## **Diagnosis of Streptococci**

## **Group A streptococci**

#### LABORATORY FEATURES

*Specimens*: Include a throat swab (avoiding saliva contamination) or swabs of pus and serous fluid depending on the site of infection, and blood for culture. Testing for ASO antibody in serum is helpful in diagnosing rheumatic fever.

#### Morphology

Streptococci are Gram positive cocci, occurring characteristically in short chains, but also in pairs and singly. Long chains are formed in fluid cultures. The organisms are non-motile. Some strains are capsulated.



Blood agar: S. pyogenes produces beta-haemolytic colonies



**Catalase test** can be used to differentiate streptococci (negative) from staphylococci (positive)



Antibiotic sensitivity: Only Streptococcus pyogenes among the group A beta streptococci is sensitive to bacitracin, as shown by a zone of inhibition.



#### PYR test

Test is designed for presumptive identification of group A streptococci (*Streptococcus pyogenes*) and enterococci by determining the presence of the enzyme *L*-pyrrolidonyl arylamidase (PYR).

This enzyme hydrolyzes the amide pyroglutamyl $\beta$ -naphthylamide to produce *L*-pyrrolidone and  $\beta$ -naphthylamine, both of which are colorless.  $\beta$ -naphthylamine will react with *p*-dimethylaminocinnamaldehyde and form a red precipitate.



**PYR DISK TEST** The disk (containing substrate)on the left was inoculated with *Streptococcus pyogenes* (PYR-positive); the disk on the right contains *Streptococcus agalactiae* (PYR-negative)

### *Group B streptococci Streptococcus agalactiae* Culture

**Blood agar:** Most strains of *S. agalactiae* produce grey mucoid colonies about 2 mm in diameter, surrounded by a small zone of *beta*-haemolysis. About 5% of strains are nonhaemolytic. Placing discs of penicillin and

gentamicin on the plate can help to identify these strains (penicillin sensitive, gentamicin resistant).



*MacConkey agar:* Most strains grow on this medium. *Neomycin blood agar:* A useful selective medium for isolating *S. agalactiae* from urogenital specimens.

# CAMP (Christie, Atkins, Munch, Peterson) test to identify presumptively *S. agalactiae*

*Streptococcus agalactiae* secretes a protein called the" CAMP" factor and when it comes into contact with a beta hemolysin produced by a specific strain of *Staphylococcus aureus* causes a synergistic, enhanced hemolysis.

The CAMP test: id of *Streptococcus agalactiae* 

- (A) Streptococcus (group B) positive test (enhanced hemolysis)
- (B) Streptococcus pyogenes (group A) negative test
- (C) Staphylococcus aureus – replaced by Clostridium perfringens in reverse CAMP test (see next slide)





## Hippurate hydrolysis test

S. agalactiae hydrolyzes hippurate.

Hippurate is the glycine conjugate of benzoic acid. When hippurate is hydrolysed by an organism, glycine and benzoic acid are formed. In the rapid test (2 hour) **Ninhydrin** is used as the indicator to detect Glycine. Ninhydrin reacts with glycine to form a deep blue or purple colour.

