

 **BD BBL™ Pneumoslides™ Test**
for *Streptococcus pneumoniae*8800621
Revised: 2001/03**INTENDED USE**

The BBL™ Pneumoslides™ Test is a serologic latex slide agglutination method for the qualitative detection of capsular antigens of *Streptococcus pneumoniae* directly from isolated colonies or pure cultures from liquid nutrient broth. Visible agglutination occurs when the *S. pneumoniae* capsular antigen reacts with the antibody-coated latex beads.

SUMMARY AND EXPLANATION

Streptococcus pneumoniae (Pneumococcus), a normal inhabitant of the human upper respiratory tract, is one of the major causative agents of bacterial pneumonia, meningitis, bacteremia and otitis media in the U.S.^{1,2} Despite the availability of effective antimicrobial therapy, *S. pneumoniae* has been implicated in infections of debilitated patients with alarming severity and case fatality. Worldwide emergence of penicillin and multiple-drug resistant strains has made accurate and rapid identification of pneumococcal isolates more important.³

The traditional methods for identification of *S. pneumoniae* are presumptive procedures based on Gram stain reaction, alpha-hemolysis, optochin susceptibility, and bile solubility. Identification of streptococci directly from blood cultures has also been evaluated.⁴ Tentatively identified pneumococcal isolates can be confirmed by serological methodology using Neufeld's quellung reaction with polyvalent antiserum.⁵ The latex agglutination procedure permits a simple and more rapid serological identification of *Streptococcus pneumoniae* isolates for the clinical laboratory.

PRINCIPLES OF THE PROCEDURE

The capsular polysaccharide antigen of *S. pneumoniae* enables the distinction of 83 serotypes.^{6,7} Specific antibodies to the 83 antigens are absorbed onto the surface of latex beads. Latex particle aggregation becomes large enough to allow rapid visualization of positive agglutination in the presence of specific pneumococcal antigens derived from saline suspensions of suspect alpha-hemolytic colonies.

REAGENTS:

Pneumoslides *Streptococcus pneumoniae* Antibody-Coated Latex Bead Suspension (2.5 mL) is a 1% suspension of latex beads with rabbit antibody specific for *S. pneumoniae* capsular antigens in glycine buffer with MIT/BND as a preservative.

Pneumoslides Polyvalent Positive Control is a lyophilized preparation of an extract of polyvalent capsular polysaccharide antigens of *S. pneumoniae* with MIT/BND as a preservative.

Pneumoslides™ Latex Negative Control (2.5 mL) is a 1% suspension of latex beads coated with normal rabbit immunoglobulins in glycine buffer with MIT/BND as a preservative.

Stirring sticks (50) and Glass Slide (1).

Precautions: For *in vitro* Diagnostic Use.

Do not use test components beyond expiration date.

Do not interchange reagents from different kit lots.

Observe established precautions against microbiological hazards throughout all procedures. After use, specimen containers and other contaminated materials must be sterilized by autoclaving. Directions for use should be followed carefully.

Storage Instructions

Upon receipt, store the Antibody-Coated Latex Bead Suspension and Latex Negative Control at 2 to 8°C. These reagents are ready for use as supplied. Cap tightly between each use. *Do not freeze.*

Upon receipt, store the Pneumoslides Polyvalent Positive Control at 2 to 8°C. Prior to use, reconstitute with 0.5 mL sterile purified water and use within six months but prior to the expiration date stated on the vial label. After reconstitution store at 2 to 8°C. Cap tightly between each use.

Product Deterioration

Do not use if positive and negative controls do not yield appropriate results.

Examine the Pneumoslides Polyvalent Positive Control restored reagent for evidence of contamination, evaporation or other signs of deterioration, such as turbidity. Examine latex reagents for signs of deterioration and do not use if not a homogeneous suspension.

SPECIMEN HANDLING

These reagents are not recommended for use directly on clinical specimens. Organisms must first be isolated on solid media or demonstrate evidence of pure growth in broth culture (i.e., by Gram stain). It is recommended that alpha-

hemolytic colonies from suspect *S. pneumoniae* culture plates first be examined for characteristic colonial morphology and proper Gram stain reaction.

PROCEDURE

Materials Provided: All materials as listed under "REAGEBNTS".

Materials Required But Not Provided: Sterile physiological saline - 0.85 percent sodium chloride, solid culture medium, such as **Trypticase™** Soy Agar with 5% Sheep Blood or Columbia Agar with 5% Sheep Blood, Liquid culture medium, such as Brain Heart Infusion, Blood culture media, such as **SEPTI-CHEK™** TSB, Pasteur pipettes, Centrifuge (blood culture method only), sterile purified water, 50 microliter pipetter and tips and bacteriological loop.

