

# Veterinary Microbiology

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# *Streptococci*

The Streptococci are a group of bacteria that can infect many animal species, causing suppurative conditions such as mastitis, metritis, polyarthritis and meningitis. The enterococci are a related group of organisms, previously classified in the genus *Streptococcus*. Strictly anaerobic Gram-positive cocci, previously placed in the genus *Peptostreptococcus*, are now classified as members of the genus *Peptoniphilus* (Ezaki *et al.*, 2001). Most pathogenic species are in the genus *Streptococcus*. These Gram-positive cocci are approximately 1.0  $\mu\text{m}$  in diameter and form chains of different lengths ([Fig. 15.1](#)).

*Streptococcus* species are catalase-negative, facultative anaerobes, which are non-motile. They are fastidious bacteria and require the addition of blood or serum to culture media. *Streptococcus pneumoniae* (pneumococcus) occurs as slightly pear-shaped cocci in pairs. Pathogenic strains have thick capsules and produce mucoid colonies. These bacteria cause pneumonia in humans, guinea-pigs and rats.

*Peptoniphilus indolicus* is an anaerobic Gram-positive coccus, which is aetiologically implicated in bovine 'summer mastitis' in association with *Arcanobacterium pyogenes* and *S. dysgalactiae*.

## Usual habitat

The streptococci are distributed worldwide. Most species live as commensals on the mucosae of the upper respiratory tract and lower urogenital tract. These fragile bacteria are susceptible to desiccation and survive for only short periods off the host.

## Key points

- Gram-positive cocci in chains
- Fastidious, requiring enriched media
- Small, usually haemolytic, translucent colonies
- Catalase-negative
- Facultative anaerobes, usually non-motile
- Commensals on mucous membranes
- Susceptible to desiccation
- Cause pyogenic infections

## Differentiation of the streptococci

Three procedures are used for conventional laboratory differentiation of the streptococci, namely type of haemolysis, Lancefield grouping and biochemical testing.

- Type of haemolysis on sheep or ox blood agar:
  - Beta-haemolysis is complete haemolysis indicated by clear zones around colonies.
  - Alpha-haemolysis is partial or incomplete haemolysis indicated by greenish or hazy zones around colonies.
  - Gamma-haemolysis denotes no observable changes in the blood agar around colonies.
- Lancefield grouping is a serological method of classification based on the group-specific C-substance. Most pathogenic streptococci possess C-substance, a cell-wall polysaccharide antigen, which differs between species or groups of species and is widely used to classify clinical isolates of *Streptococcus*. Test methods include:
  - Ring precipitation test. The C-substance is extracted by acid or heat from the *Streptococcus* species under test. This antigen extract is layered over antisera of different specificities, in narrow tubes placed in plasticine on a slide. A positive reaction is indicated by the formation of a white ring of precipitate close to the interface of the two fluids within 30 minutes ([Fig. 15.2](#)).

- Latex agglutination test. Specific C-substance antisera for groups A to G (with the exception of group E) are commercially available. Suspensions of latex particles are coated with each of the group-specific antibodies. The group antigen is extracted enzymatically from the *Streptococcus* under test. A drop of antigen is mixed on a plate with a drop of each latex-antibody suspension and rocked gently. A positive reaction, which usually occurs within 1 minute, is indicated by agglutination ([Fig. 15.3](#)).

- Biochemical testing:

- A number of commercial test systems are available for rapid biochemical identification of streptococci.
- A short range of biochemical tests is used for differentiating equine group C streptococci.

- Differentiation of streptococcal species can be carried out using molecular tests such as PCR- based techniques. Multiplex PCR methods for the detection and differentiation of pathogens causing bovine mastitis, including streptococci, have been developed (Gillespie and Oliver, 2005). Molecular typing techniques have been applied to streptococci pathogenic for animals and include a MLST scheme for *S. zooepidemicus* (Webb *et al.*, 2008), AFLP typing for *S. suis* (Rehm *et al.*, 2007), and PFGE and MLST typing for *S. uberis* (Rato *et al.*, 2008).

