Name: Date:
Group Nr.:

LABORATORY PROTOCOL Nr.3

Topic: Staphylococci, Streptococci

Task Nr.1: Identification of Staphylococci

1a) Free plasmacoagulase

Procedure:

Add several colonies of the test strain to tube with 0.5 ml of rabbit plasma.
The clot formation is evaluated after 1 hour of incubation at 37°C and then the next day.

Conclusion:

1b) Bound coagulase

Procedure:

On a slide, mix a drop of rabbit plasma with the tested strain of *Staphylococcus*.

Conclusion:

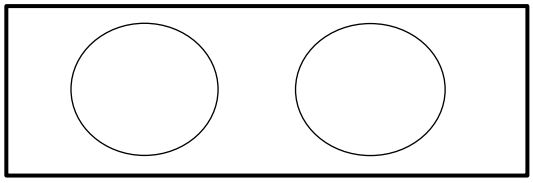
1c) Catalase test

Using for differentiation of staphylococci and streptococci.

Procedure:

After mixing the test colony with a drop of H_2O_2, staphylococci release bubbles.

Drawing:



Conclusion:

Name: Date:
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Task Nr.2: Identification of Streptococci

2a) Optochin test

Differentiation of *Streptococcus pneumoniae* from other viridans streptococci.

Procedure:

Inoculate culture of *Streptococcus pneumoniae* on half of the agar and a culture of viridans streptococci on the other half. Cultures of both strains must not overlapped !!!
Using a needle, place a disc with optochin on both inoculated strains.

Incubate for 24 hours at 37°C and check the plate.

Zone size (mm)	Strain
≥ 14mm	*Streptococcus pneumoniae*
< 14mm	viridans streptococci

Conclusion:

2b) CAMP test

Principle:

It is used to identify group B of beta-hemolytic streptococci.

Procedure:

Inoculate the line of tested streptococcal strain on blood agar and perpendicularly to this line inoculate the line of *Staphylococcus aureus.*

Incubate for 24 hours at 37°C and check the plate.

Conclusion:

2c) Bile solubility test

Principle:

Through deoxycholate, autolysin is activated in the wall of pneumococci which leads to the cell disruption. Due to the absence of autolysin, there is no change in viridans streptococci.

Procedure:

Put a drop of 10% sodium deoxycholate on examined colony. After 15 minutes, check the colonies.

Conclusion:

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2d) Latex agglutination

Principle:

Typing of beta-hemolytic streptococci according to specific type of polysaccharide C in streptococcal cell wall (Lancefield antigen).

Procedure:

From the tested strain and reagents, create an extract. Drop an extract onto plate. Drop the latex suspension with antiserum next to the drop of extract and mix it. Swing the plate gently and read the reaction within 1 minute. A positive reaction (agglutination) is visualised by precipitate.

Conclusion:

2e) Bile-esculin test

Principle:

It is used to distinguish enterococci from streptococci. Bile suppresses the growth of streptococci and enterococci hydrolyze esculin resulting to black discoloration of the medium.

Procedure:

Using a loop, inoculate the test strain to the bottom of the tube. Incubate for 24 hours at 37°C and check the growth.

Conclucion: