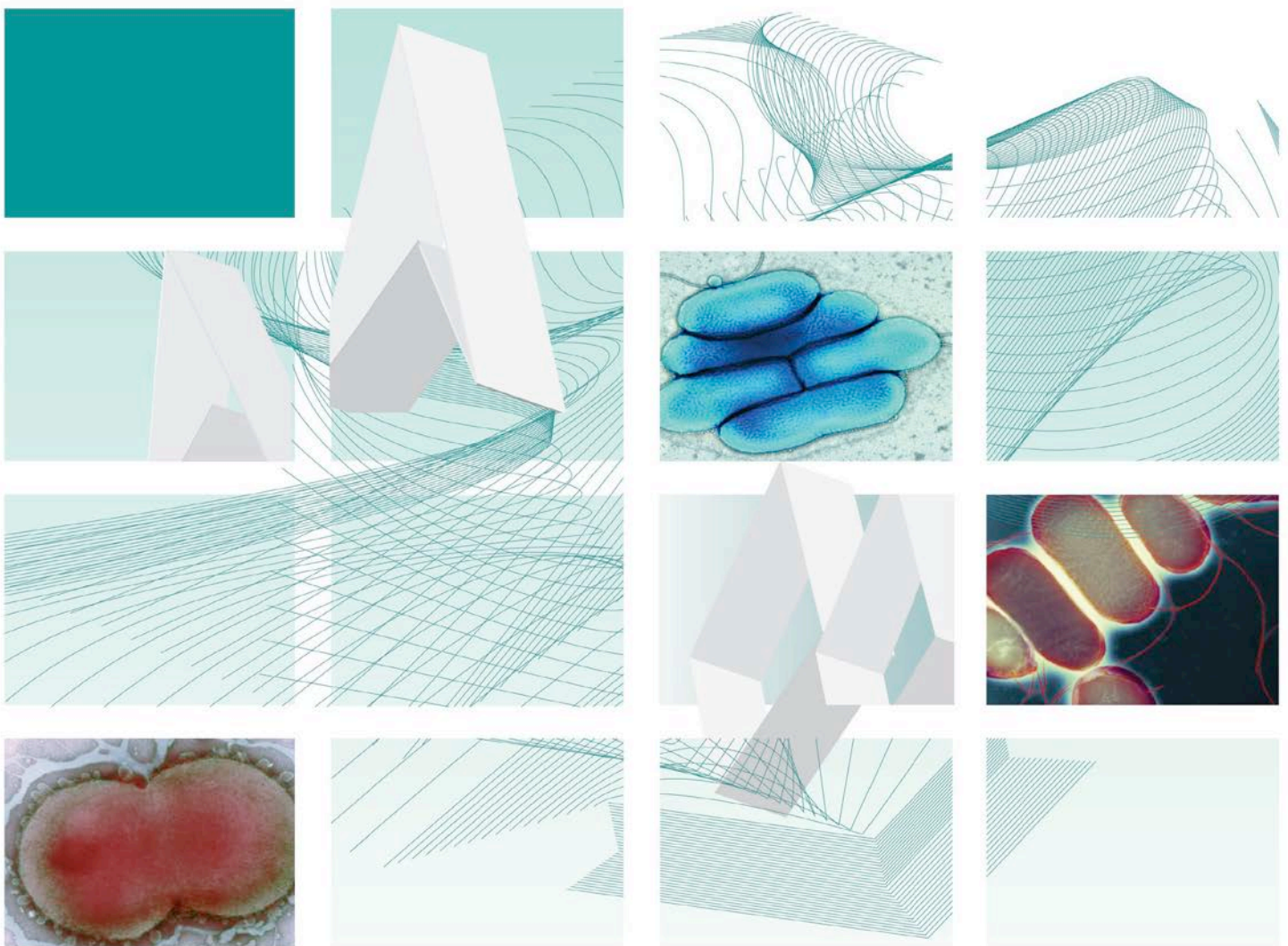




UK Standards for Microbiology Investigations

Identification of *Streptococcus* species, *Enterococcus* species and Morphologically Similar Organisms



Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (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 website <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

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

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Amendment Table

Each 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 No/Date.	10/28.10.14
Issue no. discarded.	2.3
Insert Issue no.	3
Section(s) involved	Amendment
Scope of document.	The scope has been updated with the addition of molecular methods as a means of identification of <i>Streptococcus</i> and <i>Enterococcus</i> species isolated from clinical material.
Introduction.	The taxonomy of <i>Streptococcus</i> and <i>Enterococcus</i> has been updated. More information has been added to the Characteristics section. The medically important species are mentioned. Other morphologically similar organisms that are medically important are also mentioned and their characteristics described. Section on Principles of Identification has been rearranged.
Technical Information/Limitations.	Addition of information regarding catalase test, commercial identification systems and differentiation between <i>Streptococcus</i> groups using rapid methods.
Safety considerations.	This section has been updated regarding laboratory workers.
Target Organisms.	The section on the Target organisms has been updated and presented clearly.
Identification.	Updates have been done on 3.2, 3.3 and 3.4 to reflect standards in practice. It also includes all the morphologically similar organisms apart from <i>Enterococcus</i> and <i>Streptococcus</i> species. The table in 3.4 and the footnote has been updated with references. Subsection 3.5 has been updated to include the

Identification of *Streptococcus* species, *Enterococcus* species and Morphologically Similar Organisms

	Rapid Molecular Methods.
Identification Flowchart.	Modification of flowchart for identification of <i>Enterococcus</i> and <i>Streptococcus</i> species has been done for easy guidance.
Reporting.	Subsections 5.1 has been updated to reflect reporting practice.
Referral.	The addresses of the reference laboratories have been updated.
Whole document.	Document presented in a new format.
References.	Some references updated.

