterococci possess the group D antigen although some strains can cross react with Lancefield group D and G antiserum(22).

Enterococci demonstrates both intrinsic and acquired resistance to a number of antibiotics, such as β-lactam antibiotics, aminoglycosides, vancomycin and chloramphenicol(19).

Streptococcus bovis group

S. bovis group has undergone many taxonomic changes due to DNA-DNA reassociation studies, resulting in 4 DNA clusters. DNA cluster I contains *S. bovis* and *S. equinus;* which are now combined into a single species called *S. equinus*. Cluster II includes *S. gallolyticus*, with three subspecies: subsp. *gallolyticus*, subsp. *pasteurianus* and subsp. *macedonicus*. Cluster III includes *S. infantarius*, with 2 subspecies: subsp. *infantarius* and subspecies *coli.* Cluster IV includes *S. alactolyticus*. Members of *S. bovis* group are alpha haemolytic or non-haemolytic on blood gar under aerobic conditions(2,5,6).

The *S. bovis* group may be differentiated from enterococci by a negative reaction in both PYR and arginine tests, whereas enterococci are usually positive for both.

Further biochemical and identification tests should be done for a confirmed differentiation between Lancefield group D organisms(1,7,21).

These species are small colony, Gram positive cocci, catalase negative and occur in chains. Members of the *S. bovis* group are associated with bacteremia, sepsis, and endocarditis. *S. bovis group* and *S. mutans* strains have similar phenotypic characteristics such as fermentation of mannitol and production of glucan. Most *S. bovis* strains grow in bile-aesculin agar and are unable to grow in 6.5% NaCl broth(6,23).

Streptococcus suis

Cells are Gram positive, catalase negative, coccoid and occur singly, in pairs, or (rarely) in short chains, some may rarely appear in short chains. *S. suis* are encapsulated, appear as alpha haemolytic on sheep blood agar but some species can appear as β-haemolytic on horse blood agar. Some strains are resistant to bile and all are optochin resistant and PYR negative. They do not grow at 10 or 45°C, in 6.5% NaCl or 0.04% tellurite. Some strains are able to grow in the presence of 40% bile and all are able to hydrolyse aesculin. *S. suis* is an important zoonotic pathogen which is also isolated from human cases of meningitis and bacteremias(1,6,24).

Based on phenotypic and phylogenetic results, reassortment of *S. suis* has resulted in new species: *Streptococcus parasuis* (formerly *S. suis* serotype 20, 22 and 26)(25) and *Streptococcus ruminantium* (formerly *S. suis* serotype 33)(26).

Streptococcus pneumoniae

Streptococcus pneumoniae (“pneumococci”) are Gram positive, diplococci or chains of cocci, typically lanceolate cells occurring in pairs, which may be capsulated. With aging colonies, Gram positive reaction can turn into Gram variable and some cells may appear as Gram negative. Colonies are 1 to 2mm, appear as small, greyish, mucoid, glistening and dome shaped with an entire edge after 24hr of incubation at 35 to 37°C. With age, autolysis occurs and the colony collapses. On blood agar, α-haemolysis will appear under aerobic conditions and β-haemolysis under anaerobic conditions. *S. pneumoniae* can be differentiated from other viridans streptococci by 2 tests: optochin susceptibility and bile solubility. *S. pneumoniae* are usually sensitive to optochin (ethylhydrocupreine hydrochloride), which enables rapid identification of the organism; although, resistance has been described. *S. pneumoniae* are also soluble in bile salts solution. *S. pneumoniae* may also be identified by serological methods. The 'Quellung reaction' (capsular swelling) may be used to identify the specific types of *S. pneumoniae* microscopically. Commercial agglutination tests are also available for the rapid detection of pneumococcal antigens, but these should be used with caution as cross-reactions may occur with the *S. oralis* and *S. mitis* groups(1,7,27,28).

Viridans streptococci

Viridans streptococci include 5 phenotypic groups: *S. mutans* group, *S. salivarius* group, *S. anginosus* group, *S. mitis* group and *S. bovis* group. These are catalase negative and Gram positive cocci, appearing in chains. Although the word ‘viridans’ or ‘greening’ refers to α-haemolysis, some species can be β-haemolytic or non-haemolytic. Most viridans streptococci lack distinct Lancefield antigen. These organisms can be both commensal flora and pathogens in humans. Identification of streptococci in cases of suspected endocarditis has some value in the confirmation of

the diagnosis and for epidemiological purposes. Some species of streptococci, such as *S. sanguinis* and *S. oralis* (formerly *mitior*), may account for up to 80% of all streptococcal endocarditis cases(1,7,29).

In the *Streptococcus mitis* subgroup, *Streptococcus pseudopneumoniae* has been mistaken for *S. pneumoniae* but has a number of features that allows it to be distinguished from *S. pneumoniae:*

- there is no pneumococcal capsule (and is therefore not typable)
- it is not soluble in bile
- it is sensitive to optochin when incubated in ambient air, but appears resistant or to have indeterminate susceptibility under increased CO_2
- Commercial DNA probe hybridisation tests are false positive (1,30)

Abiotrophia and *Granulicatella*

Abiotrophia and *Granulicatella* (previously referred to as nutritionally variant streptococci*,* and resembling viridans streptococci) are Gram positive cocci that grow as 'satellite' colonies around other organisms. Clinically important species are *A*. *defectivus*, *G*. *adiacens*, *G*. *balaenopterae*, and *G. elegans*. They are catalase and oxidase negative, facultatively anaerobic, non-motile and non-sporulating. No growth occurs at 10 or 45°C(7,31-33).

These species 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. They are often associated with negative blood cultures(32,34).

Genera closely related to streptococci

Aerococcus species

There are 7 species of *Aerococcus*, of which 5 are pathogenic and cause both urinary tract and invasive infections (including infective endocarditis) in humans. These are *A*. *christensenii, A. sanguinicola*, *A. urinae*, *A. urinaehominis* and *A. viridans*. *Aerococcus* species are Gram positive, catalase negative (some strains may give a weak catalase or pseudocatalase reaction), oxidase negative, non-motile, non-spore forming and facultatively anaerobic cocci. On blood agar after 24hr of incubation at 37°C colonies appear small (1 mm or less), non-pigmented (occasionally yellow pigment production by *A. viridans* strains) and show α-haemolysis. All organisms can grow well in 6.5% NaCl, and *A. viridans* grows in 10% NaCl. *Aerococcus* species have been previously misidentified due to similarities between staphylococci, streptococci and enterococci. For example, aerococci resemble streptococci in terms of colony morphology similar to that of "viridans" streptococci and appearing with α-haemolysis on culture but differ microscopically by occurring characteristically as pairs, tetrads or clusters. Tests such as PYR, LAP, MALDI TOF MS, 16s rRNA and other conventional test can be used to differentiate *Aerococcus* species from other organisms. Most strains of *A. viridans* and *A. anguinicola* give positive bile aesculin reaction and are PYR positive. *A. urinae* is bile aesculin negative and PYR negative. Growth occurs both under aerobic and anaerobic conditions (1,35).

