SSI DIAGNOSTICA

Instructions for use

PNEUMOTEST KIT

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PNEUMOTEST KIT

For *in vitro* diagnostic use

Intended use

The SSI Diagnostica Pneumotest Kit is intended for visual qualitative serogrouping and serotyping of *Streptococcus pneumoniae* (pneumococcus) by use of the Neufeld test (also named the capsular reaction test or the Quellung reaction)[1]. The Pneumotest Kit identifies the 23 vaccine related serogroups and serotypes by use of the Chessboard method (see table 1)[2]. The Chessboard method identifies and separates the vaccine related serogroups and serotypes from the non-vaccine serotypes.

The 23 vaccine related serogroups and serotypes that can be detected with the Pneumotest Kit are the serogroups 6, 7, 9, 10, 11, 12, 15, 17, 18, 19, 22, 23, 33 and the serotypes 1, 2, 3, 4, 5, 8, 14 and 20 (see table 1).

This product is for testing identified and confirmed, pure cultured isolates and strains of pneumococcus.

Description

The Pneumotest Kit contains 12 vials of 1 mL ready-to-use pneumococcus Pool antisera (Pool A, B, C, D, E, F, H and Pool P, Q, R, S, T). Each vial contains antiserum for approximately 300 tests. The pneumococcus Pool antisera in the Pneumotest Kit are for visual qualitative serogrouping and serotyping of pure cultures of capsulated pneumococci using the Neufeld test. The pneumococcus antisera are polyclonal, raised in rabbits and absorbed to eliminate cross-reacting antibodies when necessary.

The antisera may have a light yellow to brown colour. This will not affect the result of the Neufeld test.

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

Principle

The Neufeld test is performed by mixing antiserum and a pneumococcal isolate/strain. The antiserum-isolate mix is inspected in a phase contrast microscope. The pneumococcal capsule becomes visible and the pneumococci agglutinate

if the reaction is positive. The pneumococcal capsule becomes visible and appears swollen as a result of a capsular reaction which is an *in situ* immunoprecipitation (an antigen-antibody reaction) between the pneumococcal capsular polysaccharide (the antigen) and its homologous antibodies in the antiserum. The size and visibility of the capsule depend on the serotype as well as the growth conditions of the pneumococcal isolate/strain.

The Pneumotest Kit is designed in such a way that the 23 vaccine related serogroups and serotypes must react with two Pool antisera, one positive reaction in Pool A, B, C, D, E, F or H and one positive reaction in Pool P, Q, R, S or T (see the Chessboard scheme, table 1)[2]. Non-vaccine groups/types react only with one Pool serum of the Pool A, B, C, D, E, F and H (see table 1).

To determine the exact serotype within the identified serogroups by the Pneumotest Kit, SSI Diagnostica offers antisera to determine 92 serotypes specifically (see ssidiagnostica.com).

Precautions

- Before using SSI Diagnostica pneumococcus antisera, confirm that the isolate/strain is a pure culture of *Streptococcus pneumoniae*.
- Some isolates/strains, and in particular non-capsulated (rough) isolates/strains, may self-agglutinate and cause false positive reactions.
- 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 in the section “Limitations” and by the

below described method.

- Excessive amount of culture compared to antisera might cause false positive reactions.
- 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

The SSI Diagnostica Pneumotest Kit contains 12 pneumococcus Pool antisera A, B, C, D, E, F, H and P, Q, R, S and T.

The antisera are supplied in vials containing 1 mL ready-to-use antisera.

Materials required but not provided

- Serum broth, Todd Hewitt broth or 5-10% blood agar plate
- Physiological saline (0.9% NaCl)
- Pipette
- 1 µL inoculation loop
- Glass slide and cover slip
- Immersion oil

- Phase contrast microscope (100 x magnification, oil immersion lens)
- Incubator (35-37 °C)

Storage and stability

The Pneumotest Kit 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.

Pneumococcus antisera have been tested after being stored at 37 °C for up to four weeks. The antisera were still fully functional.

Preservative

The pneumococcus antisera contain less than 0.1% sodium azide (NaN_3) as a 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 that there is no damage and/or leak. In case of damage or leak, discard the vial.

