SSI DIAGNOSTICA

Instructions for use

STREPTOCOCCUS SUIS AND GROUP B ANTISERA

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STREPTOCOCCUS SUIS AND GROUP B ANTISERA

For *in vitro* diagnostic use

Intended use

The *Streptococcus suis* antisera are for visual qualitative identification and bacterial serotyping by means of the Neufeld test[1] (also named the capsular reaction test or the Quellung reaction) or the Lancefield test[2,3].

The *Streptococcus* group B antisera are intended for visual qualitative identification and bacterial serotyping by means of the Lancefield test[2,3].

It is important to use only pure culture isolates of *Streptococcus* for determination of bacterial antigens.

Description

Streptococcus antisera from SSI Diagnostica are supplied in vials with 1 mL (sodium azide as preservation).

The *Streptococcus* antisera described in this IFU are used for serotyping of Group B streptococci or serotyping of *S. suis*.

Streptococcus antisera are for serotyping directly from a blood agar plate using the Neufeld test or from an extract using the Lancefield test.

The *Streptococcus* antisera are polyclonal, raised in rabbits and absorbed to eliminate cross-reacting antibodies when necessary.

Streptococcus antisera are for use by laboratory professionals and/or healthcare professionals only.

Principle

Streptococcus antisera for serotyping using the Neufeld or the Lancefield test.

Neufeld test: by mixing specific antisera with a *Streptococcus* 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 phase contrast microscope where the capsule becomes visible and the streptococci agglutinate. 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.

Precautions

- Before using SSI Diagnostica *Streptococcus* antisera, confirm that the isolate/strain is a pure culture of *Streptococcus*.

- If an isolate is difficult to serotype this may be because the isolate did not grow well and therefore also the polysaccharide capsule was not expressed well. A well-expressed polysaccharide capsule is crucial for serotyping. In such cases try to regrow the isolate several times, grow the isolate on 10% blood agar instead of 5% blood agar, in Serum broth instead of Todd Hewitt broth or grow the isolate in air with 5% CO_2 instead of in air without additional CO_2.

- Turbidity in the antisera may occur due to lipoprotein precipitation after prolonged storage. If you experience precipitation, it can be removed by centrifugation (10,000 x g) followed by sterile filtration (0.22 µm).

- The antisera have only been validated for confirmation and serotyping with the serotypes indicated on the label and by the below described methods.

- Antisera that have accidentally been frozen should not be used.

- Do not use the antisera after the expiry date.

- Inspect the vial before use to ensure it is intact. Any damaged vials should be discarded.

Materials provided

Streptococcus antisera from SSI Diagnostica are supplied in vials with 1 mL (sodium azide as preservation).

Materials required but not provided

Neufeld test:

- 5-10% blood agar plate
- Physiological saline pH 7.4
- 1 µL inoculation loop
- Pipette
- Glass slides and cover slip
- Immersion oil
- Phase contrast microscope (100 x magnification, oil immersion lens)
- Incubator (35-37 °C)

Lancefield test:

- 5-10% blood agar plate
- Glucose broth
- 1 µL Inoculation loop
- Centrifuge
- Pipette
- 0.06N, 0.1N and 0.2N HCl
- Phenol red (indicator)
- 0.2N NaOH
- Glass tube
- Capillary tubes
- Incubator (35-37 °C)
- Water bath (100 °C)

Storage and stability

Streptococcus antisera must be stored at 2-8 °C in a dark place. Do not freeze. Stored under these conditions the antisera may be used up to the date of expiry shown on the product label.

The in-use stability is not affected by working with the antiserum on the bench throughout the day if it is stored at 2-8 °C when not in use.

Preservative

The *Streptococcus* antisera contain less than 0.1% sodium azide (NaN_3) as preservative.

Sample collection and storage

For sample collection and storage, please follow your local standard procedure.

Quality control

Before use check the vial to ensure there is no damage and/or leaks. In case of damage or leaks discard the vials.

As positive controls, *Streptococcus* strains with known serotypes should be used.

As negative controls, physiological saline or growth media (without any strains) or *Streptococcus* strains with known serotypes should be used. These negative controls should show no agglutination.

