SSI DIAGNOSTICA

Instructions for use

IMMULEX™ PNEUMOTEST KIT

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IMMULEX™ PNEUMOTEST KIT

For *in vitro* diagnostic use

Intended use

The SSI Diagnostica ImmuLex™ Pneumotest Kit is intended for visual qualitative serogrouping and serotyping of *Streptococcus pneumoniae* (pneumococcus) by use of a rapid agglutination test.[1-5]

The ImmuLex™ Pneumotest Kit identifies 92 pneumococcal serotypes using a Chessboard method (see table 1)[5]. The Chessboard method provides a quick way to divide the 92 serotypes into Pools, Groups and Types. Furthermore, the Chessboard method identifies and separates the 23 vaccine related serogroups and serotypes from the non-vaccine serogroups and serotypes.

The 23 vaccine related serogroups and serotypes that can be detected with the ImmuLex™ 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 of identified and confirmed, pure cultured isolates and strains of pneumococcus.

Description

The ImmuLex™ Pneumotest Kit contains 14 bottles of ImmuLex™ pneumococcus Pool sera, (Pool A, B, C, D, E, F, G, H, I, P, Q, R, S and T) and 50 disposable reaction cards. Each bottle contains 1.5 mL ready-to-use ImmuLex™ solution for approximately 75 tests. The ImmuLex™ solution is blue.

SSI Diagnostica ImmuLex™ solutions consist of latex particles coated with specific antiserum. All antisera have been raised in rabbits and cross-reactions have been removed by absorption when necessary to make the antisera specific.

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

Principle

The rapid agglutination test is performed by mixing a drop of ImmuLex™ solution and a drop of

pneumococcus culture solution on a reaction card. If the test is positive, agglutination will show within 10 seconds resulting in large visible aggregates (see figure 1). The aggregates consist of pneumococcal bacteria and latex particles from the ImmuLex™ solution. These aggregates are formed as a result of an antigen-antibody reaction between the pneumococcal capsule (antigen) and its homologue antibodies coated on the latex particles. No agglutination and no aggregation will show if the test is negative (see figure 1).

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The ImmuLex™ Pneumotest Kit identifies 92 pneumococcal serotypes using a Chessboard method (see table 1)[5]. The Chessboard method provides a quick way to divide the 92 serotypes into Pools, Groups and Types. Furthermore, the Chessboard method identifies and separates the 23 vaccine related pneumococcus serogroups and serotypes from the non-vaccine serogroups and serotypes. The 23 vaccine related serogroups and serotypes must react with two ImmuLex™ 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 or types react only with one ImmuLex™ Pool antiserum of the Pool A, B, C, D, E, F G, H or I (see table 1).

To determine the exact serotype within the ImmuLex™ Pneumotest Kit identified serogroups or Pool Groups, SSI Diagnostica offers antisera to determine 92 serotypes specifically (see ssidiagnostica.com and the section “Limitations”).

Precautions

- Before using SSI Diagnostica pneumococcus antisera products, confirm that the isolate/strain is a pure culture of *Streptococcus pneumoniae*.
- Before use of the ImmuLex™ solution, it is very important to bring the bottles to room temperature and to shake the solution. Use the solution immediately while the latex particles are still homogeneously dispersed in the ImmuLex™ solution.
- For the ImmuLex™ agglutination test, please make sure the result is read within 10 seconds. Therefore, do not perform more than 3 reactions simultaneously before reading the result.

- 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, grow it 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.
- The ImmuLex™ products 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 ImmuLex™ solution might cause false positive reactions.
- ImmuLex™ products that have accidentally been frozen should not be used.
- Do not use the ImmuLex™ products after the expiry date.

- Inspect the bottles before use to ensure they are intact. Any damaged bottles should be discarded.

Materials provided

The ImmuLex™ Pneumotest Kit contains 14 bottles of ImmuLex™ pneumococcus Pool sera, (Pool A, B, C, D, E, F, G, H, I, P, Q, R, S and T) and 50 disposable reaction cards. Each bottle contains 1.5 mL ready-to-use ImmuLex™ solution for approximately 75 tests.

For ordering extra disposable reaction cards, see ssidiagnostica.com.