In some commercial identification systems, *Helcococcus kunzii* may be misidentified as *A. viridans*. *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. Most aerococci are sensitive to β-lactams as well as to several other groups of antibiotics. *Aerococcus* species are sensitive to vancomycin although elevated MICs have been reported(36).

Facklamia species

There are 6 species of which 4 are pathogenic to humans: *F. hominis, F. languida, F. sourekii* and *F. ignava*. The most common human species is *Facklamia hominis*. *Facklamia* species resemble “viridans” streptococci on culture. They are Gram positive facultatively anaerobic, catalase negative, oxidase negative, LAP positive, non-spore forming, non-motile cocci occurring as pairs, groups or chains. *Facklamia* species are often misidentified as viridans streptococci, key difference between both organisms are that *Facklamia* species are positive for PYR. They grow well in 6.5% NaCl at 37°C but fail to grow at 10 or 45°C. *Facklamia languida* do not hydrolyse hippurate but all other species do, and this is a differentiating characteristic amongst them. Acid is not produced from glucose and other sugars and nitrate is not reduced(1,37).

Gemella species

There are currently 6 *Gemella* species: *G. haemolysans* (type species), *G. morbillorum* (formerly *Streptococcus morbillorum*), *G. bergeriae, G. sanguinis, G. palaticanis* and *G. cuniculi. Gemella* species are slow growing, they are catalase negative, non-motile, non-spore forming, facultatively anaerobic, Gram variable cocci, arranged in pairs, tetrads, clusters and sometimes short chains. Some strains easily decolourise on Gram staining, appearing as Gram negative. In addition, some strains may require strictly anaerobic conditions for primary isolation and become aerotolerant after transfer to laboratory media. They are either α-haemolytic or non-haemolytic on blood agar and resemble colonies of viridans streptococci. Colonies are small and greyish to colourless. They grow best at 35 and 37°C but fail to grow at 10 or 45°C. They are usually PYR and LAP positive. In some commercial identification systems, “viridans” streptococci can be misidentified as *Gemella* species(1,37-39).

Globicatella species

There are 2 species of G*lobicatella* but the species that is implicated in human infections is *G. sanguinis*. *Globicatella* species grow on 5% horse or sheep blood agar forming small viridans *Streptococcus* like colonies on blood agar plate and produce a weak α-haemolytic reaction. They are Gram positive (but sometimes stain Gram negative), non-motile, non-spore forming, facultatively anaerobic, catalase negative, cocci occurring singly, in pairs, or in short chains. Growth occurs in broth containing 6.5% NaCl. They fail to grow at 10 or 45°C. *Globicatella* species can be distinguished from aerococci by cellular morphology. Aerococci form pairs and tetrads while *Globicatella* species form short chains of cocci(1,40).

Helcococcus species

There are currently 6 species of *Helcococcus*: *Helcococcus kunzii* (type species), *H. pyogenes, H. ovis, H. sueciensis. H. seattlensis* and *H. massiliensis*(41,42).

Helcococcus species are Gram positive cocci that are catalase negative, non-motile, and facultatively anaerobic. They are arranged in irregular groups or pairs, tetrads and clusters and form small pinpoint non-haemolytic colonies on blood agar after 48hr of incubation at 35 and 37°C. They are slow growing and appear like viridans streptococci on blood agar plate and may be difficult to grow on a non-blood

containing medium, which suggests that this species is lipophilic. They are usually non-haemolytic. This differentiates them from aerococci that form larger colonies surrounded by a large zone of α haemolysis after incubation. Acid is produced, but not gas, from glucose and other sugars. There is no growth on bile-aesculin agar. These species are susceptible to vancomycin. *H. kunzii* produces tiny grey, non-haemolytic colonies; growth is stimulated by the addition of serum or Tween 80 to the basal medium. In some commercial identification systems, *Aerococcus viridans* may be misidentified as *Helcococcus kunzii,* both can be differentiated on the basis of colony size and haemolysis(1,39,43).

Lactococcus species

Lactococcus species are physiologically similar to *Enterococcus species* and they have been misidentified because of similar characteristics to both streptococci and enterococci*.* They are facultatively anaerobic, α or non-haemolytic, catalase negative, non-motile Gram positive cocci which occur singly, in pairs, or in chains, colonies are small, translucent to whitish. They inhabit animals and plants and derived products. They have been rarely isolated from human cases of the urinary tract, wound infections and from patients with endocarditis. Growth occurs between 10 to 40°C but not at 45°C(1,39).

Leuconostoc species

Leuconostoc species are Gram positive lenticular cocci occurring in pairs and chains and are characteristically vancomycin resistant and produce CO_2 from glucose. They are catalase negative, non-motile and colonies are often α-haemolytic on blood agar. They are facultative anaerobes and may be confused with the enterococci because most *Leuconostoc* species are bile aesculin positive and some may cross-react with the group D antisera. The optimum growth temperature is between 20 and 30°C, but growth may occur at 5°C. *Leuconostoc* species have been involved in a variety of opportunistic infections such as meningitis and bacteremia(1,39).

Pediococcus species

Pediococcus species may resemble viridans streptococci on culture, but microscopically they are similar to *staphylococci*. They are Gram positive cocci appearing in pairs, clusters and tetrads and are intrinsically resistant to vancomycin and moderately susceptible to β-lactam antimicrobial agents. They are facultatively anaerobic and catalase negative. All strains are non-motile and appear as non-haemolytic or α-haemolytic on blood agar plate. Optimum growth temperature is 25 to 35°C but is species dependent. They are leucine aminopeptidase positive, which distinguishes them from *Leuconostoc* species. They may be confused with enterococci because they are bile aesculin positive and cross-react with the Group D antisera(1,39,44).

Vagococcus species

Vagococcus species are Gram positive, non-spore-forming, non-pigmented, catalase negative, oxidase negative and facultatively anaerobic. Cells are coccus or oval shaped occurring singly, in pairs, or in chains. Most strains grow at 10°C but not at 45°C. Most strains are motile. The type species is *Vagococcus fluvialis* which is reported to react with Lancefield group N antisera. In addition, some isolates may give a weak reaction with Lancefield group D antiserum and may be confused with some enterococci. *Vagococcus* are isolated from different sources, mostly from animal or

animal-related products and habitats and very rarely from human clinical samples(1,45).

Principles of Identification

Isolates from primary culture are identified by colonial appearance, Gram stain, type and pattern of haemolysis, Lancefield grouping and physiological divisions such as the pyogenic division which includes *S. pyogenes*. Further identification may be possible by use of biochemical tests using commercially available kits and physiological tests to distinguish between species.

