IDENTIFICATION OF STREPTOCOCCUS SPECIES, ENTEROCOCCUS SPECIES AND MORPHOLOGICALLY SIMILAR ORGANISMS

BSOP ID 4

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections

IDENTIFICATION OF STREPTOCOCCUS SPECIES, ENTEROCOCCUS SPECIES AND MORPHOLOGICALLY SIMILAR ORGANISMS
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### AMENDMENT PROCEDURE

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<tr>
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<td>Identification of <em>Streptococcus</em> species, <em>Enterococcus</em> species and morphologically similar organisms</td>
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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

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**IDENTIFICATION OF *STREPTOCOCCUS* SPECIES, *ENTEROCOCCUS* SPECIES AND MORPHOLOGICALLY SIMILAR ORGANISMS**

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IDENTIFICATION OF STREPTOCOCCUS SPECIES, ENTEROCOCCUS SPECIES AND MORPHOLOGICALLY SIMILAR ORGANISMS

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the identification of Streptococcus and Enterococcus species isolated from clinical material to genus or species level by phenotypic 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 NSM adopts a simplified approach based on grouping organisms with similar phenotypic attributes. Further identification may be necessary where clinically or epidemiologically indicated.

INTRODUCTION

Taxonomy

In recent years the taxonomy of streptococci and related organisms 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.

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. There are currently 18 members of the genus Enterococcus. Enterococcus faecalis and Enterococcus faecium are the commonest enterococci isolated from human infections.

Characteristics

Streptococci are Gram-positive cocci (spherical or ovoid) often occurring in pairs and chains. 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 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

Streptococci are facultatively anaerobic and catalase-negative. Carbohydrates are metabolised fermentatively; lactic acid is the major metabolite. Streptococci produce the enzyme leucine aminopeptidase (LAP), which has also been called leucine arylamidase.
**Streptococcus pyogenes** (Lancefield group A)

*Streptococcus pyogenes* is a Gram-positive coccus occurring in chains. After 18 - 24 h incubation at 35°C - 37°C on blood agar colonies are approximately 0.5 mm, 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 oxygen

Lancefield group A streptococci will not grow on media containing bile.

Low concentration bacitracin susceptibility has been used for screening purposes but is unreliable. Resistance to benzylpenicillin has not been reported.

The pyrrolidonyl arylamidase (or pyrrolidonyl-aminopeptidase) 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.

Pinpoint colony forms of the *S. anginosus* group may cross react with Lancefield group A antibodies and may grow on media containing bile.

**Streptococcus agalactiae** (Lancefield group B)

*Streptococcus agalactiae* are Gram-positive cocci, occurring in chains. After 18 - 24 h incubation at 35°C - 37°C colonies tend to be slightly larger than other streptococci (approximately 1 mm) and have a less distinct zone of β-haemolysis. Some strains may be non-haemolytic.

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 NSM.

**Streptococcus dysgalactiae** subspecies *equisimilis* (Lancefield groups A, C, G and L) **Streptococcus equi** subspecies *zooepidemicus* (Lancefield group C) and Lancefield group G streptococci

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 streptococci. Group C and G strains of *S. dysgalactiae* subsp *equisimilis* are identified much more commonly in human infections than those strains which possess Group A (or L) antigens.

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 bile.

**Enterococcus species, Streptococcus bovis** group (Lancefield group D)

The genus Enterococcus and organisms of the *S. bovis* group possess Lancefield group D antigen. Microscopically the organisms are Gram-positive cocci, spherical or ovoid in shape (0.6 - 2.5 µm), usually occurring in pairs or short chains in broth culture. After 18 - 24 h incubation at 35°C - 37°C on blood agar colonies are 1 - 2 mm 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 30 minutes.

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.

