STREPTOCOCCUS

Methods for distinguishing catalase-negative streptococci are given on pages 1 to 3. Details for distinguishing species are from page 3 onwards.

Morphology

Streptococci are more or less spherical in shape although they may be pleomorphic, particularly when initially isolated. Arranged in chains which vary in length, depending to some extent on the culture medium. Some species characteristically form long chains whereas others are mainly diplococcal.

Cultural Characteristics

Growth on ordinary nutrient medium is usually poor. Pathogenic strains require enrichment of the media with blood or serum. Colonies are translucent and usually about 1 mm diameter after 24 hours growth on SBA. Many species produce zones of haemolysis on SBA. Mostly aerotolerant and catalase negative.

Classification

Because of their complexity of habitat, pathogenicity and other characteristics, a logical subdivision of the streptococci as a group has proved difficult. Most of the species which are frequent causes of disease have been accurately characterized. Others that form part of the normal flora, or are saprophytes, are less well classified or unclassified.

Criteria for Differentiation

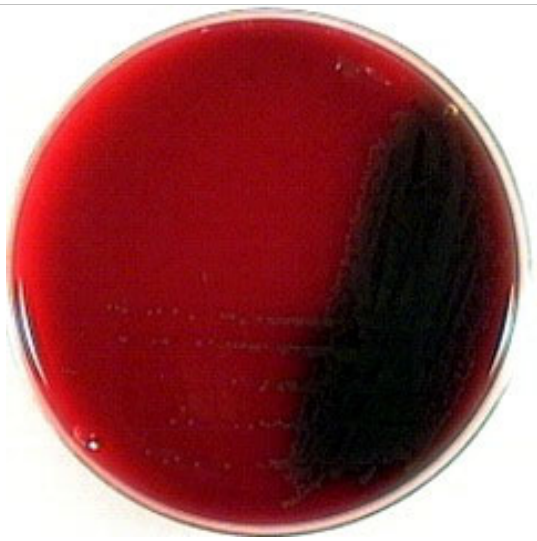
Physiological Characteristics

Some streptococci grow at extremes of temperature and pH not tolerated by more fastidious species. Use has also been made of the ability to grow on MCA, or in the presence of inhibitory substances such as 1% methylene blue or 6.5% NaCl. The fermentation of specific carbohydrates is useful for differentiating species that have been grouped together on the basis of other characteristics. For example fermentation of lactose, sorbitol and trehalose is useful for the differentiation of the equine group C streptococci.

Haemolysis

Three categories of streptococci are recognised depending on the type of haemolysis they produce on SBA or HBA.

Viridans streptococcus. SBA 48h culture



No Haemolysis: Colonies produce no observable change in red blood cells.

α-Haemolysis: A narrow hazy zone of greenish discolouration surrounds the colony. The red blood cells are not lysed but discoloured. a-haemolytic strains are often termed 'viridans' streptococci. The zone of greenish discolouration may be surrounded by a hazy partially cleared zone. The width of this area may increase on further incubation, or after storage at room temperature or in the refrigerator. However, the clearing is only partial, the margin is indistinct and there are always clumps of unlysed cells present.

Streptococcus equi ssp zooepidemicus. A prominent zone of ß-haemolysis is visible surrounding each colony.		**ß-Haemolysis**: The colony is surrounded by a sharply defined clear, colourless zone in which no intact red blood cells can be seen.

Antigenic Structure

The antigenic structure of streptococci is very complex. The cell wall is composed principally of peptidoglycan. In addition many streptococci have group specific cell-wall polysaccharides attached to the peptidoglycan and type-specific polysaccharide or protein antigens that are used in typing.

Group Specific Polysaccharides

Group specific carbohydrate antigens of all groups except D reside in the cell wall, and specificity depends on the nature of the terminal sugar residue on the oligosaccharide rhamnose side chain.

The polysaccharides can be acid extracted and purified. When injected into rabbits they are serologically active, producing specific precipitating antibody. The precipitin test for group specific polysaccharide is the basis of the Lancefield grouping system. Other serological methods such as latex agglutination, coagglutination and fluorescent antibody are also used to identify Lancefield groups. Many streptococci can be placed in groups (A to T), depending on their polysaccharide antigen. However, it is mainly the pyogenic streptococci that can be classified on the basis of their group polysaccharides. Acid-extractable polysaccharide antigens are found in other streptococci, but their distribution is less regular and they often cannot be typed by the Lancefield system.