In some instances, based on colonial morphology, clinical details and operator experience, it may be possible to omit the early steps of identification (such as Gram stain and catalase) and proceed to other tests. All identification tests should ideally be performed from non-selective agar.

If primary identification does not provide sufficient identification for clinical management, a full identification may be obtained using a commercial identification system such as MALDI-TOF MS and NAAT based methods, in conjunction with the results of sensitivity testing.

Careful consideration should be given to isolates which give an unusual identification.

If confirmation of identification is required, isolates should be sent to a reference laboratory where a referred taxonomic identification service for streptococci and other related Gram positive, catalase negative genera is available.

5 Technical information and limitations

5.1 Commercial identification systems

Some commercial kits may give unreliable results with the identification of α-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*(46).

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 system used.

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

5.2 Matrix Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS based bacterial identification systems have limitations with several Streptococcal species, including distinguishing *Streptococcus pneumoniae* from other members of the *Streptococcus mitis* group; however, combining bile solubility test with MALDI-TOF spectra results can provide accurate identification of *S. pneumoniae* (47). 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.

5.3 Catalase test

Sometimes a weak catalase or pseudocatalase reaction is produced by *Aerococcus* and *Enterococcus* species, this can be avoided by testing from MacConkey or other non-blood containing media.

6 Safety considerations

Hazard Group 2 organisms.

View current guidance on the safe handling of all organisms documented in the safety considerations section of the UK SMI scientific information.

Appropriate personal protective equipment (PPE) and techniques designed to minimise exposure of the laboratory workers should be worn and adhered to at all times.

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

Employers should ensure that personnel who are pregnant, immunocompromised or immunosuppressed should be restricted from performing work with these highly infectious microorganisms or from handling isolates for identification of these microorganisms and, in some situations, be restricted to a low-risk laboratory(48).

Laboratory acquired infections have been reported.

The above guidance should be supplemented with local COSHH and task specific risk assessments. Compliance with postal and transport packaging regulations is essential(49-70).

7 Target organisms

7.1 *Streptococcus* species reported to have caused human infection

The following streptococci cause human infections(1,5,39). Note this is not an exhaustive list:

Streptococcus pyogenes

Streptococcus agalactiae

Streptococcus dysgalactiae subspecies *equisimilis*

Streptococcus equi subspecies *zooepidemicus*

Streptococcus equi subsp. equi

***Streptococcus bovis* group**: *S. gallolyticus* subspecies*. gallolyticus, S. infantarius*, with 2 subspecies: *subsp. infantarius* and *subspecies coli. Streptococcus lutetiensis Streptococcus pasteurianus*

The “viridans” streptococci

These are divided into 5 subgroups. They are as follows:

***Streptococcus anginosus* group**

Streptococcus constellatus subspecies *constellatus*, *Streptococcus constellatus* subspecies *pharyngis*, *Streptococcus intermedius*

***Streptococcus mutans* group** - *Streptococcus mutans, Streptococcus sobrinus*

***Streptococcus mitis* group** - *Streptococcus mitis*, *Streptococcus oralis, Streptococcus sanguinis, Streptococcus gordonii, Streptococcus parasanguinis, Streptococcus cristatus, Streptococcus pneumoniae**, *Streptococcus peroris*, *Streptococcus oligofermentans*, *Streptococcus australis*, *Streptococcus infantis*, *Streptococcus sinensis*

***Streptococcus salivarius* group -** *Streptococcus salivarius, Streptococcus vestibularis*

Streptococcus suis

Abiotrophia and Granulicatella - *Granulicatella adjacens, Granulicatella elegans, Granulicatella balaenopterae* and *Abiotrophia defectiva.*

*******Taxonomically, this is shown to be within the mitis cluster but could be separated from all other species.*

7.2 *Enterococcus* species reported to have caused human infections

Enterococcus faecalis, Enterococcus faecium, Enterococcus casseliflavus, Enterococcus dispar, Enterococcus durans, Enterococcus flavescens, Enterococcus gallinarum, Enterococcus raffinosus

7.3 Other genera reported to have caused human infections

Aerococcus species, *Facklamia* species, *Gemella* species, *Globicatella* species, *Helcococcus* species, *Lactococcus* species, *Leuconostoc* species, *Pediococcus* species and *Vagococcus* species

8 Identification

8.1 Microscopic appearance

Gram stain (TP 39 – Staining Procedures)

Streptococcus, *Enterococcus* and *Lactococcus* species are Gram positive, round or ovoid cells occurring in pairs, short or long chains or sometimes in clusters.

Streptococcus pneumoniae are Gram positive, lanceolate cells occurring in pairs, often with a visible capsule.

Aerococcus, Pediococcus, Facklamia, and *Helcococcus* species are Gram positive cocci in clusters or tetrads.

Gemella, Leuconostoc species and *Vagococcus* species are Gram positive cocci occurring in pairs, clusters and short chains (*Gemella* may be easily decolourised).

8.2 Primary isolation media

Blood agar incubated in 5 to 10% CO_2 at 35 to 37°C for 16 to 24hr, or anaerobically at 35 to 37°C for 16 to 24hr for throat swabs (B 9 – Investigation of Throat Swabs).

Staph/Strep agar incubated aerobically at 35 to 37°C for 16 to 48hr.

CLED agar incubated aerobically at 35 to 37°C for 16 to 24hr.

Fastidious anaerobe agar incubated anaerobically for 16 to 48hr.

8.3 Colonial appearance

Organism "group"	**Haemolysis**	**Characteristics of growth on blood agar**
β-haemolytic streptococci	β	Different colony appearance: see full details in section 4.1.
"*S. anginosus*"	α,β or non	Colonies are small (≤0.5mm), haemolysis is variable. Some strains have a white "heaped" up colony.
Enterococci	α,β or non	Colonies are larger than those of streptococci, usually 1 to 2mm, with a wet appearance. Haemolysis is variable.
S. bovis group	α or non	Colonies are small 1mm, non-pigmented.
S. pneumoniae	α	Colonies are 1 to 2mm, small, cream, grey or white and may appear as "draughtsman" colonies. After anaerobic incubation colonies may be larger and mucoid.
"viridans" streptococci	α or non	Colonies are tiny, non-pigmented, grey, smooth or matte, 0.5 to 1.0mm, entire edged.
Abiotrophia and *Granulicatella*	α or non	Colonies are small (≤0.5 mm), require pyridoxal or cysteine for growth.
Aerococcus species	α	Resemble "viridans" streptococci.
Facklamia species	α or non	Resemble "viridans" streptococci.
Gemella species	α or non	Resemble "viridans" streptococci.
Globicatella species	α	Resemble *Aerococcus* species.
Helcococcus species	non	Resemble "viridans" streptococci.
Lactococcus species	α or non	Resemble enterococci.
Leuconostoc species	α or non	Resemble "viridans" streptococci.
Pediococcus species	α or non	Resemble "viridans" streptococci

8.4 Test procedures

8.4.1 Biochemical tests

A wide range of biochemical tests are available for the characterisation of streptococci, some common ones are listed below. 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

Bile Aesculin hydrolysis test (TP 2 – Aesculin Hydrolysis Test)

Enterococci, Lancefield Group D streptococci and Lactococci hydrolyse aesculin in the presence of 40% bile, other streptococci do not.