Materials required but not provided

- Serum broth, Todd-Hewitt broth or 5-10% blood agar plate
- Physiological saline (0.9% NaCl)
- Pipette (droplet of approximately 10 µL)
- 1 µL inoculation loops
- Mixing sticks
- Incubator (35-37 °C)
- Timer (to measure 10 seconds)
- Disposable reaction cards (if more than 50 cards are needed)

Storage and stability

The ImmuLex™ solutions of the ImmuLex™ Pneumotest Kit must be stored at 2-8 °C in a dark place. Stored under these conditions the ImmuLex™ solutions may be used up to the date of expiry shown on the product label.

Do not freeze the product. If the ImmuLex™ solutions have accidentally been frozen, they should not be used.

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

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

Preservative

The ImmuLex™ solutions of the ImmuLex™ Pneumotest Kit 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 bottles to ensure that there is no damage and/or leak. In case of damage or leak discard the bottle.

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

As negative agglutination 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.

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

Procedure

Always before use of the ImmuLex™ solution, bring the bottles to room temperature and shake the solution. Use immediately after shaking while the latex particles are still homogeneously dispersed in the ImmuLex™ solution. It is not recommended to perform more than 3 reactions simultaneously before reading the result.

After growing a pure isolate in broth or on a plate (see recommended media in the section “Materials required but not provided”), do the following to perform a rapid agglutination test (see also quick guide in figure 2).

1. Allow the ImmuLex™ solution to reach room temperature.
2. Gently shake the ImmuLex™ solution and use it immediately while the latex particles are still homogeneously dispersed in the ImmuLex™ solution.
3. Apply one drop (10 µL) of ImmuLex™ solution in one of the circles of the reaction card (one drop for each reaction). NB. Hold the bottle vertically and press gently. Place the drop on the card, it

should NOT be free falling drop (see figure 2).

4. Add a drop (10 µL) of a freshly grown broth culture next to the ImmuLex™ solution on the reaction card. Alternatively, freshly grown colonies from a blood agar plate can be suspended in physiological saline (1 µL inoculation loop of colonies resuspended well in 100 µL saline) and a drop of this solution can be used.
5. Mix the two drops (ImmuLex™ solution and culture) with a mixing stick. Use a separate mixing stick for each reaction.
6. Spread the mix to cover the area of the circle by rocking the card slowly and observe for agglutination within 5-10 seconds (see figure 1). Agglutination visible within 10 seconds from mixing start is a positive reaction (see figure 1). Any agglutination after 10 seconds is not a positive reaction.
7. Use the interpretation schemes (see table 1) to interpret the result and determine the serogroup or serotype of the isolate/strain.

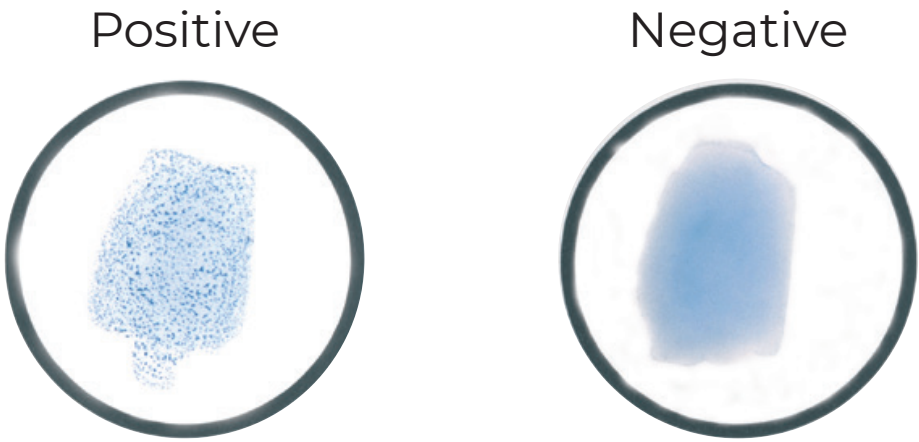
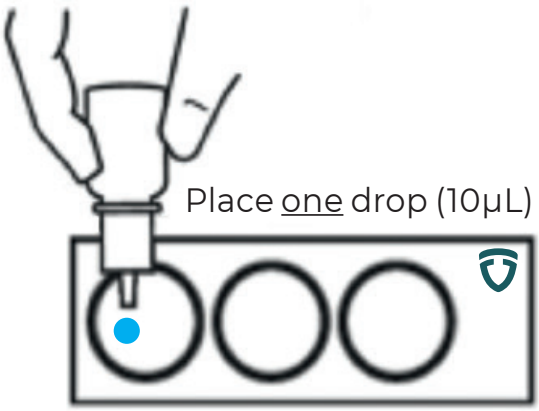