Instructions - for colonies isolated on agar

1. Perform Gram stain testing of 18 to 48h suspected alpha-hemolytic colonies from a solid medium (**Trypticase** Soy Agar with 5% Sheep Blood or Columbia Agar with 5% Sheep Blood).
2. If the Gram stain is indicative of *Streptococcus* species (i.e., gram-positive cocci in pairs or chains), prepare two separate smears of suspect colonies on the provided glass slide using a bacteriological loop. Use at least 2 to 3 colonies per smear (smear should be visible on slide).
3. Add one drop (approximately 50 microliters) of sterile physiological saline next to each of the two bacterial smears. Gradually mix the saline with the colonies using the loop (touch the loop to the saline and transfer a drop to the smear and mix). Continue this procedure until the entire drop of saline has been transferred to the smear with colonies completely emulsified into a smooth suspension.
4. Thoroughly mix the antibody-coated latex bead suspensions before each use.
5. Add one drop of the **Pneumoslide** Latex Negative Control reagent adjacent to one of the colony suspensions and mix the drops with a stirring stick provided.
6. Add one drop of **Pneumoslide** Antibody-Coated Latex Bead Suspension to the second colony suspension and mix, using the same stirring stick. **Note:** The same stirring stick must not be used for the Negative Latex Control if the Antibody-Coated Latex Bead Suspension is mixed first.
7. A positive control using **Pneumoslide** Polyvalent Positive Control should be run daily or with each batch tested using one drop (50 microliters) of the reagent. Follow steps 4 through 6 above. See User Quality Control.
8. Rock the slide for 2 to 3 minutes. (A mechanical rotator at a speed of 100 rpm may be used.) Read for agglutination using a high intensity incandescent light source. Record the test results.

Note: After 3 minutes reagents may begin to dry out and render reactions noninterpretable.

9. Appropriately disinfect and clean the glass slide before reuse or storage.

Alternate Procedures:

For 4-hour cultures in broth

1. Inoculate 5-10 colonies into 5 mL of Brain Heart Infusion (BHI) broth. Mix and incubate at 35°C for 4 hours.
2. After incubation, mix the BHI broth tube and transfer two 50 microliter drops onto separate areas of a glass slide.
3. Place a drop (approximately 50 microliters) of **Pneumoslide** Latex Negative Control reagent next to one of the drops of culture suspension. Mix with a stirring stick.
4. Add one drop of the **Pneumoslide** Antibody-Coated Latex Bead Suspension adjacent to the other drop of the BHI broth suspension and mix with a stirring stick.
5. Rock the slide and read reactions within 2 to 3 minutes. (A mechanical rotator at a speed of 100 rpm may be used.) Record test results.

For broth blood cultures

1. Perform Gram stain examinations of blood culture broth as soon as turbidity is evident. If the Gram stain is suggestive of a pure culture of *Streptococcus* species, proceed as follows.
2. Mix the blood culture broth and aseptically remove 2 to 3 mL of the culture medium. Centrifuge the aliquot for 3 minutes at 2500 rpms (approximately 1400 X g) to separate the red blood cells.
3. Perform the latex agglutination test on the supernatant as described for the 4-hour broth cultures from steps 3 through step 5.
4. A positive test provides presumptive identification of *S. pneumoniae* only. A negative test result should not be regarded as conclusive evidence that *S. pneumoniae* is not present. Confirmation of both reactions using isolated colonies is necessary.

User Quality Control

The **Pneumoslide** Polyvalent Positive Control should be tested with each batch of specimens.

The positive latex reagent will yield strong agglutination within 2 to 3 minutes. The negative control latex reagent should remain homogeneous; i.e., no agglutination.

Periodically check latex reagents for auto-agglutination by testing one drop of the latex reagents with one drop of 0.85% sodium chloride solution. Positive and negative latex reagents should exhibit no agglutination greater than a trace amount of graininess.

Periodically check latex reagents for performance by testing cultures of known pneumococci and viridans streptococci in parallel with test specimens.

NOTE: Do not use the test reagents if appropriate positive and negative control results are not obtained.

RESULTS AND INTERPRETATION**Positive Tests:**

Positive tests will exhibit significantly stronger agglutination with the *Streptococcus pneumoniae* antibody-coated latex beads within 2 to 3 minutes as compared to the negative control latex beads. The negative control latex reagent may exhibit some graininess. This does not affect interpretation of a positive reaction.