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

As negative controls, physiological saline or growth media (without any strains) and pneumococcal 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 that 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.

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 strains with known serotypes before testing of isolates/ strains with unknown serotypes.

Procedure

To perform the Neufeld test, do the following (after growing a pure isolate in broth or on a plate, see recommended media in the section “Materials required but not provided”).

1. Dispense one drop (2 - 4 µL) of a freshly grown broth culture on a glass slide. Alternatively, freshly grown colonies from a blood agar plate can be suspended in physiological saline. The concentration of bacteria should be at a level where you can clearly see single cells distributed with distance in the microscope field.
2. Add an equal amount, one drop (2 - 4 µL) of antiserum and mix.
3. Immediately place a cover slip on top of the mixture (must not dry out).
4. Examine the mixture under a phase contrast microscope within 5 minutes.

5. If the capsule becomes visible (the bacterium appears swollen) the reaction is positive (see figure 1). The bacteria may also agglutinate. Note if you only see agglutination and no swelling of the capsule, this agglutination may be a false positive reaction (see the section “Quality control”).
6. Use the interpretation chessboard scheme (see table 1) to interpret the result and determine the serogroup or serotype of the isolate/strain.

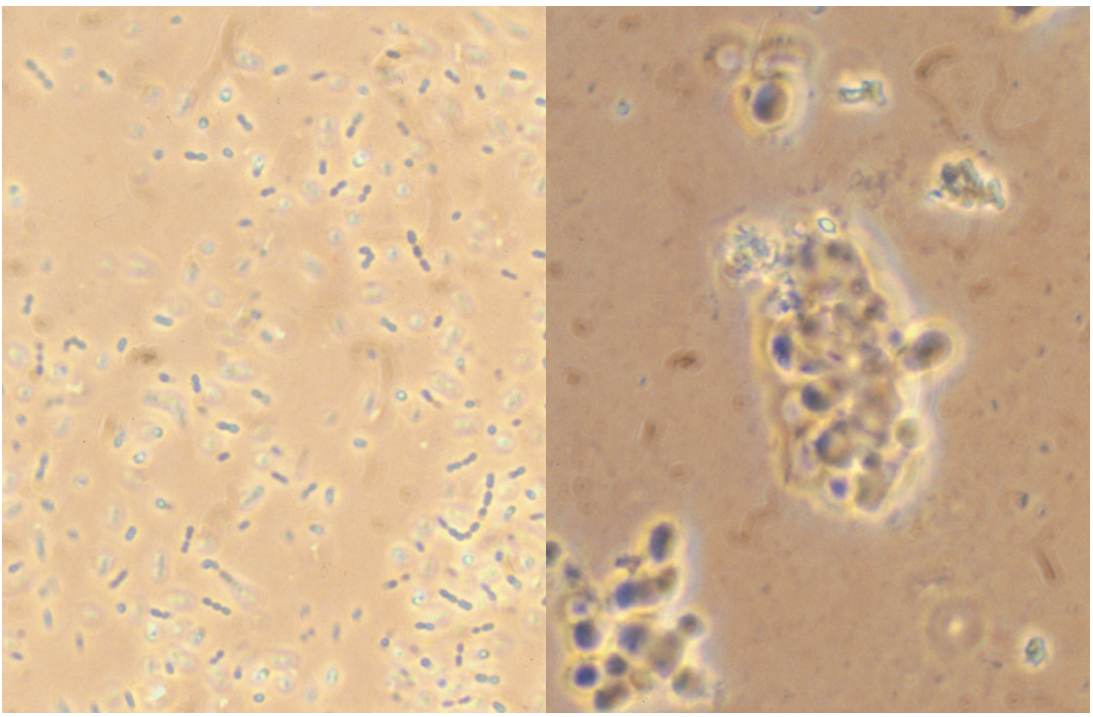

Figure 1. The Neufeld test on a pneumococcal strain serotype 3. A negative reaction is shown on the left panel. A positive reaction on the right panel.