Some strains of *Aerococcus* and *Leuconostoc* species can hydrolyse aesculin.

Optochin sensitivity test (TP 25 – Optochin Test)

S. pneumoniae is usually sensitive to optochin, other streptococci and enterococci are usually resistant.

Occasional strains of *S. oralis*, *S. mitis* and *S. pseudopneumoniae* are optochin sensitive.

Pyrrolidonyl arylamidase /PYR-aminopeptidase (PYR)

Enterococci and *S. pyogenes* are positive; *S. bovis* group and *S. anginosus* group are negative(10).

Bile solubility test (optional) **(TP 5 – Bile Solubility Test)**

S. pneumoniae is soluble in 10% bile salts, *S. pseudopneumoniae* is partially soluble and other α-haemolytic streptococci are insoluble.

Bacitracin Test

Sensitivity test used to differentiate the β-haemolytic *Streptococcus*.

8.4.2 Streptococcal grouping (commercial identification kits)

Established by Lancefield in 1933 found that the majority of pathogenic streptococci possess specific carbohydrate antigens, which permit the classification of streptococci into groups. These streptococcal group antigens can be extracted from the cells using either the acid, formamide or the enzymatic method(71-73). The use of an enzymatic extraction procedure considerably shortens the time required for antigen extraction and much improves the antigen yield, partially for Group D streptococci.

More recently commercial streptococcal grouping kits based on latex agglutination are available for routine diagnosis. 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).

8.4.3 Direct Antigen Detection of *S. pyogenes*

Rapid antigen detection tests can be used in the clinical setting for the detection of *S. pyogenes* in patients with sore throat. These tests are easy to use, low cost and

improve turnaround times with high specificity but vary in sensitivity. Due to low sensitivity, it is best practice to confirm negative direct antigen test results with culture, especially in children and adolescents. Alternatively, DNA amplification methods for direct detection are much more sensitive than antigen detection(6,74).

8.4.4 MALDI-TOF MS

MALDI-TOF MS is a simple, rapid, accurate and highly reliable identification tool for the characterisation of a diverse collection of pathogens. It combines the advantages of phenotypic assays with the rapidity and accuracy of molecular assays. Over the past years this technique has been increasingly used by the diagnostic laboratories due to its high reproducibility, speed and sensitivity of analysis and improved turnaround times compare to phenotypic and other commercial methods(75).

MALDI-TOF MS has been developed and validated to determine species and lineages of clinically relevant Gram positive coccus: *Streptococcus, Aerococcus* and *Enterococcus* species. One limitation of MALDI-TOF MS is that it cannot readily distinguish between closely related species such as *Streptococcus pneuomoniae* from other members of the *Streptococcus mitis* group. There is difficulty in differentiating among the viridans group streptococci, as well as between *Streptococcus dysgalactiae, S. pyogenes* and *S. canis.* However, curation of MALDI-TOF MS databases continues to improve identification(28,76,77). MALDI TOF MS is an effective identification technique for enterococci species compare to other automated methods which are less efficient in detecting non-*faecalis* and non-*faecium Enterococcus* species(20). This method has also been used for the identification of aerococci to the 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(78).

8.4.5 Nucleic Acid Amplification Tests (NAATs)

Real time PCR-based NAATs are rapid and reliable tests for the identification of *Streptococcus* and *Enterococcus* species. 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(79). Real time PCR have also been developed for the identification of *S. pneumoniae* from culture isolates and serum specimens(6,80).

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*, *E. gallinarum*, and *E. casseliflavus)* (81). In addition, PCR-based methods have also been developed to improve detection and surveillance of vancomycin resistant enterococci (VRE), which are serious nosocomial pathogens linked to high mortality and cost; however, sensitivity and specificity may vary(82).

8.5 Further identification and typing

Following the growth characteristics, colonial morphology, catalase test, Gram stain of the culture, serological results and biochemical identification results, if further identification is required, send isolate to the reference laboratory.

A variety of typing methods have been developed for isolates from clinical samples; these include molecular techniques such as pulsed field gel electrophoresis (PFGE), 16S rRNA gene sequencing, *atpA* gene sequence analysis, and multilocus sequence typing (MLST). All of these approaches enable subtyping of unrelated strains, but do so with different levels of accuracy, discriminatory power and reproducibility.

However, some of these methods remain accessible to reference laboratories only and are difficult to implement for routine bacterial identification in a clinical laboratory.

Pulsed Field Gel Electrophoresis (PFGE)

PFGE was a reproducible, discriminatory and effective epidemiological molecular typing method for identifying and classifying streptococci and enterococci into subtypes(83). PFGE was found to be superior for interpretation of the inter-strain relationships among enterococci but did not result in species-specific discriminative DNA bands(84). Due to its time-consuming nature (30 hr or longer to perform) and its requirement for special equipment, PFGE is not used widely outside the reference laboratories.

Multi-locus sequence typing (MLST)

Multi-locus sequence typing (MLST) is a robust and an unambiguous sequenced based typing procedure for characterising isolates of bacterial species using the sequences of internal fragments of (usually) 7 house-keeping genes(85).

A major advantage of MLST, compared with most typing procedures, is that the sequence data are unambiguous and data exchange can easily be compared between different laboratories via the Internet. Suitable web-based database and tools can be accessed on PubMLST, they contain MLST schemes for many streptococcal and enterococcal species such as *S. pyogenes*(86), *S. pneumoniae*(87) and *E. faecalis* (88).

Whole genome sequencing (WGS)

This technique determines the complete DNA sequence of an organism's genome at a single time. WGS is becoming widely used technique in research, clinical diagnostics and public health laboratories. There are several high-throughput techniques that are available and used to sequence an entire genome such as pyrosequencing, nanopore technology and sequencing by synthesis (SBS) technology.

WGS is a rapid, affordable and accurate genotyping tool that provides information on pathogen detection, identification, epidemiological typing and drug susceptibility. WGS has been used in the investigation of hospital clusters of invasive group B *Streptococcus agalactiae* infection in the UK and Ireland (89). In additions, complete genome sequencing of pathogens such as *S. pyogenes* provide valuable taxonomic and genomic references for infectious disease diagnostics, as well as references for future studies and applications within the genus *Streptococcus*(90). *Enterococcus* species such as *E. faecium* have also been studied using WGS to trace transmission events between patients in hospital wards and between hospitals(91).

Sequencing

Sequencing-based *emm* typing by the use of oligonucleotides that target the N-terminus of the M-protein coding gene is the most practical method of Group A typing because the gene coding for the group A *Streptococcus* M protein contains a

hypervariable region that is subject to many single nucleotide polymorphisms, which serves as the basis for emm typing *S. pyogenes* isolates(92).

***atpA* Gene Sequence Analysis** is used to differentiate all currently known *Enterococcus* species on the basis of their *atpA* sequences and the 16S rRNA gene is very useful for discriminating the main groups of enterococci, for example the *E. avium*, *E. casseliflavus*, *E. cecorum*, *E. faecalis*, and *E. faecium* species groups; but it fails to discriminate closely related species, including the members of *E. faecalis* and *E. faecium* species groups. *Streptococcus* species are not readily identified by sequencing of the16S rRNA gene(84,93).

8.6 Storage and referral

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

9 Reporting

9.1 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*

According to local protocols, the infection specialist should be informed of isolates of β-haemolytic streptococci of Lancefield Group B when:

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

Follow local protocols for reporting to the patients’ clinicians.

9.2 Preliminary identification

Presumptive identification can be made if appropriate growth characteristics, colonial appearance, Gram stain of the culture; catalase and serological results are demonstrated.

9.3 Confirmation of identification

Confirmation of identification and epidemiological typing for *Streptococcus pneumoniae* are undertaken by the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU) PHE Colindale.

Confirmation of identification and epidemiological typing for GAS, GBS, group G streptococci and group C streptococci are undertaken by the Staphylococcus and Streptococcus Reference Section (SSRS) AMRHAI, PHE Colindale.

9.4 Health Protection Team (HPT)

Refer to local agreements in devolved administrations.

9.5 Public Health England

Refer to current guidelines on SGSS reporting(94).

9.6 Infection prevention and control team

The hospital infection control team should be informed of Group A streptococci, glycopeptide resistant *Enterococcus* species and penicillin resistant pneumococci isolated from in-patients in accordance with local protocols. Consideration should be given to informing the relevant infection control staff of such isolates from patients currently in the community (including nursing homes) in accordance with local arrangements, notably if suspecting cross-transmission.

10 Referral to reference laboratories

All GAS, GBS, group G streptococci and group C streptococci from invasive disease should be referred for surveillance. Those from superficial infections which are associated with an infection control or cluster investigation should also be referred. Please inform the reference laboratory of investigation details.

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see the Specialist and reference microbiology: laboratory tests and services page on GOV.UK for user manuals and request forms

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.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

Staphylococcus and Streptococcus Reference Section

Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI)
National Infection Service
Public Health England
61 Colindale Avenue
London
NW9 5EQ

Enterococci

Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI)
Microbiology Services
Public Health England
61 Colindale Avenue
London
NW9 5EQ

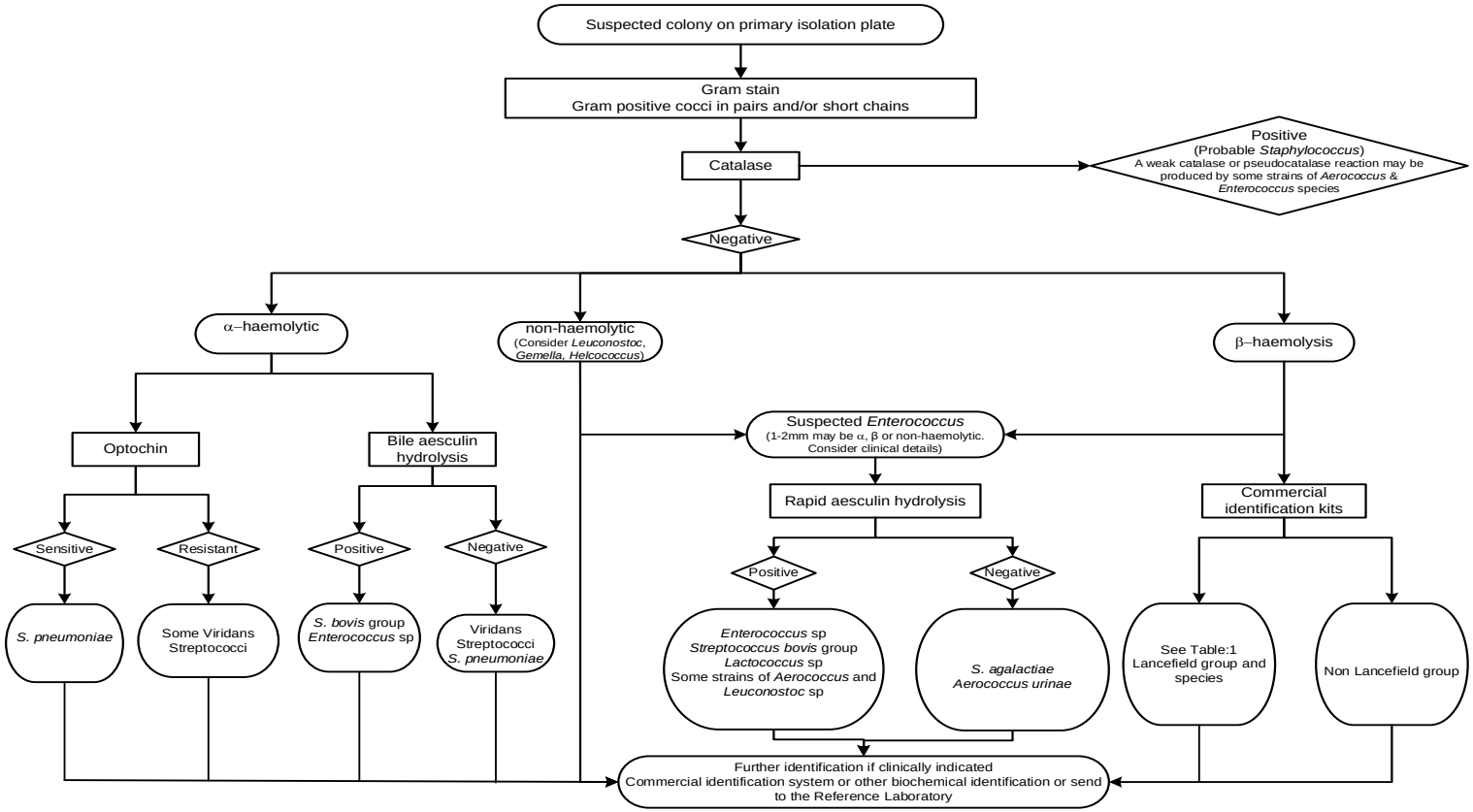
England and Wales

Scotland

Northern Ireland

Appendix 1: Identification of *Streptococcus* species, *Enterococcus* species and morphologically similar organisms

An accessible text description of this flowchart is provided with this document.



The flowchart is for guidance only and for the identification of species in cases where confirmation by an alternative technique is required or automated methods are not available.