## Virulence factors of group A Streptococcus and their role

Factor	Role
M cell wall protein	Antiphagocytic, anticomplement, and epithelial adhesion roles.
Hyaluronic acid capsule	Antiphagocytic
Streptolysin O and S	Haemolytic, Cytotoxic,
Hyaluronidase, streptokinase	Lysis of blood clots and tissue spread
DNAases A,B,C,D	Basis of anti-DNAase B assay
Erythrogenic toxins	Rash, fever, Cytotoxic effect "scarlet fever
Firbonectin-binding protein	Adhesion, and invasion
C5a peptidase	Inhibits leukocyte recruitment
Superantigens	Nonspecific immune stimulation
Protein Sic	Inhibitor of complement
Cysteine protease	Generate biologically active molecules, inflammation, shock, and tissue damage

## Comparison of Hemolytic Activity for Group A, B, G, F Streptococci





## Non-hemolytic and Hemolytic GBS

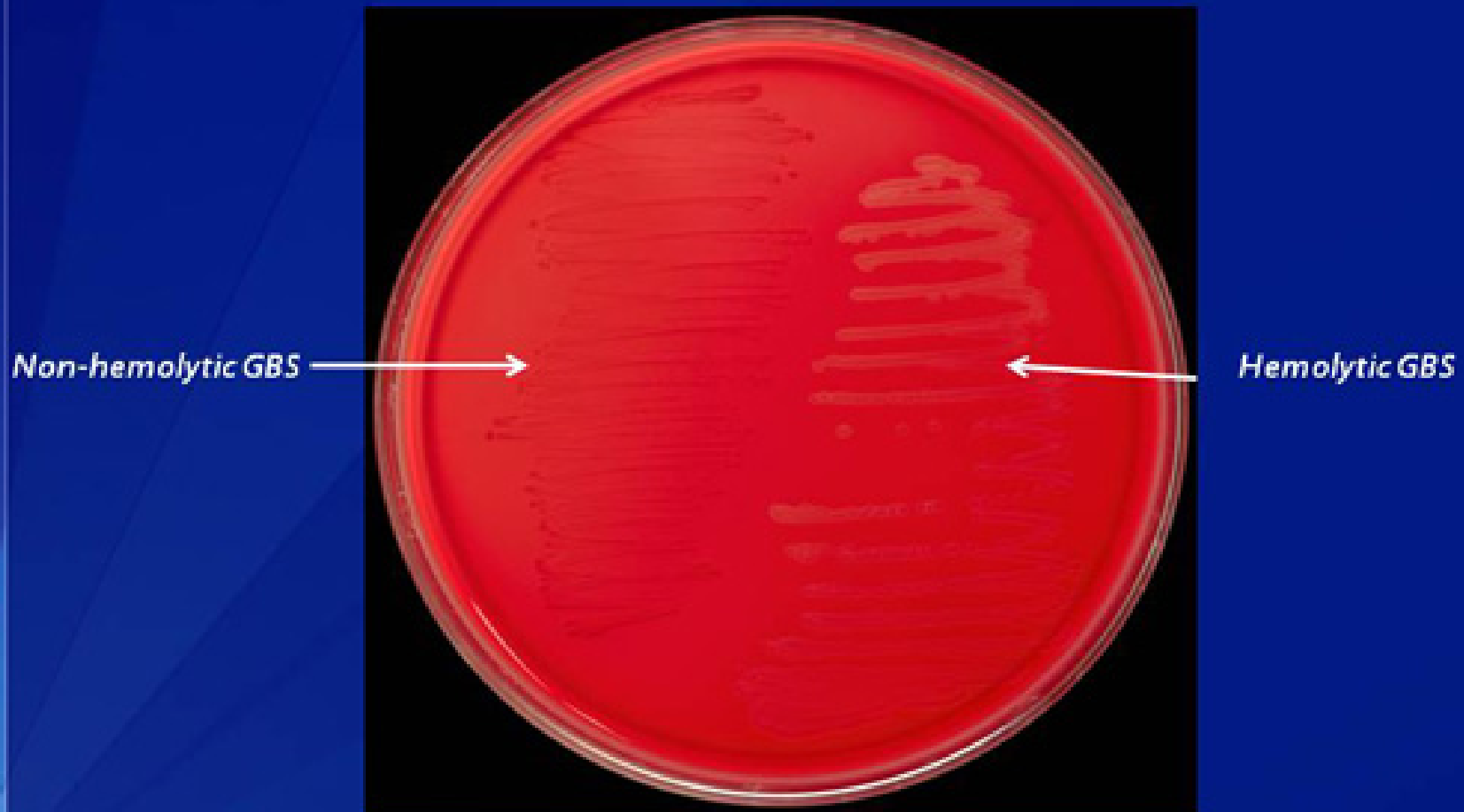
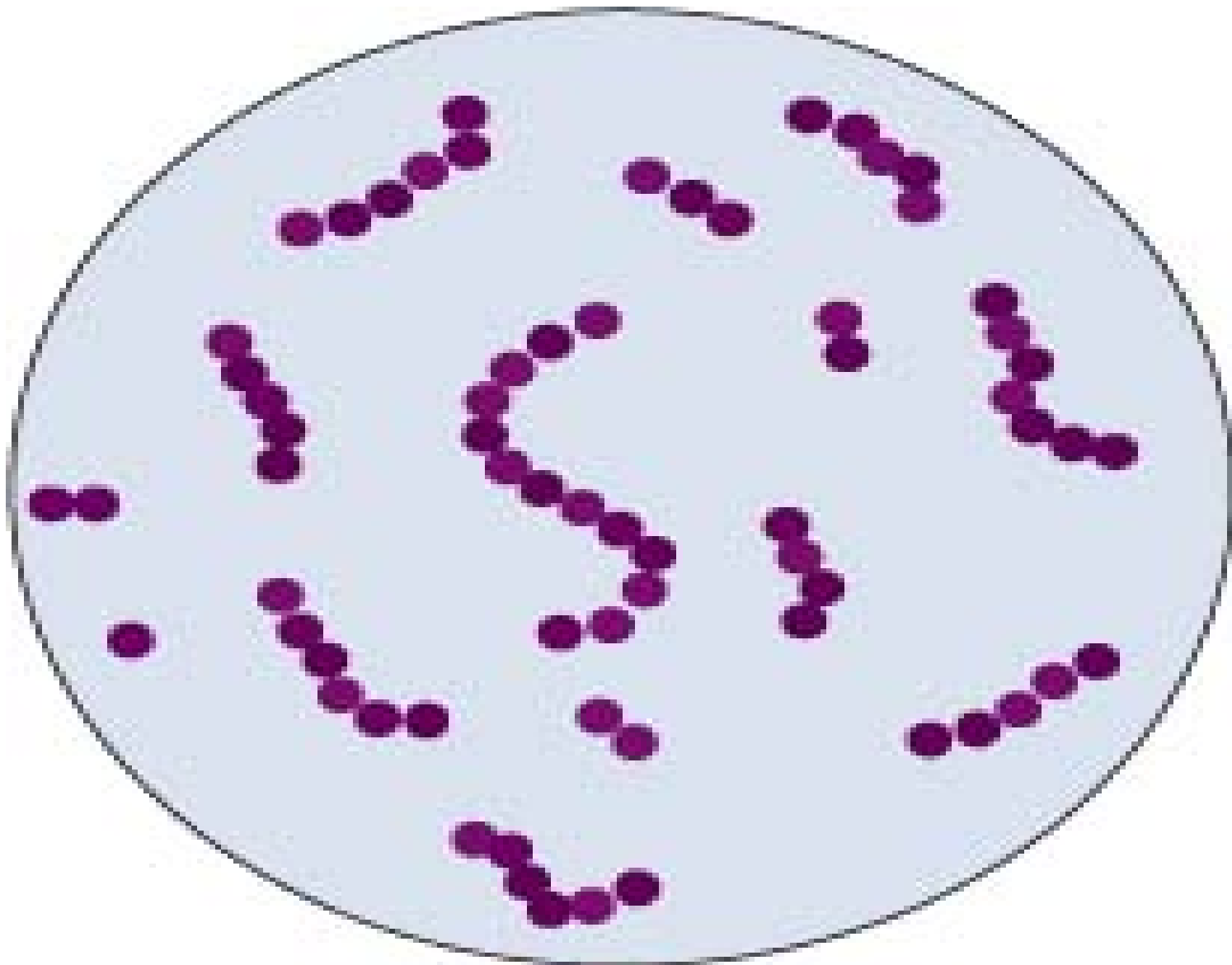
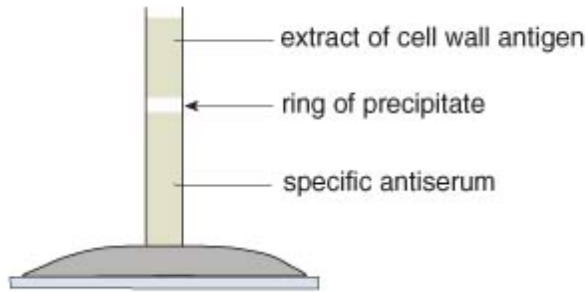
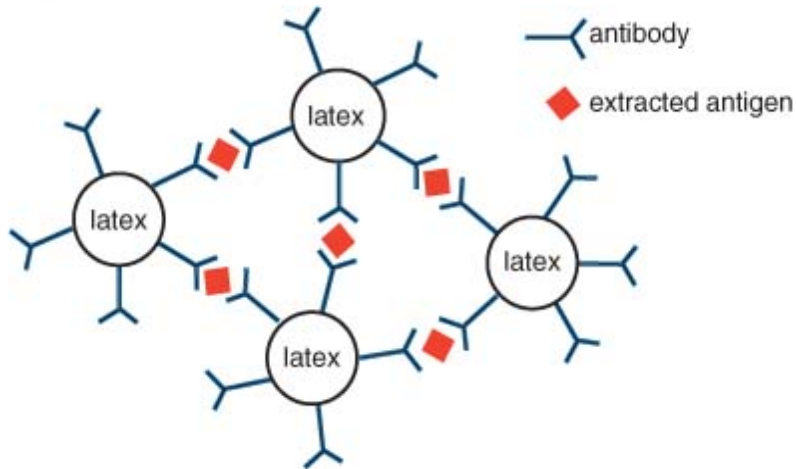


Figure 15.1 Streptococcal chains.





**Figure 15.3** Diagrammatic representation of the latex agglutination test for streptococcal identification.



## Pathogenesis and pathogenicity

Most of the streptococci that are pathogenic for animals are pyogenic and are associated with abscess formation, other suppurative conditions and septicaemia. *Streptococcus suis*, which is non-pyogenic, is a major pathogen of pigs, causing<sup>234</sup> septicaemia, meningitis and pneumonia among other conditions. Beta-haemolytic streptococci are generally more pathogenic than those producing alpha-haemolysis. Virulence factors include enzymes and exotoxins such as streptolysins (haemolysins), hyaluronidase, DNase, NADase, streptokinase and proteases. The specific action and significance of some of these factors are poorly understood. Polysaccharide capsules, which are major virulence factors

of *S. pyogenes*, *S. pneumoniae* and most strains of *S. equi*, are antiphagocytic. The cell-wall M proteins of *S. pyogenes*, *S. equi* and *S. porcinus* are also antiphagocytic. In the absence of antiphagocytic factors, these bacteria are rapidly killed by phagocytes.

## Diagnostic procedures

History, clinical signs and pathology may be indicative of certain streptococcal infections such as strangles.

- Streptococci are highly susceptible to desiccation and specimens should be cultured promptly. Pus or exudate collected on swabs should be placed in transport medium if specimens cannot be processed immediately.
- A sensitive technique using the polymerase chain reaction has been developed for detecting both viable and non-viable *S. equi* in nasal swabs (Timoney and Artiushin, 1997).
- Chains of Gram-positive cocci may be demonstrable in smears from specimens.
- Specimens should be cultured on blood agar, selective blood agar and MacConkey agar. Plates are incubated aerobically at 37°C for 24 to 48 hours.
- Identification criteria for isolates:
  - Small, translucent colonies, some of which may be mucoid
  - Type of haemolysis on blood agar
  - Chains of Gram-positive cocci
  - No growth on MacConkey agar with the exception of *Enterococcus* species
  - Negative catalase test
    - Lancefield grouping
    - Biochemical test profile.

**Table 15.2** Differentiation of equine group C streptococci by sugar fermentation.

	Trehalose	Sorbitol	Lactose	Maltose
<i>S. equi</i>	-	-	-	+
<i>S. zooepidemicus</i>	-	+	+	+(-)
<i>S. equisimilis</i>	+	-	v	+

v, variable reactions.

(-), a few strains are negative.