Enterococci are also heat resistant (60°C/30 mins) and PYR-positive which differentiates them from *S. bovis* and *S. gallolyticus*. **IDENTIFICATION OF STREPTOCOCCUS SPECIES, ENTEROCOCCUS SPECIES AND MORPHOLOGICALLY SIMILAR ORGANISMS**
There are six species included in the *S. bovis* group: *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. lutetensis*. Microscopically these species are Gram-positive cocci, occurring in chains after 18 - 24 h incubation at 35°C - 37°C in CO₂ or anaerobically. Colonies are usually non-haemolytic on blood agar and 1 - 2 mm 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 colon. 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.

Enterococci are facultative anaerobes. Two species within the genus, *Enterococcus cassilfalus* 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 G antiserum.

*E. faecalis* are very rarely resistant to ampicillin. 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 species.

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

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 - 24 h at 35°C - 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 levels.

Organisms of this group may possess the Lancefield group A, C, F or G antigen or be ungroupable. *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* group.

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.

**Streptococcus pneumoniae**

*Streptococcus pneumoniae* (“pneumococci”) are Gram-positive typically lanceolate cells occurring in pairs, which may be capsulate. Colonies are 1 - 2 mm, α-haemolytic and may appear as ‘draughtsmen’ colonies due to autolysis of the organisms after incubation in 5 - 10% CO₂ at 35°C - 37°C for 16 - 24 h. 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*. Commercial agglutination tests are also available for the rapid detection of pneumococcal antigen, but these should be used with caution because cross reactions may occur with the *S. oralis* and *S. mitis* groups.

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**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.

**Other streptococci ("viridans" streptococci)**

These species are Gram-positive cocci occurring in chains, which are indistinguishable by Gram stain from β-haemolytic streptococci. Colonies are 0.5 - 1.0 mm and may be α or non-haemolytic on blood agar after anaerobic incubation at 35°C - 37°C in CO₂ for 16 - 24 h. “Viridans” is derived from the Latin word *viridis*, meaning green.

Generally these streptococci would not require further identification, other than as an α or non-haemolytic streptococcus, 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 cases.

**Nutritionally Variant Streptococci (NVS)**

NVS have now been reclassified as *Granulicatella adiacens*, *Granulicatella elegans* and *Abiotrophia defectiva*²⁰,²¹. NVS require media supplemented with either pyridoxal or cysteine for growth.

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 10 mg/L pyridoxal hydrochloride.

Recognition of these species is important for deep seated infections (notably endocarditis) to ensure the most appropriate antimicrobial therapy²².

**Aerococcus species**

Aerococci resemble “viridans” streptococci on culture but differ microscopically by characteristically occurring as tetrads or clusters, similar to staphylococci. Sometimes a weak catalase or pseudocatalase reaction is produced. Some strains of *Aerococcus viridans* are bile aesculin-positive and PYR-positive. *Aerococcus urinae* is bile aesculin-negative and PYR-negative. In some commercial identification systems *Helcococcus kunzii* may be mis-identified as *A. viridans*.

**Facklamia species**²³

Facklamia resemble “viridans” streptococci on culture but differ microscopically by characteristically occurring as pairs, tetrads or clusters. There are six species, but the most common human species is *Facklamia hominis*. They are weakly α haemolytic and usually hydrolyse urea.

**Gemella species**

There are four species: *Gemella haemolysans*, *Gemella morbillorum* (formerly *Streptococcus morbillorum*), *Gemella bergeriae* and *Gemella sanguinis*²⁴. These bacteria easily decolourise on Gram staining, occurring as Gram-negative cocci in pairs, tetrads, clusters or short chains. *Gemella* species are α or non-haemolytic on blood agar and resemble colonies of viridans streptococci. In some commercial identification systems, “viridans” streptococci can be mis-identified as *Gemella* species.

**Globicatella species**²⁵

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These organisms resemble enterococci both morphologically and microscopically in being Gram-positive cocci in pairs and short chains. However, they do not produce leucine aminopeptidase.

*Helcococcus kunzii*

*Helcococcus kunzii*, similar to *Aerococcus* species, has recently been described. *H. kunzii* produces tiny grey, slightly α-haemolytic colonies; growth is stimulated by the addition of serum or Tween 80 to the basal medium. In some commercial identification systems *A. viridans* may be mis-identified as *Helcococcus kunzii*.