The antigens defining Lancefield groups D and N are special, in that they are not polysaccharides, but glycerol teichoic acids containing glucose and D-alanine.

In general, group is closely related to both host and disease specificity in streptococcal diseases.

Type Specific Antigens

As well as group specific polysaccharide antigens, some members of the Lancefield groups also possess type-specific polysaccharide antigens, either as part of the cell wall structure or situated more superficially eg. groups B, D, E, F, L. In some Lancefield groups there are type-specific protein antigens which may be of several classes including the M, T and R antigens of *S. pyogenes* (group A). The M and R antigens of group A streptococci are located together with a lipoteichoic acid component in hair-like surface fibrils.

Tests to Identify the Genus

Gram reaction — Positive

Shape — Coccus

Growth in air — Positive

Growth anaerobically — Positive

Catalase — Negative

Oxidase — Negative

Glucose (acid) — Positive

OF — Fermentative

Susceptibility to Antibiotics

Pyogenic streptococci are all highly sensitive to benzyl penicillin. They tend to become resistant to aminoglycosides. Sensitive to sulphonamides, erythromycin, cephalosporins, lincomycin, and tetracyclines. Acquired resistance to tetracyclines has been reported.

Categories of Streptococci and Related Organisms

Pyogenic

Usually ß-haemolytic, possess polysaccharide group antigens (although these polysaccharides may occasionally be found in otherwise unrelated strains), don't grow at extremes of temperature or pH. This group contains most of the pathogenic species including:

S. agalactiae, S. canis, S. dysgalactiae subsp. dysgalactiae, S. dysgalactiae subsp. equisimilis. S. equi subsp. equi, S. equi subsp. zooepidemicus, S. pneumoniae, S. porcinus, S. pyogenes.

Oral

Oral streptococci are commonly found in the oral cavity and URT of humans and animals. Opportunist pathogens which are associated with dental caries and endocarditis in humans. They are usually a-haemolytic and are often referred to as viridans streptococci. Includes species such as: *S. salivarus, S. mutans.*

Enterococci

Enterococci occur in the intestinal tracts of humans and animals. Show variable haemolysis, are heat resistant and grow over a wide range of temperature and pH and possess Group N antigen. Opportunist pathogens in the genus *Enterococcus*.

Lactic

Lactic streptococci occur in milk and milk products. Non pathogenic, α- or non- haemolytic, grow at low temperatures possess group N antigen. Included in the genus *Lactococcus*.

Anaerobic

Anaerobic streptococci occur in the alimentary and respiratory tracts. Opportunist pathogens, non haemolytic. Includes *Peptostreptococcus indolicus, Peptococcus* sp.

Other

Other streptococci have little in common except a series of negative characteristics. Serologically heterogeneous, contains species which don't fit in the above categories and includes — *S. bovis, S. equinus, S. uberis, S. suis.*

Streptococcus agalactiae

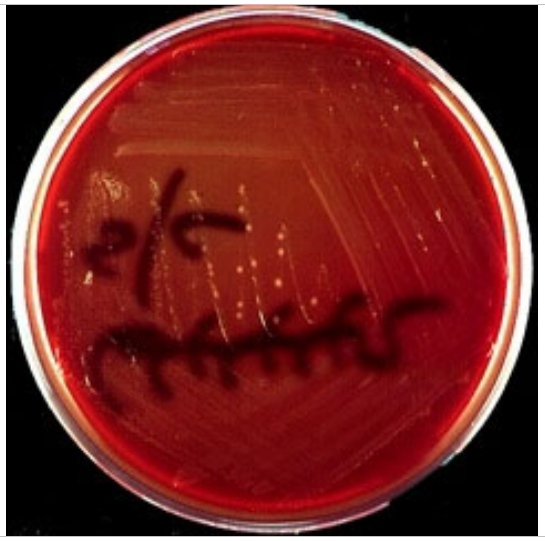
Collection of Specimens

Milk samples should be aseptically collected and processed as rapidly as possible after collection.