**Table 15.3** Differentiation of streptococci which cause bovine mastitis.

	Haemolysis on blood test agar	CAMP test	Aesculin hydrolysis (Edwards medium)	Growth on MacConkey agar	Lancefield group
<i>Streptococcus agalactiae</i>	$\beta$ ( $\alpha, \gamma$ )	+	-	-	B
<i>S. dysgalactiae</i>	$\alpha$	-	-	-	C
<i>S. uberis</i>	$\alpha$	-	+	-	Not assigned
<i>Enterococcus faecalis</i>	$\alpha$	-	+	+	D

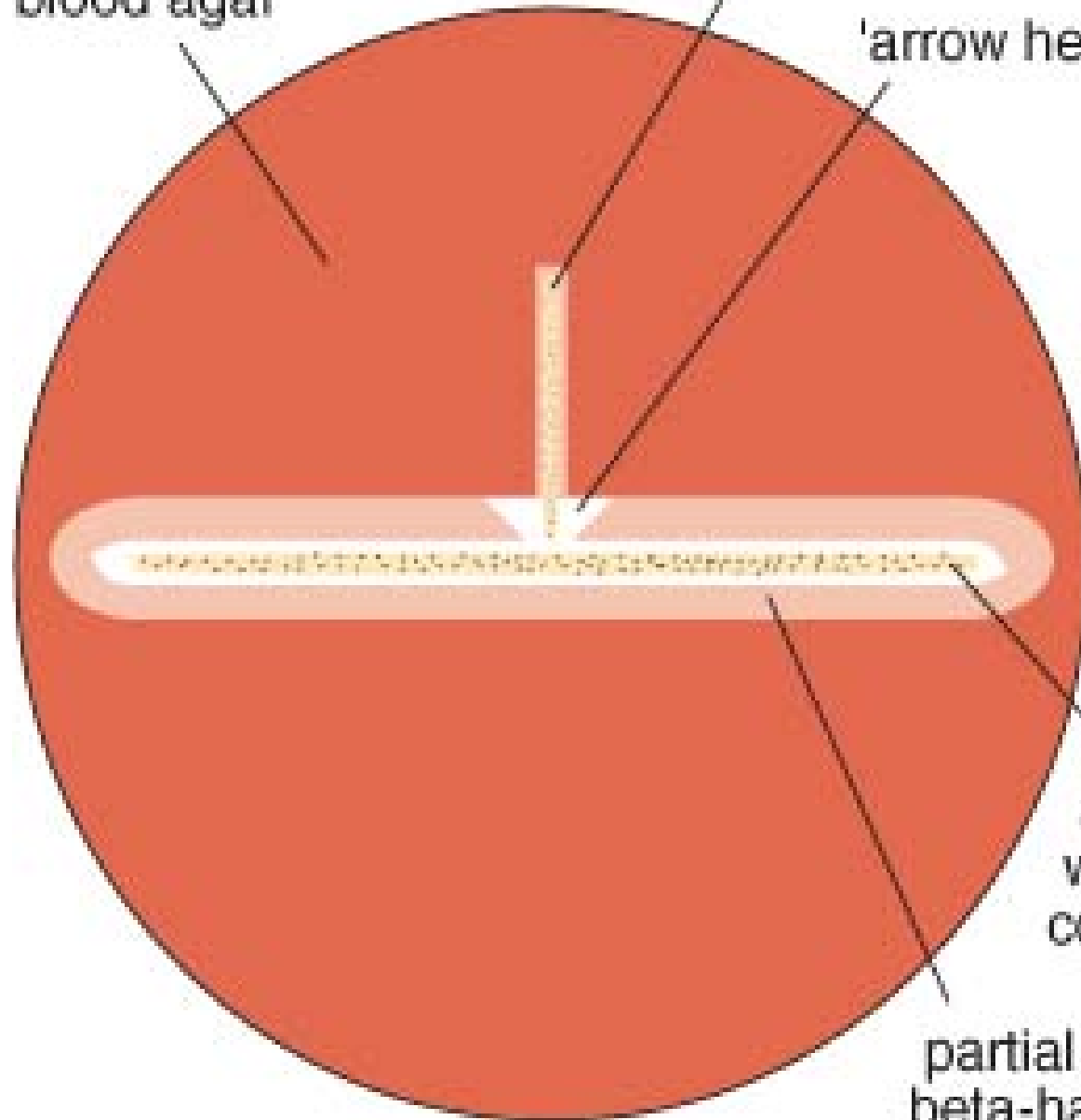
## CAMP Test

CAMP factor is a diffusible, heat-stable protein produced by group B streptococci. This is a synergistic test between *Staphylococcus aureus* and *Streptococcus agalactiae*. *S. agalactiae* produces CAMP factor. *S. aureus* produces sphingomyelinase C, which binds to red blood cell membranes. The two bacteria are streaked at 90° angles of one another, then incubated at 37°C for 24 hours. They do NOT touch. The CAMP factor produced by *S. agalactiae* enhances the beta-hemolysis of *S. aureus* by binding to already damaged red blood cells. As a result, an arrow of beta-hemolysis is produced between the two streaks. The test is presumptive for *S. agalactiae* that produces CAMP factor.

sheep or ox  
blood agar

streak of *Streptococcus agalactiae*

'arrow head' of clear haemolysis



streak of  
*Staphylococcus aureus*  
with narrow zone of  
complete haemolysis

partial haemolysis caused by  
beta-haemolysin of *S. aureus*

# CAMP Test Comparison

Positive zone of enhanced hemolytic activity.



Positive for GBS

Negative reaction of non-enhanced zone of hemolytic activity.



Not Positive for GBS



*Staphylococcus aureus*

zone of  $\beta$ -hemolysis  
of *S.aureus*

*Streptococcus agalactiae*



*Corynebacterium pseudotuberculosis*

**CAMP test**  
**reverse CAMP test**

Species	Lancefield groups	Haemolysis on blood agar <sup>a</sup>	Hosts	Consequences of infection	Usual habitat
<i>S. pyogenes</i>	A	β	Humans	Scarlet fever, septic sore throat, rheumatic fever	Mainly upper respiratory tract