*Lactococcus* species

*Lactococcus* species are physiologically similar to Enterococci. They are 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 antigen.

*Leuconostoc* species

*Leuconostoc* species are Gram-positive lenticular cocci occurring in pairs and chains and are characteristically vancomycin resistant. They may be confused with the enterococci because most *Leuconostoc* species are bile aesculin-positive, produce gas from glucose and some cross-react with the group D antisera.

*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 vancomycin resistant. They may be confused with enterococci because they are bile aesculin-positive and cross-react with the Group D antisera.

**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. 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. 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. Careful consideration should be given to isolates which give an unusual identification. Some commercial kits may give unreliable results with the identification of α-haemolytic streptococci. 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. All identification tests should ideally be performed from non-selective agar.

**TECHNICAL INFORMATION**

N/A
1 SAFETY CONSIDERATIONS

Hazard Group 2 organisms.

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

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

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\textsuperscript{2,6,8-12,14,16,18-20,24}

Streptococci possessing Lancefield group antigens A-G

**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**
Enterococcus species (see below)
Streptococcus bovis
Streptococcus gallolyticus

**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)

Other streptococci
Streptococcus pneumoniae
Streptococcus suis

**Streptococcus anginosus group**
Streptococcus anginosus
Streptococcus constellatus subspecies constellatus
Streptococcus constellatus subspecies pharyngis
Streptococcus intermedius

**Streptococcus mutans group**
Streptococcus mutans
Streptococcus sobrinus
Streptococcus sanguinis group
Streptococcus cristatus
Streptococcus gordonii
Streptococcus sanguis
Streptococcus parasanguinis

Streptococcus mitis group
Streptococcus mitis
Streptococcus oralis

Streptococcus salivarius group
Streptococcus salivarius
Streptococcus vestibularis

Nutritionally variant streptococci
Granulicatella adjacens
Abiotrophia defectiva
Granulicatella elegans

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 (see BSOPTP 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°C – 37°C for 16 – 48 h, or anaerobically at 35°C - 37°C for 16 - 24 h for throat swabs (see BSOP 9 - Investigation of Throat Swabs)

Staph/Strep agar incubated in air at 35°C – 37°C for 16 - 48 h.

CLED agar incubated in air at 35°C – 37°C for 16 - 24 h.

Fastidious anaerobe agar incubated anaerobically for 16 - 48 h.

### 3.3 **COLONIAL APPEARANCE**

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

Catalase test (see BSOPTP 8 - Catalase Test)
Streptococci and morphologically similar organisms are usually catalase-negative.

Bile Aesculin hydrolysis test (see BSOPTP 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 species can hydrolyse aesculin.

Optochin sensitivity test (see BSOPTP 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.

Streptococcal grouping (commercial kit)
For Lancefield groups A, B, C, D, F and G. Cross reactions may occur.

PYR11 - pyrrolidonyl arylamidase (PYR-aminopeptidase)
Enterococci and S. pyogenes are positive, S. bovis group and S. anginosus group are negative.

Bile solubility test (optional) (see BSOPTP 5 - Bile Solubility Test) S. pneumoniae is soluble in 10% bile salts, S. pseudopneumoniae is partially soluble and other α-haemolytic streptococci are insoluble.