1. Some *S. pneumoniae* may be resistant to optochin: if there is a clinical suspicion of pneumococcal infection, confirm by performing bile solubility test.
2. Occasional strains of *S. oralis*, *S. mitis* and *S. pseudopneumoniae* may be optochin sensitive:
   S. pseudopneumoniae optochin resistant when incubated in increased CO_2
3. Some strains of *Aerococcus* and *Leuconostoc* species can hydrolyse aesculin

References

For the information for the evidence grade ratings given, refer to the scientific information section on the UK SMI website.

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. De la Maza LM Color atlas of medical bacteriology. Washington, DC, Hoboken, NJ: ASM Press, Wiley; 2020. **++**

3. Tian Z, Lu S, Jin D, Yang J, Pu J, Lai XH et al. *Streptococcus chenjunshii* sp. nov. isolated from feces of Tibetan antelopes. International journal of systematic and evolutionary microbiology 2019;69:1237-43.**2+** 10.1099/ijsem.0.003303

4. Foster G, Kirchner M, Muchowski J, Duggett N, Randall L, Knight HI et al. *Streptococcus caledonicus* sp. nov., isolated from sheep. International journal of systematic and evolutionary microbiology 2020;70:2611-5.**2+** 10.1099/ijsem.0.004081

5. Facklam R. What happened to the streptococci: overview of taxonomic and nomenclature changes. Clinical microbiology reviews 2002;15:613-30.**+** 10.1128/cmr.15.4.613-630.2002

6. Carroll KC, Pfaller MA Manual of clinical microbiology. Washington, DC: ASM Press; 2019. **++**

7. Delost MD Introduction to Diagnostic Microbiology for the Laboratory Sciences. Burlington, UNITED STATES: Jones & Bartlett Learning, LLC; 2020. **++**

8. Abraham T, Sistla S. Identification of *Streptococcus pyogenes* - Phenotypic Tests vs Molecular Assay (spy1258PCR): A Comparative Study. Journal of clinical and diagnostic research : JCDR 2016;10:Dc01-3.**3+** 10.7860/jcdr/2016/20053.8093

9. Ferretti JJ, Stevens DL, Fischetti VA. *Streptococcus pyogenes*: Basic Biology to Clinical Manifestations Oklahoma City (OK): University of Oklahoma Health Sciences Center; 2016 2016.**+**

10. Facklam RR, Thacker LG, Fox B, Eriquez L. Presumptive identification of streptococci with a new test system. J ClinMicrobiol 1982;15:987-90.**2+**

11. Brandt CM, Haase G, Schnitzler N, Zbinden R, Lütticken R. Characterization of blood culture isolates of *Streptococcus dysgalactiae subsp. equisimilis* possessing Lancefield's group A antigen. Journal of clinical microbiology 1999;37:4194-7.**3+** 10.1128/JCM.37.12.4194-4197.1999

12. Thompson T, Facklam R. Cross-reactions of reagents from streptococcal grouping kits with *Streptococcus porcinus* 1997;35:1885-6.**2+**

13. Shakir SM, Gill R, Salberg J, Slechta ES, Feldman M, Fritsche T et al. Clinical Laboratory Perspective on *Streptococcus halichoeri*, an Unusual Nonhemolytic, Lancefield Group B *Streptococcus* Causing Human Infections. Emerging infectious diseases 2021;27:1309-16.**2+** 10.3201/eid2705.203428

14. Pinho MD, Foster G, Pomba C, Machado MP, Baily JL, Kuiken T et al. *Streptococcus canis* Are a Single Population Infecting Multiple Animal Hosts Despite the Diversity of the Universally

Present M-Like Protein SCM. Frontiers in microbiology 2019;10:631-.**2+** 10.3389/fmicb.2019.00631

15. Matsue M, Ogura K, Sugiyama H, Miyoshi-Akiyama T, Takemori-Sakai Y, Iwata Y et al. Pathogenicity Characterization of Prevalent-Type *Streptococcus dysgalactiae* subsp. *equisimilis* Strains. Frontiers in microbiology 2020;11:97.**2++** 10.3389/fmicb.2020.00097

16. Alves-Barroco C, Roma-Rodrigues C, Raposo LR, Brás C, Diniz M, Caço J et al. *Streptococcus dysgalactiae subsp. dysgalactiae* isolated from milk of the bovine udder as emerging pathogens: In vitro and in vivo infection of human cells and zebrafish as biological models. Microbiologyopen 2019;8:e00623-e.**2+** 10.1002/mbo3.623

17. Jensen A, Hoshino T, Kilian M. Taxonomy of the Anginosus group of the genus *Streptococcus* and description of *Streptococcus anginosus* subsp. whileyi subsp. nov. and *Streptococcus constellatus* subsp. *viborgensis* subsp. nov. IntJSystEvolMicrobiol 2012.**2+** ijs.0.043232-0 [pii];10.1099/ijs.0.043232-0 [doi]

18. Jiang S, Li M, Fu T, Shan F, Jiang L, Shao Z. Clinical Characteristics of Infections Caused by *Streptococcus Anginosus* Group. Scientific Reports 2020;10:9032.**2++** 10.1038/s41598-020-65977-z

19. Růžičková M, Vítězová M, Kushkevych I. The Characterization of *Enterococcus* Genus: Resistance Mechanisms and Inflammatory Bowel Disease. Open Med (Wars) 2020;15:211-24.**2++** 10.1515/med-2020-0032

20. Fang H, Ohlsson AK, Ullberg M, Ozenci V. Evaluation of species-specific PCR, Bruker MS, VITEK MS and the VITEK 2 system for the identification of clinical *Enterococcus* isolates. EurJClinMicrobiolInfectDis 2012;31:3073-7.**2+** 10.1007/s10096-012-1667-x [doi]

21. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. ClinMicrobiolRev 2000;13:686-707.**+**

22. Birch BR, Keaney MG, Ganguli LA. Antibiotic susceptibility and biochemical properties of *Streptococcus faecalis* strains reacting with both D and G antisera. J ClinPathol 1984;37:1289-92.**3+**

23. Schlegel L, Grimont F, Ageron E, Grimont PAD, Bouvet A. Reappraisal of the taxonomy of the *Streptococcus bovis/Streptococcus equinus* complex and related species: description of *Streptococcus gallolyticus* subsp. *gallolyticus* subsp. nov., *S. gallolyticus* subsp. *macedonicus* subsp. nov. and S*. gallolyticus* subsp. *pasteurianus* subsp. nov. International journal of systematic and evolutionary microbiology 2003;53:631-45.**2+** 10.1099/ijs.0.02361-0

24. Rayanakorn A, Goh B-H, Lee L-H, Khan TM, Saokaew S. Risk factors for *Streptococcus suis* infection: A systematic review and meta-analysis. Scientific reports 2018;8:13358-.**2++** 10.1038/s41598-018-31598-w