Microscopic Examination of Tissues

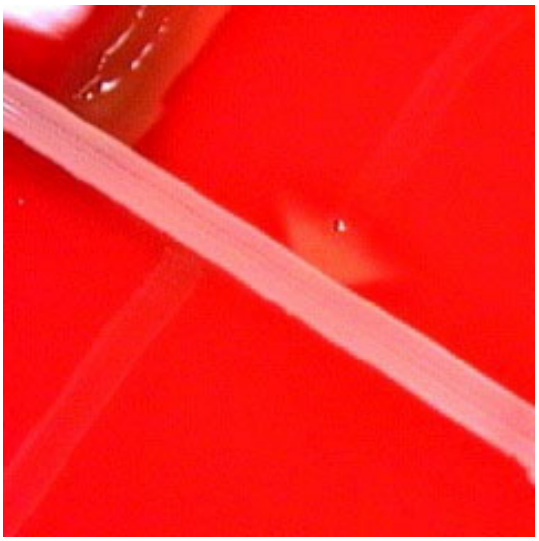
Usually appear in long chains in mastitic milk. May be numerous and easily found or very difficult to find, even though milk is grossly abnormal.

Cultural Examination

Streptococcus agalactiae on SBA. 48 hr culture		Inoculate SBA and MCA plates and incubate at 37° C in air. Grows readily on SBA. Small (~ 1 mm diam), smooth, glistening, round, translucent colonies with entire edges. May be ß-haemolytic but with a much smaller zone of complete hemolysis than other ß-streptococci. Other strains are α-haemolytic or non-haemolytic. Edward's medium is a selective differential medium for isolation of streptococci from mastitic milk. Aesulin splitters (*S. uberis)* produce a dark discolouration.Some growth occurs on MCA which is unusual for a pyogenic *Streptococcus* sp. Gram smear of *S. agalactiae* culture shows typical Gram-positive cocci.

Identification

Lancefield Group — B; Hippurate — Positive; Aesculin — Negative; CAMP-Test — Positive

CAMP Test to help identify *Streptococcus agalactiae*.		A ß-haemolysin producing strain of staphylococcus is streaked across the centre of a SBA plate. Strains of streptococci to be tested are then streaked at right angles to the staphylococcus streak. The plate is incubated at 37° C in air. CAMP-positive organisms are indicated by an area of clear haemolysis in the adjoining zone where diffusing products from the two species overlap. Only one of the 3 streptococcal cultures shown in picture is CAMP positive.

Serological Testing

Not used in routine diagnosis.

Agglutinins for hematoxylin-stained *S. agalactiae* can be detected in milk from infected cows by a milk ring test.

An enzyme-linked immunosorbent assay (ELISA) has also been used to measure antibody levels in milk and as an aid in the detection of latent and subclinical carriers of infection.

Streptococcus bovis

Cultural Examination

Inoculate SBA and MCA plates and incubate at 37° C in air.

Somewhat larger than other streptococcal colonies on the surface of blood agar (0.5 to 1.0 mm). Less opaque, raised, and gray to gray-white.

Non haemolytic.

Lancefield Group D.

Strptococcus canis

Cultural Examination

Inoculate SBA and MCA plates and incubate at 37° C in air.

Selective enrichment can be achieved using broth containing blood, azide, and crystal violet or penicillin and salt.

Closely related to the group C streptococci, they produce wide zones of ß-hemolysis on horse-blood agar

Specimen Collection

Bovine milk samples should be aseptically collected and processed as rapidly as possible after collection.

Microscopic Examination of Milk

Preparation of smears from milk is described in Bacto Mycology Methods database.

Newman's stain may be used to demonstrate organisms in milk

Chains are usually short or medium in length

Streptococcus dysgalactiae subsp. *dysgalactiae*

Cultural Examination

Inoculate SBA and MCA plates and incubate at 37° C in air.

Edward's medium is a selective differential medium for isolation of streptococci from mastitic milk. Aesulin splitters (*S. uberis*) produce a dark discolouration.

Grows readily on SBA. Small (~ 1 mm diam), smooth, glistening, round, translucent colonies with

entire edges. Colonies are usually non hemolytic and often produce a distinct greenish discoloration.

No growth MCA.

Differs from *S. agalactiae* only in minor cultural features.

Identification

S. dysgalactiae does not always coagulate milk in 48 hours when incubated at 37°C.

Methylene blue milk, however, is regularly reduced.

The final pH in glucose broth varies between 5.3 and 5.0. It never goes below 5.0.

Serological Testing

Not used in routine diagnosis.

Streptococcus dysgalactiae subsp. *equisimilis*

Microscopic Examination of Tissues

Gram-positive cocci. Chains are usually short or medium in length.

Cultural Examination

Inoculate SBA and MCA plates and incubate at 37° C in air.