Commercial identification kit
### Summary of test results

<table>
<thead>
<tr>
<th>Possess Lancefield grouping antigen (Commercial kit)</th>
<th>Optochin sensitivity test</th>
<th>Catalase</th>
<th>Bile Aesculin hydrolysis (BSOP TP 2)</th>
<th>PYR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B, C, F and G</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>-</td>
<td>S</td>
<td>-</td>
<td>d</td>
</tr>
<tr>
<td>Group D</td>
<td>+</td>
<td>R</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Enterococci</td>
<td>+</td>
<td>R</td>
<td>v</td>
<td>+</td>
</tr>
<tr>
<td>S. bovis</td>
<td>+</td>
<td>R</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Aerococcus species</td>
<td>-</td>
<td>R</td>
<td>v</td>
<td>v</td>
</tr>
<tr>
<td>Group A</td>
<td>+</td>
<td>R</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>S. anginosus group</td>
<td>v</td>
<td>R</td>
<td>-</td>
<td>v</td>
</tr>
<tr>
<td>“viridans” streptococci</td>
<td>-</td>
<td>v</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Facklamia species</td>
<td>-</td>
<td>R</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Gemella species</td>
<td>-</td>
<td>R</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>Globicatella species</td>
<td>-</td>
<td>R</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Helcococcus species</td>
<td>R</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Leuconostoc species</td>
<td>R</td>
<td>-</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Pediococcus species</td>
<td>cr</td>
<td>R</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

v = variable  
R = resistant  
S = sensitive  
cr = cross reacts  
d = 6 - 84% strains positive  
w = weak reaction

3.5 **Further Identification**

N/A

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 STREPTOCOCCI AND ENTEROCOCCI – FLOW CHART

Clinical specimen
Primary isolation plate
(blood, CLED, Staph/Strep medium or fastidious anaerobe agar)

Gram stain
Gram-positive cocci in pairs and/or short chains

Catalase

Positive
(Probable Staphylococcus)
A weak catalase or pseudocatalase reaction may be produced by some strains of Aerococcus & Enterococcus species

Negative

Suspected Enterococcus
(β-haemolytic, Enterococcus sp.)

Suspected Strentococcus
(α-haemolytic, Streptococcus sp.)

α-haemolytic (Consider Gram stain)

Optochin

Sensitive

S. pneumoniae
Some S. pneumoniae may be resistant to optochin if there is a clinical suspicion of pneumococcal infection, confirm by performing bile solubility

“viridans” Streptococci
Occasional strains of S. oralis may be optochin sensitive
S. pneumoniae optochin sensitive, bile solubility inconclusive

β-haemolytic

Enterococcus spp
(PYR & LAP positive)
PYR positive, LAP positive consider Aerococcus, Lactococcus, Faciibacter, Doseislaginum,
PYR positive, LAP negative consider Globistella, Aerococcus, viridans, Doseislaginum

Further identification if clinically indicated
Commercial identification system or other biochemical identification or send to the Reference Laboratory

Lancefield Group

ABC, DFG
(A-E), consider Sanguinous group

PYR

Nongroupable (repeal, consider Enterococcus, check previous testing)

S. anginosus group

Positive

Group A

Reference no: BSOPID 42.1
This SOP should be used in conjunction with the series of other SOPs
www.evaluations-standards.org.uk
Email: standards@hpa.org.uk
5 REPORTING

5.1 PRESUMPTIVE IDENTIFICATION
If appropriate growth characteristics, colonial appearance, Gram stain of the culture, catalase and serological results are demonstrated.

5.2 CONFIRMATION OF IDENTIFICATION
Following biochemical/serological identification and/or the Reference Laboratory report.

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 protocol’s, 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 card 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 CENTRE FOR INFECTIONS
Refer to current guidelines on CDC and CoSURV reporting.
5.6 **INFECTION CONTROL STAFF**

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 Laboratories**

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory refer to:

http://www.hpa.org.uk/cfi/reference_tests_index.htm

Streptococcus and Diphtheria Reference Unit  
Respiratory and Systemic Infection Laboratory  
Health Protection Agency Centre for Infection  
61 Colindale Avenue  
London  
NW9 5HT  

Enterococci:  
Laboratory of Hospital Infection  
Centre for Infections  
61 Colindale Avenue  
London  
NW9 5HT  

Contact Centre for Infections main switchboard: Tel. +44 (0) 20 8200 4400

7 **ACKNOWLEDGMENTS AND CONTACTS**

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology
The National Standard Methods are issued by Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency London.

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REFERENCES