Interpretation of results

For serogroup and serotype determination of a pneumococcal isolate/strain, test the isolate with pneumococcus Pool antisera provided in the Pneumotest Kit using the Neufeld test.

1. First, test the isolate in pneumococcus Pool A, B, C, D, E, F and H antisera.
2. Proceed by testing the isolate in pneumococcus Pool P, Q, R, S and T antisera.
3. Interpret the result on the chessboard scheme (see table 1). If the isolate is positive in Pool A and Pool P and negative in all other Pool antisera, the isolate is a serotype 1. Is the isolate positive in Pool F and Pool S, and negative in all other Pool antisera, the isolate is a serogroup 17 (see table 1).

POOL	P	Q	R	S	T	Non-vaccine groups/ types
A	**1**	**18** (18F, 18A, 18B, **18C**)	**4**	**5**	**2**	
B	**19** (**19F**, **19A**, 19B, 19C)	**6** (6A, **6B**, 6C,6D)	**3**	**8**		
C	**7** (**7F**, 7A, 7B, 7C)				**20**	24 (24F, 24A, 24B) 31, 40
D			**9** (9A, 9L **9N**, **9V**)		**11** (11F, **11A**, 11B, 11C, 11D)	16 (16F, 16A) 36, 37
E			**12** (**12F**, 12A, 12B)	**10** (10F, **10A**, 10B, 10C)	**33** (**33F**, 33A, 33B, 33C, 33D(	21, 39
F				**17** (**17F**, 17A)	**22** (**22F**, 22A)	27 32 (32F, 32A) 41 (41F, 41A)
H	**14**	**23** (**23F**, 23A, 23B)		**15** (15F, 15A, **15B**, 15C)		13 28 (28F, 28A)

Table 1. Chessboard for identification of pneumococcus serogroups/serotypes[2]. Boldface indicates the 23 vaccine related serogroups and serotypes.
( ) states serotypes within a serogroup.

Disposal

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

Limitations

- The culture must be confirmed *Streptococcus pneumoniae* before serotyping using antisera from SSI Diagnostica.
- The Pneumotest Kit is intended for the serogrouping and serotyping of pure cultures of capsulated pneumococci only.
- The pneumococcus antisera products have been validated with the following 92 serotypes: 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 7F, 7A, 7B, 7C, 8, 9A, 9L, 9N, 9V, 10F, 10A, 10B, 10C, 11F, 11A, 11B, 11C, 11D, 12F, 12A, 12B, 13, 14, 15F, 15A, 15B, 15C, 16F, 16A, 17F, 17A, 18F, 18A, 18B, 18C, 19F, 19A, 19B, 19C, 20, 21, 22F, 22A, 23F, 23A, 23B, 24F, 24A, 24B, 25F, 25A, 27, 28F, 28A, 29, 31, 32F, 32A, 33F, 33A, 33B, 33C, 33D, 34, 35F, 35A, 35B, 35C, 36, 37, 38, 39, 40, 41F, 41A, 42, 43, 44, 45, 46, 47F, 47A, 48.

Performance

Sensitivity, specificity and repeatability

Pool antisera in the Pneumotest Kit		
	Percent (number positive/ actual positive)	**95% confidence interval**
Sensitivity	100% (62/62)	94%-100%
Specificity	100% (60/60)	94%-100%
Repeatability	100% (183/183)	98%-100%

Table 2: Sensitivity, specificity and repeatability for pneumococcus antisera included in the Pneumotest Kit.

Reproducibility

The reproducibility of the Pool antisera in the Pneumotest Kit is 100% (confidence interval 99%-100%). Therefore, all produced Pool 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.

Certificate of analysis can be downloaded from our website: ssidiagnostica.com

Presafe
Presafe Denmark A/S

Quality System
DS/EN
ISO 13485

REF
36158

IVD

CE

References

1. Austrian R. The Quellung Reaction, A neglected Microbiologic Technique. The Mount Sinai Journal of Medicine, 43:669-09, 1976

2. Sørensen U.B.S., Typing of Pneumococci by Using 12 Pooled Antisera, J. Clin. Microbiol., 31: 2097-100, 1993

Information and ordering

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