UK Standards for Microbiology Investigations[#]: Scope and Purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests.
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>.

Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

[#]Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

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The development of SMIs are subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>. The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

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The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested Citation for this Document

Public Health England. (2014). Identification of *Streptococcus* species, *Enterococcus* species and Morphologically Similar Organisms. UK Standards for Microbiology Investigations. ID 4 Issue 3. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

Scope of Document

This SMI 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 SMI adopts a simplified approach based on grouping organisms with similar phenotypic attributes¹. Further identification may be necessary where clinically or epidemiologically indicated.

This SMI should be used in conjunction with other SMIs.

Introduction

Taxonomy

In recent years, the taxonomy of streptococci and related organisms has been strikingly resistant to satisfactory classification and has undergone extensive revision, largely following the introduction of molecular identification methods. There are also some differences in opinion on the nomenclature of some of the streptococci between identification systems in the UK and USA. There are currently 99 recognised species of *Streptococcus*, many of which are associated with disease in humans and animals².

The genus name *Enterococcus*, originally suggested in 1903 for bacteria previously called *Streptococcus faecalis* and *Streptococcus faecium*, was revived in 1984 when other bacteria were transferred to the genus^{1,3}. There are currently 48 members of the genus *Enterococcus* which are published. *Enterococcus faecalis* and *Enterococcus faecium* are the commonest enterococci isolated from human infections⁴.

Characteristics

Streptococci are Gram positive cocci (spherical or ovoid) often occurring in pairs and chains. Streptococci are facultatively anaerobic and catalase negative¹. Carbohydrates are metabolised fermentatively; lactic acid is the major metabolite. Streptococci produce the enzyme leucine aminopeptidase (LAP), which has also been called leucine arylamidase.

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.

Haemolysis on blood agar:

- α -haemolysis - partial lysis of the red blood cells surrounding a colony causing a greenish discolouration of the medium
- β -haemolysis - complete lysis of the red blood cells surrounding a colony causing a clearing of the blood from the medium

- non-haemolytic or (previously called γ -haemolysis) - no colour change or clearing of the medium
- α -prime (α) or “wide zone” α - haemolysis - a small zone of intact red blood cells are seen adjacent to the colony with a zone of complete haemolysis surrounding the zone of intact red blood cells. This type of haemolysis can be confused with β -haemolysis

Lancefield grouping:

Beta-hemolytic streptococci are further characterised via Lancefield serotyping, which describes specific carbohydrates present on the bacterial cell wall⁵. There are 20 described serotypes, named Lancefield groups A to V (excluding I and J).

Lancefield group A

Streptococcus pyogenes

Streptococcus pyogenes occurs in chains. After 18-24hr incubation at 35-37°C on blood agar colonies are approximately 0.5mm, domed, with an entire edge. Some strains may produce mucoid colonies. Haemolysis is best observed by growing the culture under anaerobic conditions because the haemolysins are more stable in the absence of oxygen⁶.

Lancefield group A streptococci will not grow on media containing bile. Pinpoint colony forms of the *S. anginosus* group may cross react with Lancefield group A antibodies and may grow on media containing bile⁷.

Bacitracin susceptibility has been used presumptively for screening purposes but is unreliable because it is not highly specific and methods vary between laboratories⁸⁻¹².

Resistance to benzylpenicillin has not, at the time of writing, been reported.

The pyrrolidonyl aminopeptidase (which has also been called the pyrrolidonyl arylamidase or PYR) test is positive for Group A streptococci and negative for most other groupable streptococci, although some human strains of groups C and G may be positive. Enterococci are also PYR positive^{7,9}.

Lancefield group B

Streptococcus agalactiae

Streptococcus agalactiae occurs in chains. After 18-24hr incubation at 35-37°C colonies tend to be slightly larger than other streptococci (approximately 1mm) and have a less distinct zone of β -haemolysis. Some strains may be non-haemolytic¹³.

Lancefield group B streptococci will grow on media containing bile.

Islam's medium, to detect orange pigment production, may be useful for primary isolation and presumptive identification, but is not recommended in this SMI¹³.

Lancefield groups A, C, G and L

***Streptococcus dysgalactiae* subspecies *equisimilis* (Lancefield groups A, C, G and L)⁵**

***Streptococcus equi* subspecies *zooepidemicus*¹ (Lancefield group C)**

***Streptococcus canis* (Lancefield group G streptococci)¹⁴**

Microscopically these species are Gram positive cocci, occurring in chains. Large colony forms of Lancefield groups C and G streptococci (≥ 0.5 mm) produce similar colonies to Group A streptococci¹⁵. Group C and G strains of *S. dysgalactiae* subspecies *equisimilis* are identified much more commonly in human infections than those strains which possess Group A (or L) antigens⁵.

Lancefield groups C and G streptococci will not grow on media containing bile. Pinpoint colony forms of the *S. anginosus* group can cross react with the Lancefield groups C and G antibodies and may grow on media containing bile⁹.

Lancefield group A, C, F or G

***Streptococcus anginosus* group:**

***Streptococcus anginosus*, *Streptococcus anginosus* subspecies *whileyi*, *Streptococcus constellatus* subspecies *constellatus*, *Streptococcus constellatus* subspecies *pharyngis*, *Streptococcus constellatus* subspecies *viborgensis*, *Streptococcus intermedius* (formerly the “*Streptococcus milleri*” group)¹⁶**

Microscopically these species are Gram positive cocci, occurring in chains. Colonies on blood agar are small (≤ 0.5 mm) and may exhibit α , β or no haemolysis after 16-24hr at 35-37°C. Incubation conditions may be of some value for the presumptive identification of the *S. anginosus* group as growth is enhanced by a low oxygen tension and raised CO₂ levels¹⁷.

Organisms of this group may possess the Lancefield group A, C, F or G antigen or be ungroupable¹⁷. *S. intermedius* possesses no group antigen. *S. constellatus* may express group C, or F and *S. anginosus* may express group A, C, F or G antigens. Human isolates of streptococci which express the group F antigen are highly likely to be members of the *anginosus* group. Streptococci in this group will grow on media containing bile although they are not salt tolerant.

Resistance to sulphonamides and bacitracin may be used as screening tests for organisms of the *S. anginosus* group¹⁸.

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.

Lancefield group D

***Enterococcus* species, *Streptococcus bovis* group**

The genus *Enterococcus* and organisms of the *S. bovis* group possess Lancefield group D antigen. Lancefield group D streptococci will grow on media containing bile and may be differentiated from other streptococci by rapid hydrolysis of aesculin in the presence of 40% bile.

Microscopically the enterococci are Gram positive cocci, spherical or ovoid in shape (0.6-2.5µm), usually occurring in pairs or short chains in broth culture. After 18-24hr incubation at 35-37°C on blood agar colonies are 1 - 2mm and may be α, β or non-haemolytic on horse blood agar. Most species will grow on nutrient agar at 45°C. A few will grow at 50°C, at pH 9.6 and in 6.5% NaCl. They can also survive at 60°C for 30min and are PYR positive which differentiates them from *S. bovis* and *S. gallolyticus*.

Enterococci are facultative anaerobes. Two species within the genus, *Enterococcus cassiflavus* and *Enterococcus gallinarum*, are motile. Enterococci are oxidase negative and ferment carbohydrates. Most species are catalase negative, but some strains produce a pseudocatalase.

Most enterococci possess the group D antigen although some strains can cross react with Lancefield group D and G antiserum¹⁹.

E. faecalis are very rarely resistant to ampicillin¹⁸. However, vancomycin or glycopeptide resistant enterococci (V/GRE) are becoming increasingly common and this spread of resistance is thought to be due to transposons and plasmids moving between bacterial species²⁰. The resistance of vancomycin in enterococci is mediated by *van* genes that encode enzymes for the synthesis of low-affinity precursors that modify the vancomycin-binding target. Currently, there are eight known vancomycin resistant phenotypes: *vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG*, *vanL*, and *vanM*. There are 2 types of glycopeptide resistance in enterococci, intrinsic and acquired. The strains with acquired resistance are the only ones aimed to control and reported in VRE surveillance programmes.

Acquired resistance is primarily found in *Enterococcus faecium* and *Enterococcus faecalis* and is typically encoded by the *vanA* and *vanB* genes. Strains that harbour the *vanA* gene display high levels of resistance to vancomycin and teicoplanin, whereas strains that harbour the *vanB* gene have variable levels of resistance to vancomycin only²¹.

In the *S. bovis* group, there are six species and they include: *S. bovis*, *S. equinus*, *S. gallolyticus* (formerly *S. bovis* biotype I), *S. infantarius* (formerly *S. bovis* biotype II/1), *S. pasteurianus* (formerly *S. bovis* biotype II/2) and *S. lutetiensis*⁵.

Microscopically these species are Gram positive cocci, occurring in chains. After 18-24hr incubation at 35°C-37°C in CO₂ or anaerobically, colonies are usually non-haemolytic on blood agar and 1-2mm in diameter. Members of the *S. bovis* group may be misidentified as enterococci because many strains share the group D antigen. It is important to identify *S. bovis* group organisms from clinical material especially in cases of bacteraemia, because *S. gallolyticus* and *S. pasteurianus* are associated with chronic bowel disease, particularly adenocarcinoma of the colon²². 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.

Streptococcus suis

S. suis is β-haemolytic on horse blood agar, optochin resistant and PYR negative. They are commonly associated with the Lancefield groups R, S and T. *S. suis* I is associated with group S and *S. suis* II with group R. They do not grow in 6.5% NaCl broth. Some strains are able to grow in the presence of 40% bile and all are able to hydrolyse aesculin.

Non-Lancefield groups

Streptococcus pneumoniae

Streptococcus pneumoniae ("pneumococci") are typically lanceolate cells occurring in pairs, which may be capsulate. Colonies are 1-2mm, α -haemolytic and may appear as 'draughtsman' colonies due to autolysis of the organisms after incubation in 5-10% CO₂ at 35-37°C for 16-24hr. Under anaerobic conditions colonies may appear larger and more mucoid.

S. pneumoniae are usually sensitive to optochin (ethylhydrocupreine hydrochloride), which enables rapid identification of the organism, but 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 microscopically to identify the specific types of *S. pneumoniae*^{17,18}. Commercial agglutination tests are also available for the rapid detection of pneumococcal antigens, but these should be used with caution because cross-reactions may occur with the *S. oralis* and *S. mitis* groups.

Viridans streptococci

"Viridans" is derived from the Latin word *viridis*, meaning green. These species are Gram positive cocci occurring in chains, which are indistinguishable by Gram stain from β -haemolytic streptococci. Colonies are 0.5-1.0mm and may be α or non-haemolytic on blood agar after anaerobic incubation at 35-37°C in CO₂ for 16-24hr. They possess no Lancefield antigens and are resistant to optochin. They are also not soluble in bile.