25. Nomoto R, Maruyama F, Ishida S, Tohya M, Sekizaki T, Osawa R. Reappraisal of the taxonomy of *Streptococcus suis* serotypes 20, 22 and 26: *Streptococcus parasuis* sp. nov. International journal of systematic and evolutionary microbiology 2015;65:438-43.**2+** 10.1099/ijs.0.067116-0

26. Tohya M, Arai S, Tomida J, Watanabe T, Kawamura Y, Katsumi M et al. Defining the taxonomic status of *Streptococcus suis* serotype 33: the proposal for *Streptococcus ruminantium* sp. nov. International journal of systematic and evolutionary microbiology 2017;67:3660-5.**2+** 10.1099/ijsem.0.002204

27. Reller LB, Weinstein MP, Werno AM, Murdoch DR. Laboratory Diagnosis of Invasive Pneumococcal Disease. Clinical Infectious Diseases 2008;46:926-32.**+** 10.1086/528798 %J Clinical Infectious Diseases

28. Harju I, Lange C, Kostrzewa M, Maier T, Rantakokko-Jalava K, Haanperä M. Improved Differentiation of *Streptococcus pneumoniae* and Other S. mitis Group Streptococci by MALDI Biotyper Using an Improved MALDI Biotyper Database Content and a Novel Result Interpretation Algorithm. Journal of clinical microbiology 2017;55:914-22.**2+** 10.1128/JCM.01990-16

29. Doern CD, Burnham C-AD. It's not easy being green: the viridans group streptococci, with a focus on pediatric clinical manifestations. Journal of clinical microbiology 2010;48:3829-35.**+** 10.1128/JCM.01563-10

30. Keith ER, Podmore RG, Anderson TP, Murdoch DR. Characteristics of *Streptococcus pseudopneumoniae* isolated from purulent sputum samples. JClinMicrobiol 2006;44:923-7.**2+** 44/3/923 [pii];10.1128/JCM.44.3.923-927.2006 [doi]

31. Téllez A, Ambrosioni J, Llopis J, Pericàs JM, Falces C, Almela M et al. Epidemiology, Clinical Features, and Outcome of Infective Endocarditis due to *Abiotrophia* Species and *Granulicatella* Species: Report of 76 Cases, 2000-2015. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2018;66:104-11.**1+** 10.1093/cid/cix752

32. Collins MD, Lawson PA. The genus *Abiotrophia* (Kawamura et al.) is not monophyletic: proposal of *Granulicatella* gen. nov., *Granulicatella adiacens* comb. nov., *Granulicatella elegans* comb. nov. and *Granulicatella balaenopterae* comb. nov. Int J Syst EvolMicrobiol 2000;50 Pt 1:365-9.**+**

33. Cargill JS, Scott KS, Gascoyne-Binzi D, Sandoe JA. *Granulicatella* infection: diagnosis and management. J Med Microbiol 2012;61:755-61.**2+** jmm.0.039693-0 [pii];10.1099/jmm.0.039693-0 [doi]

34. Giuliano S, Caccese R, Carfagna P, Vena A, Falcone M, Venditti M. Endocarditis caused by nutritionally variant streptococci: a case report and literature review. InfezMed 2012;20:67-74.**2+**

35. Rasmussen M. *Aerococcus*: an increasingly acknowledged human pathogen. Clinical Microbiology and Infection 2016;22:22-7.**+** https://doi.org/10.1016/j.cmi.2015.09.026

36. M R. Aerococci and aeococcal infections. Journal of Infection 2013:1-8.**+**

37. Parvataneni KC, Iyer S, Khatib R, Saravolatz LD. *Facklamia Species* and *Streptococcus pneumoniae* Meningitis: A Case Report and Review of the Literature. Open Forum Infect Dis 2015;2:ofv029-ofv.**2+** 10.1093/ofid/ofv029

38. Elsayed S, Zhang K. *Gemella bergeriae* endocarditis diagnosed by sequencing of rRNA genes in heart valve tissue. J ClinMicrobiol 2004;42:4897-900.**3+** 42/10/4897 [pii];10.1128/JCM.42.10.4897-4900.2004 [doi]

39. Facklam R, Elliott JA. Identification, classification, and clinical relevance of catalase-negative, gram-positive cocci, excluding the streptococci and enterococci. ClinMicrobiolRev 1995;8:479-95.**+**

40. Miller AO, Buckwalter SP, Henry MW, Wu F, Maloney KF, Abraham BK et al. Globicatella sanguinis Osteomyelitis and Bacteremia: Review of an Emerging Human Pathogen with an Expanding Spectrum of Disease. Open Forum Infect Dis 2017;4:ofw277-ofw.**2+** 10.1093/ofid/ofw277

41. Fall NS, Raoult D, Sokhna C, Lagier JC. '*Helcococcus massiliensis*' sp. nov., a new bacterial species isolated from the vaginal sample of a woman with bacterial vaginosis living in Dielmo, Senegal. New microbes and new infections 2018;25:27-9.**2+** 10.1016/j.nmni.2018.06.002

42. Collins MD, Facklam RR, Rodrigues UM, Ruoff KL. Phylogenetic analysis of some *Aerococcus*-like organisms from clinical sources: description of *Helcococcus kunzii* gen. nov., sp. nov. IntJSystBacteriol 1993;43:425-9.**2+**

43. Chow S-K, Clarridge JE, 3rd. Identification and clinical significance of *Helcococcus* species, with description of *Helcococcus seattlensis* sp. nov. from a patient with urosepsis. Journal of clinical microbiology 2014;52:854-8.**2+** 10.1128/JCM.03076-13

44. Facklam R, Hollis D, Collins MD. Identification of gram-positive coccal and coccobacillary vancomycin-resistant bacteria. JClinMicrobiol 1989;27:724-30.**2+**

45. Altintas I, Andrews V, Larsen MV. First reported human bloodstream infection with Vagococcus lutrae. New microbes and new infections 2020;34:100649.**2+** https://doi.org/10.1016/j.nmni.2020.100649

46. Ikryannikova LN, Filimonova AV, Malakhova MV, Savinova T, Filimonova O, Ilina EN et al. Discrimination between *Streptococcus pneumoniae* and *Streptococcus mitis* based on sorting of their MALDI mass spectra. ClinMicrobiolInfect 2012.**3+** 10.1111/1469-0691.12113 [doi]

47. Slotved HC, Facklam RR, Fuursted K. Assessment of a novel bile solubility test and MALDI-TOF for the differentiation of *Streptococcus pneumoniae* from other mitis group streptococci. Sci Rep 2017;7:7167.**2+** 10.1038/s41598-017-07772-x