Colony morphology is similar to *S. zooepidemicus*, although the zone of ß-haemolysis is less.

Identification

Glucose — Poitive

Lactose (Equine Strains) — Negative

Lactose (Cattle, Pig Strains) — Positive

Sorbitol — Negative

Trehalose — Positive

Aesculin — Negative

Hippurate — Negative

6.5% NaCl — Negative

Streptococcus equi subsp. *equi*

Specimen Collection

Needle aspirates of pus can be aseptically collected from lymph nodes of head and neck of horses with suspected strangles.

Alternatively, swabs of purulent nasal discharge can be collected.

Microscopic Examination of Tissues

Prepare thin smear of pus and stain by Gram's method

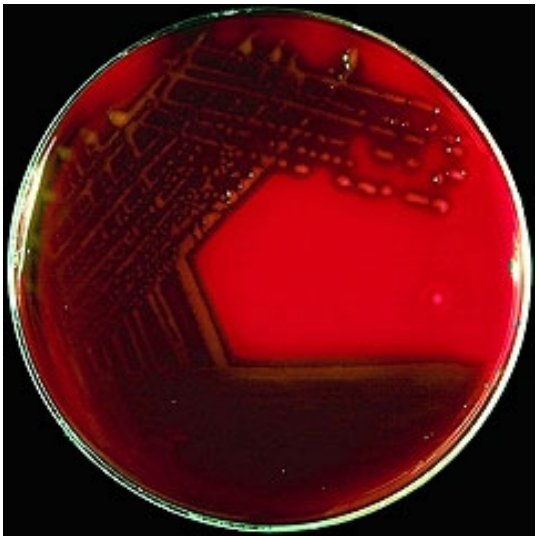
Usually occurs as long chains in exudates. Occasionally seen as short chains.

Sometimes the chains are surrounded by definite capsular material.

Readily stained with the usual dyes and is Gram positive when cultures are young. Old cultures retain Gram's stain poorly.

Cultural Examination

Inoculate SBA and MCA plates and incubate at 37° C in air.

Streptococcus equi subsp *equi* 24 hr culture on horse blood agar showing ß-haemolysis and mucoid colonies.		Strongly ß-haemolytic and produces either a mucoid colony or matt colony on primary isolation. Mucoid growth is due to the presence of the hyaluronic acid capsule, while the matt colony is due to absence of the capsule. The matt colony results from the action of a phage-controlled hyaluronidase during the early stages of growth. Mucoid colonies are usually about 3 mm in diameter after 24 hr and adjacent colonies tend to run together. Matt colonies exhibit irregular surface folding and look dried out. All colonies are mucoid in this picture.

Usually occurs as long chains in fluid cultures. Occasionally seen as short chains.

Identification

Haemolysis — ß

Glucose — Positive

Sucrose — Positive

Maltose — Positive

Galactose — Positive

Lactose — Negative

Sorbitol — Negative

Trehalose — Negative

Lancefield Group — C

Serological Testing

Not used in routine diagnosis.

Streptococcus equi subsp. *zooepidemicus*

Specimen Collection

Aspirates of pus can be aseptically collected from lesions. Alternatively swabs of discharge can be collected. Special guarded swabs are used to avoid contamination when collecting specimens for uterine culture in the mare.

Microscopic Examination of Tissues

Prepare thin smear of pus and stain by Gram's method

Occurs in exudates and in fluid cultures in the form of long chains and occasionally in short chains.

Sometimes the chains are surrounded by definite capsular material.

Cultural Examination

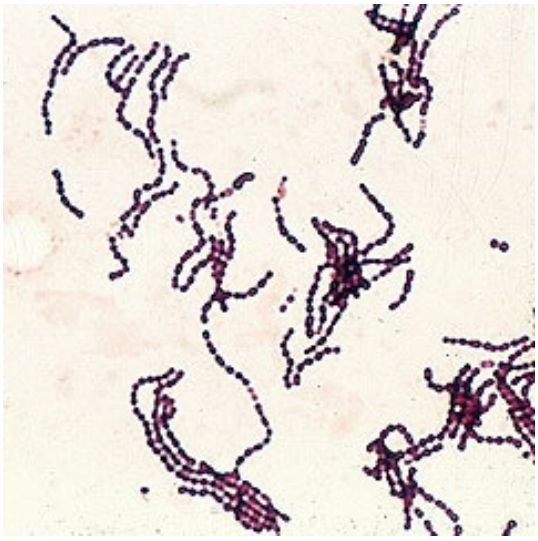
Inoculate SBA and MCA plates and incubate at 37° C in air.

