STREPTOCOCCAL ANTISERA

for *in vitro* diagnostic use

Application
The diagnostic streptococcal antisera from SSI Diagnostica are intended for qualitative identification and typing of streptococci by means of the Neufeld test[1] and the Lancefield test[2,3].

Description
Streptococcal antisera from SSI Diagnostica are raised in rabbits. Streptococcal antisera can be used for identification of Group A, B, C, D, F, G and L streptococci, serotyping of Group B streptococci and serotyping of *S. suis*. The antisera are supplied in 1 mL vials (sodium azide as preservation). Cross-reactions have been removed by absorption.

Principle
Neufeld test: by mixing specific antisera with a streptococci culture, the capsular antigens are determined. The capsular reaction is a result of an *in situ* immune precipitation between the streptococcal capsular polysaccharide and its homologous antibody. A positive reaction is seen by use of a microscope where the capsule becomes visible and the streptococci agglutinates. The size of the capsule depends on the serotype as well as the growth conditions.
Lancefield test: when an acid antigen extract is mixed with a specific antiserum directed against bacterial surface components, the cells are bound together through antigen-antibody bonds to form aggregates (precipitation). This is visible to the naked eye as snow in the capillary tube.

Materials Required but not Provided
Neufeld test:
- 5% blood agar plate
- 1 µL inoculation loop
- Pipette or any other utility that can make a droplet
- Glass slides and cover slip
- Immersion oil
- Saline, pH 7.4
- Phase contrast microscope (100 X magnification, oil immersion lens)

Lancefield test:
- 5-10% blood agar plate
- Glucose broth
- 1 µL Inoculation loop
- Centrifuge
- Pipette or any other utility that can make a droplet
- 0.06N, 0.1N and 0.2N HCl
- Water bath (100 °C)
- Phenol red (indicator)
- 0.2N NaOH
- Capillary tubes

Procedure
General
Each serotype will react positive either in the Lancefield test or the Neufeld test (the preferred method will be indicated on the certificate). The result is often more evident when compared with a negative control.

Neufeld test
- The streptococci is grown overnight on a 5% blood agar plate.
- Apply a small drop (3-6 µL) of saline on a glass slide.
- Transfer a small amount of culture from the blood agar plate with an inoculating loop and mix well.
- An equal amount of antiserum is added and mixed thoroughly with the droplet.
- Immediately place a cover slip on top of the mixture (must not dry out).
- Examine the mixture under a phase contrast microscope. The reaction is stable for half an hour (provided no dry out).
- If the capsule becomes visible (the bacterium appears swollen) the reaction is positive.

Lancefield test (modified version)
- The streptococci is grown overnight on a 5% blood agar plate.
- Add a few colonies into 6 mL glucose broth and incubate at 35-37 °C overnight.
- Centrifuge the suspension for 10 min at 3000 rpm and remove the supernatant.
- Add 0.1 mL of either 0.06N, 0.1N or 0.2N HCl to the bacteria pellet (the preferred method will be indicated on the label).
- The acid suspension is placed in a water bath (100 °C) for 15 minutes.
- Cool the acid suspension under tab water.
- The pH-value is adjusted to approximately 7 by addition of droplets of 0.2N NaOH until the colour is brown/orange (use phenol red as a pH-indicator, red (pH > 8.2) - yellow (pH < 6.4)).
- Centrifuge the suspension for 10 min at 3000 rpm and transfer the supernatant (acid antigen extract) to a new glass.
- Equally amounts of the antiserum (first) and the acid antigen extract (second) are sucked up with the capillary tube. The antiserum must be in the upper part of the capillary tube to diffuse through the acid extract.
- Precipitation will occur if positive. Read the result against a light source.

Storage and Shelf Life
Store at 2-8 °C in a dark place. Expiry date is printed on the package. Turbidity due to lipoprotein precipitation is sometimes seen after prolonged storage. Precipitation and/or contamination can be removed by centrifugation (10,000g) followed by sterile filtration (0.22 µm).

References

1. Austrian R. The Quelling Reaction, A neglected Microbiologic Technique. The Mount Sinai Journal of Medicine 1976; 43, 669-709.
2. Lancefield, R. C. A Serological Differentiation of Specific Types of Bovine hemolytic streptococci (group B). J. Exp Med. 1934; 59:441-458.
3. Slotved HC, Sauer S, Konradsen HB. False-negative results in typing of group B streptococci by the standard ILncefield antigen extraction method. J Clin Microbiol. 2002 May;40(5):1882-3.

Information and ordering

SSI Diagnostica A/S
Herredsvejen 2
3400 Hillerød
Denmark
Tel.: +45 4829 9100
Fax: +45 4829 9179
info@ssidiagnostica.com
ssidiagnostica.com

Presafe Denmark A/S

Quality System DS/EN ISO 9001

Quality System DS/EN ISO 13485

CE

3rd Edition • December 2016 • 64239