Negative Tests:

Negative tests will exhibit absence of, or questionable agglutination in both the **Pneumoslide** Latex Negative Control and the **Pneumoslide** *Streptococcus pneumoniae* Antibody-Coated Latex Bead Suspension reagents within 3 minutes.

Noninterpretable Tests:

Agglutination with both latex reagents renders the test noninterpretable.

LIMITATIONS OF THE PROCEDURE

The **BBL Pneumoslide** *S. pneumoniae* latex bead suspension is coated with omniserum pneumococcal antiserum. Omni-serum is a pool of equal amounts of nine polyvalent sera (A to I). Each of these nine sera react with 7 to 11 types covering all 83 known pneumococcal capsular types. Occasional strains of non-pneumococcal alpha-hemolytic and non-hemolytic streptococci cross-react with omni-serum to produce false-positive results.⁸⁻¹⁰ False-positive reactions using **Pneumoslide** have been produced by strains of *S. mitis* and *S. sanguis* II. These reactions, however, are generally weaker in nature as compared to true positive reactions which are strong and intense. If culture identification is in doubt, additional tests should be performed; i.e., optochin susceptibility using **Taxo**™ P discs, bile solubility, and/or biochemical testing for gram-positive cocci.

Pneumococcal strains not possessing a capsular antigen cannot be identified with immunologic techniques. In addition, false-negative reactions may occur in the 4-hour broth test due to insufficient growth. In such cases, the colony test should be performed using a fresh 18h subculture.

Strains producing noninterpretable reactions in the colony test should be checked for culture purity. Use of bacterial colonies greater than 48h old or incomplete emulsion of colonies in saline prior to testing may cause noninterpretable reactions.

Broth cultures with or without added blood which exhibit heavy growth may yield false-negative or noninterpretable reactions as a result of the acidity developed in the broth. Dilution of the broth in physiological saline, pH 7.0, has been shown to overcome these adverse conditions.

The blood culture test has been successfully performed with a variety of culture media. However, the use of thioglycollate media is not recommended as auto-agglutination may occur. Best results are obtained when cultures are less than 72h old. The blood culture test provides presumptive identification only; positive and negative results must be confirmed using the colony test.

SPECIFIC PERFORMANCE CHARACTERISTICS**Testing of Colonies Isolated on Agar¹¹**

Evaluation of 515 specimens of fresh clinical isolates and stock cultures was conducted which consisted of 312 strains of *Streptococcus pneumoniae* and 203 strains of non-pneumococcal alpha-hemolytic streptococci. Isolates were identified as pneumococci or viridans streptococci based on colony morphology, Gram stain, optochin susceptibility, and bile solubility tests. The quellung test, the reference method of identification, was used to confirm the identification of those isolates which produced questionable optochin susceptibility and/or bile solubility results. **Pneumoslide** produced a sensitivity of 98.4% (303/308) and a specificity of 93% (179/192) for the procedure utilizing isolated colonies. Fifteen organisms (4 pneumococci and 11 viridans streptococci) produced noninterpretable results.

Testing of Broth Cultures¹¹

Of the 515 isolates tested by the isolated colony procedure, 431 were also tested by the 4-hour broth procedure, including 257 isolates of pneumococci and 174 isolates of viridans streptococci. Using the broth procedure, **Pneumoslide** produced a sensitivity of 95.7% (246/257) and a specificity of 88% (143/162). Twelve viridans streptococci produced noninterpretable results.

Testing of Blood Cultures¹¹

Testing of 64 blood cultures positive for *Streptococcus pneumoniae* and 142 blood cultures positive for other gram-positive cocci produced a sensitivity and specificity of 87% (53/61) and 97% (128/132), respectively. Three pneumococci and 10 non-pneumococcal gram-positive cocci produced noninterpretable results.

Testing of Pneumococcal Serotypes

A total of 25 known serotypes have been detected with the **Pneumoslide** latex reagents.¹¹ These include types 1, 3, 4, 6, 7, 8, 9, 14, 18, 19, 23, and 25, the most frequently isolated serotypes in the U.S.¹² Although detection of type 3 antigen has reportedly presented some difficulties in direct CSF testing with omni-serum,¹³ an external investigational trial has shown 100% detection of 16 strains of serotype 3 with the **Pneumoslide** test reagent (by both colony and 4-hour broth methods).¹¹

AVAILABILITY

Cat. No.	Description
240840	BBL™ Pneumoslide ™ Test for <i>Streptococcus pneumoniae</i> , 50 Tests.

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