Colonies are surrounded by a wide zone of ß-hemolysis

Produces either a mucoid or matt colony on primary isolation

Mucoid growth is due to the presence of the hyaluronic acid capsule, while the matt colony is due to absence of the capsule

Mucoid colonies are usually about 3 mm in diameter after 24 hours and adjacent colonies tend to run together. Matt colonies exhibit irregular surface folding and look dried out.

Gram-stain of 24 hr broth culture x1000. *Streptococcus equi* subsp *zooepidemicus* in long chain arrangement.		Readily stained with the usual dyes and is Gram positive when cultures are young. Old cultures retain Gram's stain poorly.

Identification

Growth SBA (aerobic) — Positive

Growth MCA — Negative

Haemolysis — ß

Glucose — Positive

Lactose — Positive

Sorbitol — Positive

Trehalose — Negative

6.5% NaCl — Negative

Lancefield Group — C

Serological Testing

Not used diagnostically.

Streptococcus porcinus

Microscopic Examination of Tissues

Immunofluorescence is reported to be the most effective procedure for detection of *S. porcinus* in tonsillar smears.

Cultural Examination

Inoculate SBA and MCA plates and incubate at 37° C in air.

Selective enrichment can be achieved using broth containing blood, azide, and crystal violet or penicillin and salt.

Small, elevated, entire colonies after 24 h incubation.

Slowly developing ß- haemolysis is visible on SBA in about 48 h.

Streptococcus pyogenes

Cultural Examination

After 18 to 24 h incubation on SBA, colonies of group A streptococci typically are about 0.5 mm diam, transparent or translucent, and domed, smooth or semi-matt surface and entire edge.

Surrounded by a well-defined zone of complete haemolysis, usually 2 to 4 times the diam of the colony; however, considerable variations occur.

The appearance of the colonies depends greatly on the medium used and to some extent on the atmosphere of incubation.

All colonial characteristics are not manifested on a single medium or atmosphere.

Streptococcus suis

Specimen Collection

Depends on type of lesion. Fresh brain, synovial membrane, lung. If septicaemia fresh liver, kidney, spleen, heart blood.

Microscopic Examination of Tissues

Smear of meninges or synovial fluid stained Gram.

Gram-positive cocci occurring singly, in pairs and rarely in short chains in clinical materials.

Elongated diplobacilli that form short chains in fluid cultures.

Cultural Examination

Inoculate SBA and MCA plates and incubate at 37° C in air. The colonies are flat, mucoid, weakly haemolytic, and about 1 to 2 mm in diameter.

Some strains produce greenish discoloration in blood agar or an incomplete haemolysis and may exhibit a CAMP reaction.

Identification

Lancefield Group — D

Serological Testing

Not used in routine diagnosis.

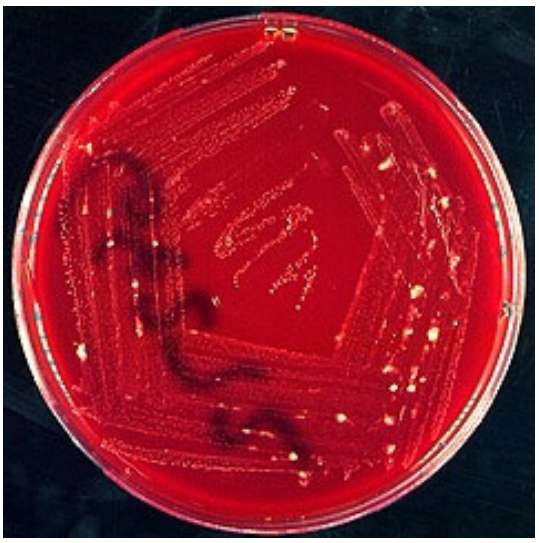
Streptococcus uberis

Microscopic Examination of Tissues

Gram-positive cocci. Chains are usually short or medium in length.

Cultural Examination

Inoculate SBA and MCA plates and incubate at 37° C in air.

Streptococcus uberis. 24 hr culture on SBA		Colonies of *S. uberis* are non-hemolytic but may be a slight greenish discoloration on blood agar. A few strains are CAMP positive.

Identification

Lancefield Group — Variable

Serological Testing

Not used in routine diagnosis.

Susceptibility to Antibiotics

More resistant to penicillin than other streptococci.