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 when incubated in 5% carbon dioxide
- Commercial DNA probe hybridization tests are falsely positive²³

Generally these streptococci would not require further identification, other than as an α or non-haemolytic streptococci, when isolated from sites where they are considered normal flora. 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, eg *Streptococcus sanguinis* and *Streptococcus oralis* (formerly *mitior*), may account for up to 80% of all streptococcal endocarditis cases²⁴.

Nutritionally Variant Streptococci (NVS)

NVS have now been reclassified as *Granulicatella adiacens*, *Granulicatella elegans* and *Abiotrophia defectiva*²⁵. NVS require media supplemented with either pyridoxal or cysteine for growth^{26,27}.

NVS colonies are small, 0.2-0.5mm in diameter and colonies at the outer edge of the zone becomes enlarged after 24hr because of the nutrients in the surrounding medium. They can be either non-haemolytic or α -haemolytic. NVS are catalase

negative, oxidase negative and facultatively anaerobic. NVS should be suspected when Gram positive cocci resembling streptococci are seen in positive blood cultures, which subsequently fail to grow on subculture. Repeat subculture of suspect broth should include a blood agar plate with a *Staphylococcus aureus* streak which is examined for satellitism of NVS around the staphylococcus. Alternatively, media may be supplemented with 10mg/L pyridoxal hydrochloride.

Recognition of these species is important for deep seated infections (notably endocarditis) to ensure the most appropriate antimicrobial therapy and they are often associated with negative blood cultures²⁷⁻²⁹.

Unusual *Streptococcus* species

Streptococcus acidominimus

Streptococcus acidominimus belongs to the *Streptococcus viridans* group and microscopically, it occurs in short chains. They are α -haemolytic and catalase negative. They do not hydrolyse aesculin or arginine but ferments sucrose and glucose. They possess no Lancefield antigens and are bile insoluble and optochin resistant.

A few cases of deep-seated human infections by *S. acidominimus* have been reported^{30,31}. They are generally quite sensitive to β -lactam antibiotics.

Genera closely related to streptococci

***Aerococcus* species**

There are seven species of *Aerococcus*, of which five are pathogenic and cause both urinary tract and invasive infections (including Infective Endocarditis) in humans³²⁻³⁴. They are *Aerococcus christensenii*, *Aerococcus sanguinicola*, *Aerococcus urinae*, *Aerococcus urinaehominis* and *Aerococcus viridans*.

Aerococci resemble “viridans” streptococci on culture but differ microscopically by characteristically occurring as pairs, tetrads or clusters, similar to staphylococci. Sometimes a weak catalase or pseudocatalase reaction is produced. These relatively slow-growing organisms produce small, well-delineated, translucent, alpha-haemolytic colonies on blood agar. Some strains of *Aerococcus viridans* are bile aesculin positive and PYR positive. *Aerococcus urinae* is bile aesculin negative and PYR negative. Growth occurs both under aerobic and anaerobic conditions.

In some commercial identification systems, *Helcococcus kunzii* may be mis-identified as *A. viridans*. Both the API and Vitek also misidentify *A. sanguinicola* as *A. viridans*. This makes the reports of infections caused by *A. viridans* problematic when identification is based on these methods³⁵.

Most aerococci are sensitive to beta-lactams as well as to several other groups of antibiotics. *Aerococcus* species are sensitive to vancomycin although elevated MICs have been reported³⁵.

***Facklamia* species**

There are six species of which four are from humans (*Facklamia hominis*, *Facklamia languida*, *Facklamia sourekii* and *Facklamia ignava*). The most common human species is *Facklamia hominis*. *Facklamia* species resemble “viridans” streptococci on culture. They are Gram positive occurring as pairs, groups or chains. The *Facklamia* species are facultatively anaerobic and grow best in an atmosphere of increased

carbon dioxide. They are weakly α -haemolytic and usually hydrolyse urea and not aesculin³⁶. They are catalase and oxidase negative but positive for pyrrolidonyl arylamidase and leucine aminopeptidase. 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 them³⁷. Acid is not produced from glucose and other sugars and nitrate is not reduced³⁸.

All *Facklamia* species are sensitive to amoxicillin and some species strains were resistant to cefotaxime and cefuroxime³⁶.

***Gemella* species**

There are currently five species isolated from human sources that are recognised: *Gemella haemolysans*, *Gemella morbillorum* (formerly *Streptococcus morbillorum*), *Gemella bergeriae*, *Gemella sanguinis* and *Gemella asaccharolytica* species nov³⁹⁻⁴².

Gemella species are catalase negative, facultatively anaerobic, Gram variable cocci, arranged in pairs, tetrads, clusters and sometimes short chains. Some strains easily decolourise on Gram staining, occurring as Gram negative. In addition, some strains may require strictly anaerobic conditions for primary isolation and become aerotolerant after transfer to laboratory media⁴³. They are either α -haemolytic or non-haemolytic on blood agar and resemble colonies of viridans streptococci. Colonies are small and greyish to colourless.

In some commercial identification systems, “viridans” streptococci can be mis-identified as *Gemella* species. The only difference between the viridans” streptococci and the *Gemella* species is the cellular arrangement and the requirement by the viridans” streptococci for pyridoxal for growth⁴³.

***Globicatella* species**

There are two species of *Globicatella* but the species that is implicated in human infections is *Globicatella sanguinis*⁴⁴⁻⁴⁶.

Globicatella species form small viridans streptococcus - like colonies on blood agar plate and produce a weak α -haemolytic reaction. Microscopically, they are Gram positive cocci occurring singly, in pairs or short chains. They are facultatively anaerobic and catalase negative. However, they do not produce leucine aminopeptidase⁴⁴. They are susceptible to vancomycin.

Globicatella species can be distinguished from aerococci by cellular morphology. Aerococci form pairs and tetrads while *Globicatella* species form short chains of cocci.