48. Sewell DL. Laboratory-associated infections and biosafety. ClinMicrobiolRev 1995;8:389-405.**+**

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

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

51. 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. 1-14. **++**

52. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102.**+**

53. Department for Transport, Maritime and Coastguard Agency, HSENI, Civil Aviation Authority. Transport of infectious substances UN2814, UN2900 and UN3373 Guidance note number 17/2012 (revision 7). 2013. **++**

54. Department of Health. Health Protection Legislation (England) Guidance. 1-112. 2010. **++**

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

56. 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. **++**

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

58. 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. **++**

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

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

61. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books 2003. **++**

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

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

64. 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. 1-37. **++**

65. Public Health England. Laboratory reporting to Public Health England: a guide for diagnostic laboratories. PHE. 2020. 1-31. **++**

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

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

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

69. Tyrrell KL, Citron DM, Leoncio ES, Goldstein EJ. Comparison of the Copan eSwab System with an Agar Swab Transport System for Maintenance of Fastidious Anaerobic Bacterium Viability. J Clin Microbiol 2016;54:1364-7.**2+** 10.1128/JCM.03246-15

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

71. Lancefield RC. A serological differentiation of human and other groups of hemolytic streptococci. JExpMed 1933;57:571-95.**2+**

72. Fuller AT. The formamide method for the extraction of polysaccharides from haemolytic streptococci. BrJExpPathol 1938:130-9.**3+**

73. Maxted WR. Preparation of streptococcal extracts for Lancefield grouping. Lancet 1948;2:255.**3+**

74. Pritt BS, Patel R, Kirn TJ, Thomson RB, Jr. Point-Counterpoint: A Nucleic Acid Amplification Test for *Streptococcus pyogenes* Should Replace Antigen Detection and Culture for Detection of Bacterial Pharyngitis. Journal of clinical microbiology 2016;54:2413-9.**2+** 10.1128/JCM.01472-16

75. Oviaño M, Bou G. Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for the Rapid Detection of Antimicrobial Resistance Mechanisms and Beyond. Clinical microbiology reviews 2018;32:e00037-18.**+** 10.1128/CMR.00037-18

76. Nybakken EJ, Oppegaard O, Gilhuus M, Jensen CS, Mylvaganam H. Identification of *Streptococcus dysgalactiae* using matrix-assisted laser desorption/ionization-time of flight mass spectrometry; refining the database for improved identification. Diagnostic Microbiology and Infectious Disease 2021;99:115207.**2+** https://doi.org/10.1016/j.diagmicrobio.2020.115207

77. Schulthess B, Brodner K, Bloemberg GV, Zbinden R, Böttger EC, Hombach M. Identification of Gram-positive cocci by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry: comparison of different preparation methods and implementation of a practical algorithm for routine diagnostics. Journal of clinical microbiology 2013;51:1834-40.**2+** 10.1128/JCM.02654-12

78. Senneby E, Nilson B, Petersson AC, Rasmussen M. Matrix-assisted laser desorption ionization-time of flight mass spectrometry is a sensitive and specific method for identification of aerococci. JClinMicrobiol 2013;51:1303-4.**2+** JCM.02637-12 [pii];10.1128/JCM.02637-12 [doi]

79. Shin JH, Pride DT. Comparison of Three Nucleic Acid Amplification Tests and Culture for Detection of Group B *Streptococcus* from Enrichment Broth. Journal of clinical microbiology 2019;57:e01958-18.**2+** 10.1128/JCM.01958-18

80. Ganaie FA, Govindan V, Ravi Kumar KL. Standardisation and evaluation of a quantitative multiplex real-time PCR assay for the rapid identification of *Streptococcus pneumoniae*. Pneumonia 2015;6:57-66.**2+** 10.15172/pneu.2015.6/559

81. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. JClinMicrobiol 1995;33:1434.**2+**

82. Seo JY, Kim PW, Lee JH, Song JH, Peck KR, Chung DR et al. Evaluation of PCR-based screening for vancomycin-resistant enterococci compared with a chromogenic agar-based culture method. Journal of medical microbiology 2011;60:945-9.**2+** 10.1099/jmm.0.029777-0

83. Patterson J, Kelly C. Pulsed-field gel electrophoresis as an epidemiological tool for enterococci and streptococci. Methods in cell science 1998;20:233-9.**2+**

84. Naser S, Thompson FL, Hoste B, Gevers D, Vandemeulebroecke K, Cleenwerck I et al. Phylogeny and identification of Enterococci by atpA gene sequence analysis. Journal of Clinial Microbiology 2005;43:2224-30.**2+** 10.1128/jcm.43.5.2224-2230.2005

85. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res 2018;3:124-.**2++** 10.12688/wellcomeopenres.14826.1

86. Enright MC, Spratt BG, Kalia A, Cross JH, Bessen DE. Multilocus sequence typing of *Streptococcus pyogenes* and the relationships between emm type and clone. InfectImmun 2001;69:2416-27.**2+** 10.1128/IAI.69.4.2416-2427.2001 [doi]

87. Enright MC, Spratt BG. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. Microbiology 1998;144 (Pt 11):3049-60.**3+**

88. Ruiz-Garbajosa P, Bonten MJM, Robinson DA, Top J, Nallapareddy SR, Torres C et al. Multilocus sequence typing scheme for *Enterococcus faecalis* reveals hospital-adapted genetic

complexes in a background of high rates of recombination. Journal of clinical microbiology 2006;44:2220-8.**2+** 10.1128/JCM.02596-05

89. Collin SM, Groves N, O'Sullivan C, Jauneikaite E, Patel D, Cunney R et al. Uncovering Infant Group B Streptococcal (GBS) Disease Clusters in the United Kingdom and Ireland Through Genomic Analysis: A Population-based Epidemiological Study. Clinical Infectious Diseases 2021;72:e296-e302.**2++** 10.1093/cid/ciaa1087

90. Salvà-Serra F, Jaén-Luchoro D, Jakobsson HE, Gonzales-Siles L, Karlsson R, Busquets A et al. Complete genome sequences of *Streptococcus pyogenes* type strain reveal 100%-match between PacBio-solo and Illumina-Oxford Nanopore hybrid assemblies. Scientific Reports 2020;10:11656.**2+** 10.1038/s41598-020-68249-y

91. Bayjanov JR, Baan J, Rogers MRC, Troelstra A, Willems RJL, van Schaik W. *Enterococcus faecium* genome dynamics during long-term asymptomatic patient gut colonization. Microbial Genomics 2019;5.**2+** https://doi.org/10.1099/mgen.0.000277

92. Beall B, Facklam R, Thompson T. Sequencing emm-specific PCR products for routine and accurate typing of group A streptococci. JClinMicrobiol 1996;34:953-8.**2+**

93. Srinivasan R, Karaoz U, Volegova M, MacKichan J, Kato-Maeda M, Miller S et al. Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. PLoS One 2015;10:e0117617.**2+** 10.1371/journal.pone.0117617

94. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories. Public Health England 2016. 1-29. **++**