<i>S. agalactiae</i>	B	β (α,γ)	Cattle, sheep, goats Humans, dogs	Chronic mastitis Neonatal septicaemia	Milk ducts Vagina
<i>S. dysgalactiae</i>	C	α (β,γ)	Cattle Lambs	Acute mastitis Polyarthritis	Buccal cavity, vagina, environment
<i>S. equisimilis</i> ( <i>S. dysgalactiae</i> subsp. <i>equisimilis</i> )	A, C, G, L <sup>b</sup>	β	Horses Pigs, cattle, dogs, birds	Abscesses, endometritis, mastitis Suppurative conditions	Skin, vagina
<i>S. equi</i> ( <i>S. equi</i> subsp. <i>equi</i> )	C	β	Horses	Strangles, suppurative conditions, purpura haemorrhagica	Upper respiratory tract, guttural pouch
<i>S. zooepidemicus</i> ( <i>S. equi</i> subsp. <i>zooepidemicus</i> )	C	β	Horses Cattle, lambs and pigs Dogs	Mastitis, pneumonia, navel infections Suppurative conditions, septicaemia Haemorrhagic pneumonia	Mucous membranes Skin, mucous membranes
<i>Enterococcus faecalis</i>	D	α (β,γ)	Many species	Suppurative conditions following opportunistic invasion	Intestinal tract
<i>S. suis</i>	D	α (β)	Pigs Cattle, sheep, horses, cats Humans	Septicaemia, meningitis, arthritis, bronchopneumonia Suppurative conditions Septicaemia, meningitis	Tonsils, nasal cavity
<i>S. porcinus</i>	E, P, U, V <sup>b</sup>	β	Pigs	Submandibular lymphadenitis	Mucous membranes

## Enterococcus species

1. These enteric organisms are found in the intestinal tract of animals and humans.
2. They are opportunistic pathogens and differ phenotypically from the Streptococcus species in two important respects:
  - a) They tolerate bile salts and grow on MacConkey agar as red, pin-point colonies.
  - b) Some isolates are motile.
3. *E. faecalis* is the species most frequently isolated from domestic animals and poultry. *E. faecium* is also found in the intestinal tract of many species.
4. Enterococcus species cause opportunistic infections such as wound infections in all species, mastitis in cattle, and urinary tract and ear infections in dogs.
5. They are intrinsically resistant to many antimicrobial agents, which can cause problems relating to the selection of antimicrobial drugs.

**thank you for Listening**