***Helcococcus* species**

There are currently three species of *Helcococcus* species isolated from humans. They are *Helcococcus kunzii*, *Helcococcus pyogenes* and *Helcococcus sueciensis*⁴⁷⁻⁴⁹.

Helcococcus species are Gram positive cocci that are catalase negative and facultatively anaerobic. They are arranged in pairs, tetrads and clusters. They are slow growing and appear like viridans streptococci on blood agar plate. They are usually non-haemolytic which differentiates them from aerococci which form large 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 mis-identified as *Helcococcus kunzii*⁴³.

H. pyogenes produces pinpoint greyish white non-haemolytic colonies and does not hydrolyse aesculin. This demonstrates relative vancomycin resistance like *Pediococcus* species⁴⁸.

H. sueciensis produces pinpoint grey, non-haemolytic colonies after 48hr anaerobic incubation. This species was unidentified using commercial API biochemical kits⁴⁹.

Lactococcus species

There are seven species of the *Lactococcus* currently recognised. *Lactococcus* species are physiologically similar to Enterococci and they have been misidentified because they show many of the characteristics of both streptococci and enterococci. They are facultatively anaerobic, α or non-haemolytic, Gram positive cocci which occur singly, in pairs or chains. They are bile aesculin positive, but do not possess group D antigen⁴³.

Leuconostoc species

The genus *Leuconostoc* consists of the following species (including re-classified and synonymous species): *Leuconostoc mesenteroides* (type species), *L. amelibiosum*, *L. argentinum*, *L. carnosum*, *L. citreum*, *L. cremoris*, *L. dextranicum*, *L. durionis*, *L. fallax*, *L. ficulneum*, *L. fructosum*, *L. garlicum*, *L. gasicomitatum*, *L. gelidum*, *L. holzapfelii*, *L. inhae*, *L. kimchii*, *L. lactis*, *L. miyukkimchii*, *L. oeni*, *L. palmae*, *L. paramesenteroides*, *L. pseudoficulneum* and *L. pseudomesenteroides*. *Leuconostoc mesenteroides* has been implicated in human infections^{50,51}.

Of these species, four of them (*L. ficulneum*, *L. fructosum*, *L. durionis* and *L. pseudoficulneum*) have been re-classified and transferred as belonging to the genus *Fructobacillus*. They are now *Fructobacillus ficulneum*, *Fructobacillus fructosum*, *Fructobacillus durionis* and *Fructobacillus pseudoficulneum* respectively. These are non-pathogenic species and they prefer fructose but not glucose as growth substrate. They are found in fructose-rich niches such as flowers, fruits, and fermented foods and more recently in the gastrointestinal tracts of animals consuming fructose⁵².

Leuconostoc mesenteroides has been divided into 4 subspecies; 2 of which were reclassified (*L. cremoris* and *L. dextranicum* have been renamed as *Leuconostoc mesenteroides* subspecies *cremoris* and *Leuconostoc mesenteroides* subspecies *dextranicum* respectively), *Leuconostoc mesenteroides* subspecies *mesenteroides* and then a more recent addition, *Leuconostoc mesenteroides* subspecies *suionicum*⁵³.

Leuconostoc species are Gram positive lenticular cocci occurring in pairs and chains and are characteristically vancomycin resistant and produce CO₂ from glucose. They are catalase negative and colonies often are α -haemolytic on blood agar. They are facultatively anaerobic and may be confused with the enterococci because most *Leuconostoc* species are bile aesculin positive and some cross-react with the group D antisera⁴³.

***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 beta-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. They are leucine aminopeptidase-positive, which distinguishes them from *Leuconostoc* species⁵⁴. They may be confused with enterococci because they are bile aesculin positive and cross-react with the Group D antisera⁴³.

Principles of Identification

Isolates from primary culture are identified by colonial appearance, Gram stain, catalase test, Lancefield grouping and optochin sensitivity. Further identification may be possible by use of biochemical or other tests to distinguish among species.

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

If Lancefield grouping does not provide sufficient identification for clinical management, a full identification may be obtained using a commercial identification system, 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 (charged for) taxonomic identification service for streptococci and other related Gram positive, catalase negative genera is available.

Technical Information/Limitations

Commercial Identification Systems

At the time of writing, 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*⁵⁵.

Another group that are difficult to differentiate are species belonging to the *S. mitis* and *S. sanguinis* groups which are often regarded as a single group, they give discordant results due to the low quality of the identification system used.

MALDI-TOF MS

One limitation of MALDI-TOF MS is that it cannot readily distinguish between *Streptococcus pneumoniae* from other members of the *Streptococcus mitis* group. Despite this limitation, subtyping of *Streptococcus pneumoniae* strains by MALDI-TOF MS can be reliably performed, even for immunologically non-typeable or non-encapsulated strains⁵⁶.

Catalase test

Sometimes a weak catalase or pseudocatalase reaction is produced by *Aerococcus* and *Enterococcus* species.

1 Safety Considerations⁵⁷⁻⁷³

Hazard Group 2 organisms.

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

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

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 requesting for identification of these microorganisms and, in some situations, be restricted to a low-risk laboratory⁷⁴.

Laboratory acquired infections have been reported⁷⁵.

The above guidance should be supplemented with local COSHH and task specific risk assessments.

Compliance with postal and transport packaging regulations is essential.

2 Target Organisms

Streptococcus species Reported to have Caused Human Infection^{43,76}

Streptococci possessing Lancefield group antigens A-G¹

Group A

Streptococcus pyogenes (*Streptococcus anginosus* and *Streptococcus constellatus* subspecies *constellatus* may cross react with the Lancefield group A antigen).

