

PNEUMOTEST

Pneumococcal antisera
for serotyping of *Streptococcus pneumoniae*

for in vitro diagnostic use



STATENS
SERUM
INSTITUT

Artillerivej 5
2300 Copenhagen S
Denmark

INTENDED USE

Pneumotest from Statens Serum Institut is intended for qualitative typing and/or grouping of 90-95% of the pneumococci commonly isolated from blood and cerebrospinal fluid by means of the capsular reaction test using the "Chessboard method" (4). This is a fast and easy-to-perform method that requires only standard microbiological equipment such as microscope, inoculating loops, glass slides and cover slips.

SUMMARY AND EXPLANATION

Pneumotest is a kit containing selected pneumococcal diagnostic antisera. The Pneumotest kit contains 12 pooled sera (A to F plus H, and P to T). The Pneumotest Plus contains the 12 pooled sera and a positive control, consisting of a lyophilized *Streptococcus pneumoniae* type 1 and its homologous Type serum 1. The 12 pool sera, the Pool sera G and I and the positive control can be purchased individually as well.

PRINCIPLE (capsular reaction test)

The capsular reaction is a result of the interaction between pneumococcal capsular polysaccharide and its homologous antibody (2). If the capsule becomes visible, the reaction is positive. A positive reaction is the result of an in situ immunoprecipitation leading to a change in the refractive index. In addition the bacteria agglutinate. The capsular reaction is also called the quellung reaction and has been described in detail elsewhere (1).

REAGENT SPECIFICATIONS

Pneumococcal antisera from Statens Serum Institut are raised in rabbits. They are manufactured under aseptic conditions and contain no agents known to be infectious to humans.

Each vial in the kit contains 1 ml antiserum preserved with 15 mM sodium azide (0,0975% sodium azide).

All antisera are carefully controlled and cross reactions are eliminated by absorption (2).

Minimum titre when used in capsular reaction test (4): Pool sera 8
Type serum 1 16

PRECAUTIONS

The diagnostic antisera are intended for use by qualified professionals who are familiar with their use and trained in good laboratory procedures. Direct contact with the reaction test should be avoided. Use gloves or wash hands after performing the test. To avoid contamination, the antisera should be handled under sterile conditions.

The type 1 culture consists of lyophilized bacteria and must be handled as potentially infectious.

The diagnostic antisera contain sodium azide which is a poison. Sodium azide may react with copper and lead plumbing systems to form explosive metal azides. Always dispose of materials containing azide by flushing with large quantities of water.

SPECIMEN PREPARATION

Prior to the typing, the pneumococcus culture (as well as the positive control, if any) must be incubated overnight at 36°C either in a serum broth (broth medium with 5% serum) or on an agar plate with 10% horse blood added.

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 (0.22µ).

PROCEDURE

Materials provided (kit contents)

Pneumotest: 1 vial of each of the Pool sera: A-B-C-D-E-F-H-P-Q-R-S and T
Pneumotest Plus: 1 vial of each of the Pool sera: A-B-C-D-E-F-H-P-Q-R-S and T
1 vial *S. pneumoniae* type 1 culture (lyophilized)
1 vial Type serum 1 (for control purposes)

Materials required but not provided

- Phosphate buffered saline, pH 7.4, for negative control and for preparing a bacterial suspension from an agar plate culture
- Serum broth or agar plate with horse blood added for the lyophilized culture
- Pipette or any other utility that can make a droplet
- Inoculation loop
- Glass slide
- Cover slip
- Immersion oil
- Phase contrast microscope (magnification x 100, oil immersion lens)

Capsular reaction test

1. 1 droplet (1-4 µl) of a broth culture is placed on a glass slide.
If the culture is taken from an agar plate, a small amount of bacterial culture is transferred from the plate and mixed into a droplet of phosphate buffered saline which has been placed on the glass slide.
It is preferable to have relatively few organisms per microscope field.
2. An equal amount of antiserum is added and mixed thoroughly with the droplet on the slide.
3. Immediately place a cover slip on top of the mixture. It is important that the preparation does not dry out.
4. Examine the mixture under a phase contrast microscope using an oil immersion lens, magnification x 100. The reaction is stable for half an hour provided the preparation does not dry out.
5. If the capsule becomes visible (the bacterium appears swollen), the reaction is **positive** and the pneumococcus in question has been mixed with an antiserum containing the homologous antibody.
The size of the capsule depends on the type as well as the growth conditions. The result is often more evident when compared with a control.

Typing strategy

Pneumococci can be typed by a capsular reaction test using the "Chessboard Method" (4).