To confirm that an observed agglutination is not a false positive reaction, make a control on the isolate/strain for self-agglutination. The self-agglutination test is done by using physiological saline instead of antiserum in the Neufeld test. If a strain/isolate agglutinates when only saline is added, it is self-agglutinating and may cause false positive reactions. To consider self-agglutinating isolates/strains as true positive the capsule should become visible in the Neufeld test (see figure 1).

Before using a new lot, or a new shipment of the same lot or the product is used by a new operator, please perform quality control testing with positive and negative controls of *Streptococcus* strains with known serotypes before testing of isolates/strains.

Procedure

For best result it is recommended to use the Neufeld test for *Streptococcus suis*.

The result is often more evident when compared with a negative control.

Neufeld test:

1. The streptococci are grown overnight at 35-37 °C on a 5-10% blood agar plate.

2. Apply a small drop (3-6 µL) of saline on a glass slide.

3. Transfer a small amount of culture from the blood agar plate with an inoculating loop and mix well.

4. An equal amount of antiserum is added and mixed thoroughly with the droplet.

5. Immediately place a cover slip on top of the mixture (must not dry out).

6. Examine the mixture under a phase contrast microscope. The reaction is stable for half an hour (provided no dry out).

7. If the capsule becomes visible (the bacterium appears swollen) the reaction is positive.

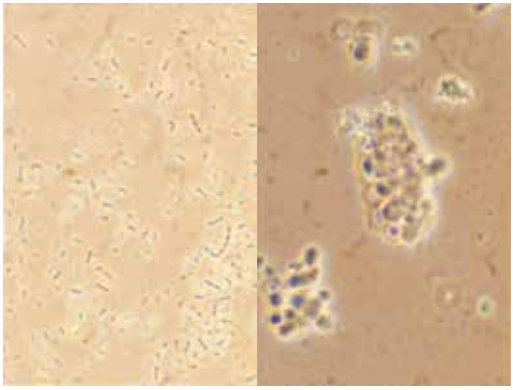

Figure 1. The Neufeld test. Negative (left) and positive reaction (right).

Lancefield test

1. The streptococci are grown overnight at 35-37 °C on a 5-10% blood agar plate.

2. Add a few colonies into 6 mL glucose broth and incubate at 35-37 °C overnight.

3. Centrifuge the suspension for 10 minutes at 3,000 rpm and remove the supernatant.

4. 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 antiserum label).

5. The acid suspension is placed in a water bath (100 °C) for 15 minutes.

6. Cool the acid suspension under tab water.

7. The pH-value is adjusted to approximately 7 by addition of droplets of 0.2N NaOH until the color is brown/orange (use phenol red as pH-indicator, red (pH > 8.2) - yellow (pH < 6.4)).

8. Centrifuge the suspension for 10 minutes at 3.000 rpm and transfer the supernatant (acid antigen extract) to a new glass.

9. Equal amount 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 down through the acid extract.

10. Read the result against a light source.

11. Precipitation looking like snowfall will occur if positive.

Figure 2. The Lancefield test. Positive reaction.

Interpretation of results

Neufeld test

If the capsule becomes visible (the bacterium appears swollen) the reaction is positive.

Lancefield test

Precipitation looking like snowfall will occur if positive.

Disposal

Follow your local procedures and/or national guidelines for disposal of biological materials.

Limitations

- The culture must be confirmed *Streptococcus* before serotyping using antisera from SSI Diagnostica.

Performance

Sensitivity, specificity and repeatability

	Percent (number positive/ actual positive)	95% confidence interval
Sensitivity	100% (46/46)	92%-100%
Specificity	100% (180/180)	98%-100%
Repeatability	100% (339/339)	99%-100%

Table 1: Sensitivity, specificity and repeatability for streptococcus antisera

Reproducibility

The reproducibility within the different groups of antisera and all antisera combined is 100% (confidence interval 99.4%-100%). Therefore, all produced antisera have a high level of reproducibility throughout time and lots.

Incident reporting

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or patient is established.

Quality certificate

SSI Diagnostica's development, production and sales of *in vitro* diagnostics are quality assured and certified in accordance with ISO 13485.

REF IVD CE

For the list of products, see our website:
https://ssidiagnostica.com/international/solutions/antisera/streptococcus-antisera/streptococcus-for-precipitation/

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 Lancefield antigen extraction method. J Clin Microbiol. 2002 May;40(5):1882-3.

Information and ordering

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