Group B

Streptococcus agalactiae

Group C

Streptococcus dysgalactiae subspecies *equisimilis*, *Streptococcus equi* subspecies *equi*, *Streptococcus equi* subspecies *zooepidemicus* (*Streptococcus anginosus* and *Streptococcus constellatus* subspecies *pharyngis* may cross react with the Lancefield group C antigen).

Group D^{3,15,19}

Enterococcus species (see below)

Streptococcus bovis group

Taxonomy of the *S.bovis* group is still an unsolved problem¹⁴

Streptococcus bovis, *Streptococcus gallolyticus*⁷⁷ (*Streptococcus gallolyticus* subsp *gallolyticus* (*S. bovis* biotype I), *Streptococcus gallolyticus* subsp *pasteurianus*

(*S. bovis* biotype II/2), *Streptococcus gallolyticus* subsp *macedonicus*), *Streptococcus equinus*, *Streptococcus infantarius* (formerly *S. bovis* biotype II/1), *Streptococcus pasteurianus*, *Streptococcus lutetiensis*

Group F

Streptococcus anginosus, *Streptococcus constellatus* subspecies *constellatus*

Group G

Group G streptococci (*Streptococcus anginosus* and *Streptococcus constellatus* subspecies *constellatus* may cross react with the Lancefield group G antigen).

The “viridans” streptococci

These are divided into 5 subgroups⁵. They are as follows:

***Streptococcus anginosus* group** (also known as the *S. milleri* group)^{16,18}

Streptococcus anginosus, *Streptococcus anginosus* subspecies *whileyi*, *Streptococcus constellatus* subspecies *constellatus*, *Streptococcus constellatus* subspecies *pharyngis*, *Streptococcus constellatus* subspecies *viborgensis*, *Streptococcus intermedius*

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

***Streptococcus mitis* group**² - *Streptococcus mitis*⁷⁸, *Streptococcus oralis*, *Streptococcus sanguinis*, *Streptococcus gordonii*, *Streptococcus parasanguinis*, *Streptococcus cristatus*, *Streptococcus massiliensis*⁷⁹, *Streptococcus pneumoniae*^{*14}, *Streptococcus pseudopneumoniae*²³, *Streptococcus peroris*^{14,80}, *Streptococcus oligofermentans*¹⁴, *Streptococcus australis*¹⁴, *Streptococcus infantis*^{14,80}, *Streptococcus sinensis*⁸¹

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

Other streptococci (with uncertain grouping or unknown genetic relationship) - *Streptococcus suis*, *Streptococcus acidominimus*⁸²

Nutritionally variant streptococci^{27,29,83} - *Granulicatella adjacens*, *Granulicatella elegans*, *Abiotrophia defectiva*.

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

Enterococcus species Reported to have Caused Human Infections

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

Other genera Reported to have Caused Human Infections

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

3 Identification

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

3.2 Primary Isolation Media

Blood agar incubated in 5-10% CO₂ at 35–37°C for 16–24hr, or anaerobically at 35–37°C for 16-24hr for throat swabs ([B 9 - Investigation of Throat Swabs](#))

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

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

Fastidious anaerobe agar incubated anaerobically for 16-48hr.

3.3 Colonial Appearance

Organism “group”	Haemolysis	Characteristics of growth on blood agar after incubation at 35-37°C for 16–24hr
β-haemolytic streptococci	β	Approximately 0.5mm, entire edged, may have a dry appearance, colonies may be difficult to pick off the plate.
“viridans” streptococci	α or non	Colonies are 0.5-1.0mm, entire edged.
Enterococci	α,β or non	Colonies are larger than those of streptococci, usually 1–2mm, with a wet appearance. Haemolysis is variable.
<i>S. pneumoniae</i>	α	Colonies are 1 – 2mm and may appear as “draughtsman” colonies. After anaerobic incubation colonies may be larger and mucoid.
“ <i>S. anginosus</i> ”	α,β or non	Colonies are small (≤0.5mm), haemolysis is variable. Some strains have a white “heaped” up colony
NVS	α or non	Colonies are small (≤0.5mm), require pyridoxal or cysteine for growth.
<i>Aerococcus</i> species	α	Resemble “viridans” streptococci.
<i>Facklamia</i> species	α or non	Resemble “viridans” streptococci
<i>Gemella</i> species	α or non	Resemble “viridans” streptococci

<i>Globicatella</i> species	α	Resemble <i>Aerococcus</i> species
<i>Helcococcus</i> species	non	Resemble “viridans” streptococci.
<i>Lactococcus</i> species	α or non	Resemble enterococci
<i>Leuconostoc</i> species	α or non	Resemble “viridans” streptococci.
<i>Pediococcus</i> species	α or non	Resemble “viridans” streptococci

3.4 Test Procedures

3.4.1 Biochemical tests

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

Streptococci and morphologically similar organisms are usually catalase negative.

Sometimes a weak catalase or pseudocatalase reaction is produced by *Aerococcus* and *Enterococcus* species.

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 are usually resistant.

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

Pyrrolidonyl arylamidase /PYR-aminopeptidase (PYR)⁹

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

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.

Summary of test results

	Possess Lancefield grouping antigen	Optochin sensitivity test ²⁴	Catalase test	Aesculin hydrolysis test	PYR
	(Commercial kit)	(TP 25)	(TP 8)	(TP 2)	
Group B, C, F and G	+	R	-	-	-
<i>S. pneumoniae</i>	-	S	-	-	d
Group D	+	R	-	+	-
Enterococci	+	R	v	+	+
<i>S. bovis</i>	(v) #	R	-	+	-
<i>Aerococcus</i> species	-	R	v	v	D
Group A	+	R	-	-	+
<i>S. anginosus</i> group	v	R	-	v	-
“viridans” streptococci	-	v	-	ND	ND
<i>Facklamia</i> species	-	R	-	-	+
<i>Gemella</i> species	-	R	w	-	+
<i>Globicatella</i> species	-	R	-	+	+
<i>Helcococcus</i> species	-	R	-	-	+
<i>Leuconostoc</i> species	-	R		d	D
<i>Pediococcus</i> species	cr	R	-	+	-

v-variable R-resistant S-sensitive cr-cross reacts d-6–84% strains positive
w-weak reaction ND- no data + -

- In the *S. bovis* group, *Streptococcus infantarius* (formerly *S. bovis* biotype II/1) and *S. lutetiensis* both give variable results with the Lancefield grouping of antigens⁵.

These test results are consistent with taxonomy from three widely published systems^{1,9,84}

3.4.2 Streptococcal grouping (Commercial Identification Kits)

Lancefield showed 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 method⁸⁵⁻⁸⁷. 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*.

Most commercial latex tests are now based on modified nitrous reagents, which will rapidly extract the group antigens without the need for any incubation. Latex test particles are sensitised with group specific antibody and will agglutinate in the presence of homologous antigen. The group specific antigens are extracted from streptococci by using an instant room temperature nitrous acid extraction procedure. The extract is then neutralized and the antigens are identified by agglutination. For Lancefield groups A, B, C, D, F and G, cross reactions may occur. 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.

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

MALDI-TOF has been developed and validated to determine species and lineages of *Streptococcus* and *Enterococcus* species⁸⁸. This has been shown to be a rapid and powerful tool because of its reproducibility, speed and sensitivity of analysis. The advantage of MALDI-TOF as compared with PFGE is that the results of the analysis are available within a few hours rather than several days. One limitation of MALDI-TOF is that it cannot readily distinguish between *Streptococcus pneumoniae* from other members of the *Streptococcus mitis* group. Despite this limitation, subtyping of *Streptococcus pneumoniae* strains by MALDI-TOF MS can be reliably performed, even for immunologically non-typeable or non-encapsulated strains⁵⁶. This has also been used for identification of aerococci to the species level but however, the accuracy of MALDI-TOF MS identification of bacterial species that are uncommon in clinical samples, such as aerococci, needs to be further evaluated⁸⁹.

3.4.4 Nucleic Acid Amplification Tests (NAATs)

PCR is now established as a rapid, reliable and reproducible technique for 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 will be performed.

Multiplex PCR is a rapid and convenient assay that allows simultaneous amplification of more than one locus in the same reaction and this has provided a reliable and rapid alternative to phenotypic testing and monoplex PCRs for the detection of five potential virulence genes (aggregation substance, gelatinase, cytolyisin, enterococcal surface protein and, very recently, hyaluronidase) in enterococci, capsule type determination of *Streptococcus agalactiae*, for pneumococcal capsular serotypes etc⁹⁰⁻⁹².

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*)⁹³.

3.5 Further Identification

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.

Rapid Methods

A variety of rapid 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 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.

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* isolates⁹⁴.

***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, ie the *E. avium*, *E. casseliflavus*, *E. cecorum*, *E. faecalis*, and *E. faecium* species groups; but it fails to discriminate closely related species, ie the members of *E. faecalis* and *E. faecium* species groups and *Streptococcus* species are not readily identified by the sequencing of the 16S rRNA gene⁴.

Pulsed Field Gel Electrophoresis (PFGE)

PFGE is a highly reproducible, discriminatory and effective epidemiological molecular typing method for identifying and classifying streptococci and enterococci into subtypes that is considered the reference standard⁹⁵. However, PFGE was found to be superior for interpretation of the interstrain relationships among enterococci but did not result in species-specific discriminative DNA bands⁴. However, due to its time-consuming nature (30hr 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 highly discriminatory tool that is widely used for phylogenetic typing of bacteria as well as to study the molecular epidemiology and population genetic structure of microorganisms. MLST is based on PCR amplification and sequencing of internal fragments of a number (usually 6 or 7) of essential or housekeeping genes spread around the bacterial chromosome. MLST measures the DNA sequence variations in a set of housekeeping genes directly and characterises strains by their unique allelic profiles.

The principle of MLST is simple: the technique involves PCR amplification followed by DNA sequencing. Nucleotide differences between strains can be checked at a variable number of genes depending on the degree of discrimination desired. Due to the sequence conservation in housekeeping genes, MLST sometimes lacks the discriminatory power to differentiate bacterial strains, which limits its use in epidemiological investigations. Its advantages are that it is unambiguous and highly portable and sequence data can be compared readily between laboratories and data stored in a central database is easily accessible via the internet to produce a powerful resource for global epidemiology⁹⁶.

This has been used successfully in the typing and investigation of the population structure of *Streptococcus agalactiae* (Lancefield group B streptococcus, GBS)

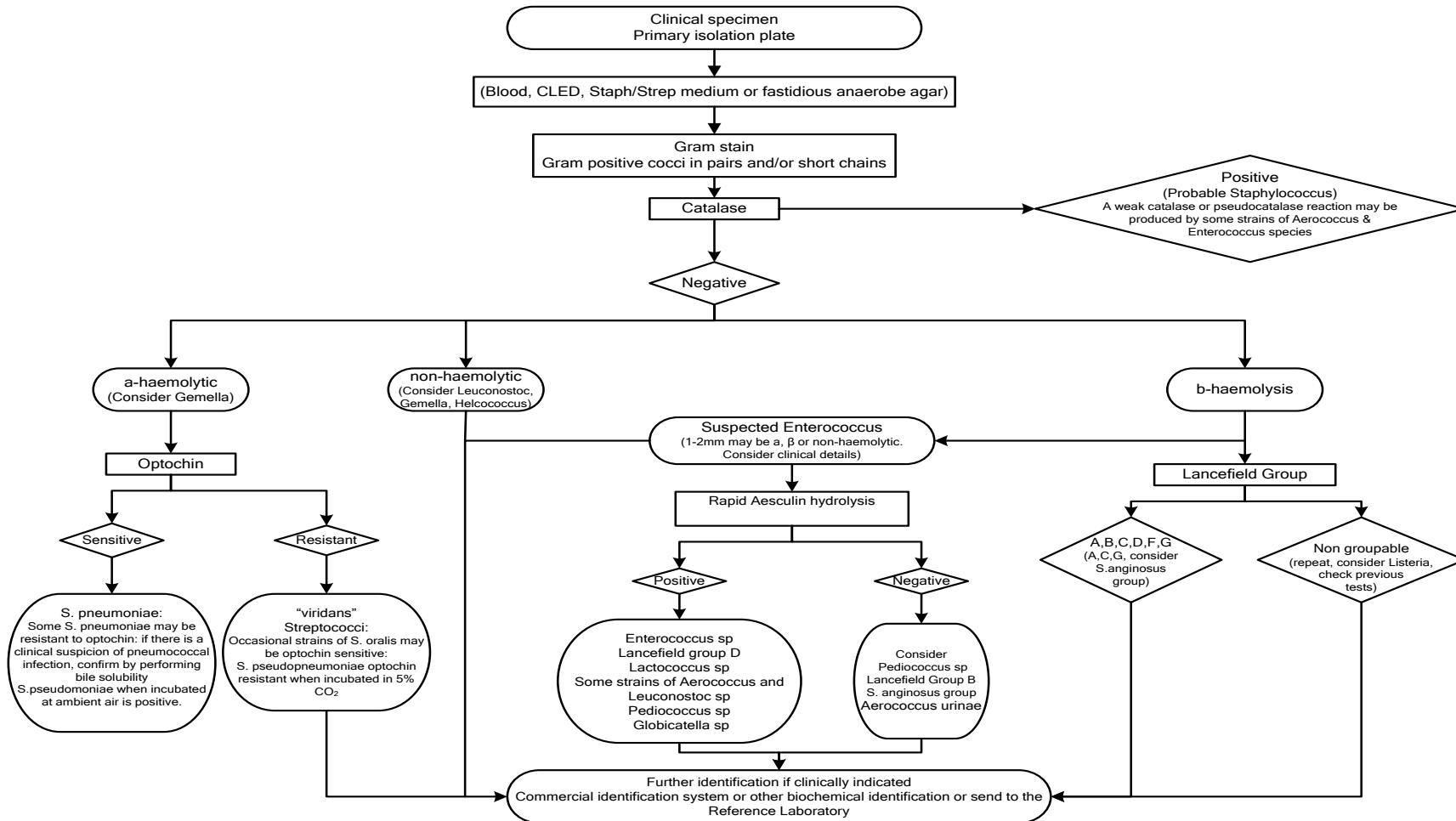
strains⁹⁷. This has also been used to identify the major clones associated with serious invasive pneumococcal disease and for characterising *Streptococcus pyogenes* (Lancefield group A streptococcus, GAS) isolates for epidemiological purposes by using this method together with its dedicated web-based database and tools which can be accessed on <http://pubmlst.org/mlst>^{98,99}.

However, the drawbacks of MLST are the substantial cost and laboratory work required to amplify, determine, and proofread the nucleotide sequence of the target DNA fragments, making the method hardly suitable for routine laboratory testing.

3.6 Storage and Referral

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

4 Identification of *Streptococcus* species, *Enterococcus* species and Morphologically Similar Organisms



The flowchart is for guidance only.

5 Reporting

5.1 Presumptive Identification

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

5.2 Confirmation of Identification

Confirmation of identification and toxigenicity are undertaken only by the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU) PHE Colindale.

5.3 Medical Microbiologist

Inform the medical microbiologist 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 medical microbiologist in accordance with local protocols, along with “large colony” isolates which possess Lancefield Group C or G antigens.

According to local protocols, consideration should also be given to informing the medical microbiologist when the request bears relevant or additional information suggestive of invasive or severe streptococcal infection eg:

- 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 medical microbiologist should be informed of isolates of β -haemolytic streptococci of Lancefield Group B when:

- The patient is pregnant, immediately post-partum or newborn

Follow local protocols for reporting to the patients’ clinicians.

5.4 CCDC

Refer to local Memorandum of Understanding.

5.5 Public Health England¹⁰⁰

Refer to current guidelines on CIDSC and COSURV reporting.

5.6 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.

6 Referrals

6.1 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:

Streptococci

Streptococcus and Diphtheria Reference Section
WHO Global Collaborating Centre for Streptococcal and Diphtheria Infections
Respiratory and Vaccine Preventable Bacteria Reference Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London
NW9 5EQ

<https://www.gov.uk/rvpbru-reference-and-diagnostic-services>

Enterococci

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

<https://www.gov.uk/amrhai-reference-unit-reference-and-diagnostic-services>

Contact PHE's main switchboard: Tel. +44 (0) 20 8200 4400

England and Wales

<https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services>

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

<http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm>

7 Notification to PHE^{100,101} or Equivalent in the Devolved Administrations¹⁰²⁻¹⁰⁵

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](#)^{102,103}, [Wales](#)¹⁰⁴ and [Northern Ireland](#)¹⁰⁵.

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