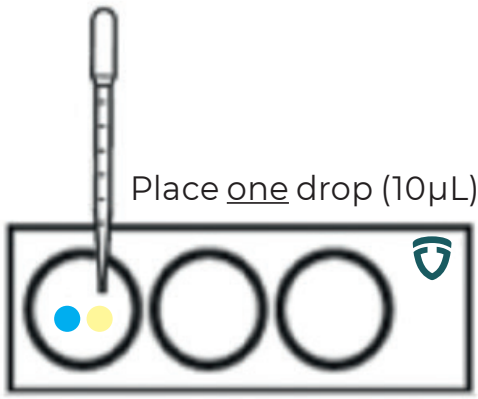
Figure 1. Positive and negative ImmuLex™ agglutination reactions on a reaction card. The positive reaction to the left shows ImmuLex™ solution agglutinating and forming large visible aggregates. The negative reaction to the right shows the ImmuLex™ solution with no agglutination.

Quick guide

Apply latex*



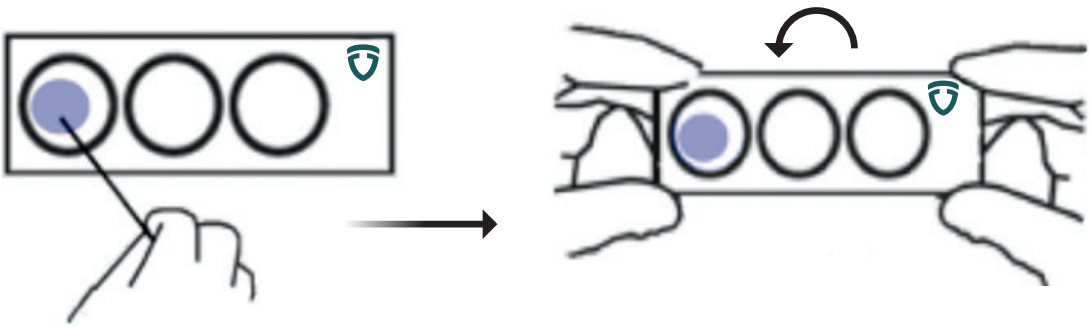
Apply sample



Mix and interpret

Mix the two drops quickly

Rock back and fourth gently



Interpret **within** 10 sec.

*How to **apply latex** solution. Precaution, the drop should be placed on the card by contact. NOT free falling. To do that hold the bottle vertically and press gently:

Figure 2, Quick guide to ImmuLex™

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.

1. First, test the isolate in pneumococcus ImmuLex™ Pool A, B, C, D, E, F, G, H and I.
2. Proceed by testing the isolate in pneumococcus ImmuLex™ Pool P, Q, R, S and T.
3. The test is positive when agglutination occurs within 10 seconds (see figure 1).
4. Interpret the result on the chessboard scheme (see table 1). If the isolate is positive in ImmuLex™ 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, 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			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)
G	**14**	**23** (**23F**, 23A, 23B)		**15** (15F, 15A, **15B**, 15)		13 28 (28F, 28A)

Table 1. Chessboard scheme 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 ImmuLex™ pneumococcus antisera from SSI Diagnostica.
- The ImmuLex™ Pneumotest Kit is intended for the serogrouping and serotyping of pure cultures of capsulated pneumococci only.
- The ImmuLex™ Pneumotest Kit product 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

ImmuLex™ pool antisera in the ImmuLex™ Pneumotest Kit		
	Percent (number positive/ actual positive)	**95% confidence interval**
Sensitivity	100% (204/ 204)	98%-100%
Specificity	100% (272/ 272)	99%-100%
Repeatability	100% (714/ 714)	99%-100%

Table 2, Sensitivity, specificity and repeatability for ImmuLex™ pool antisera

Reproducibility

The reproducibility of ImmuLex™ Pool antisera is 100% (confidence interval 99%-100%). Therefore, the ImmuLex™ Pneumotest Kit has 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
51823

IVD

CE

References

1. Slotved, H-C. et al., Journal of Clinical Microbiology, 42(6):2518-2522, 2004.
2. Slotved, H-C., Kerrn, M.B., Journal of Microbiological Methods, 61:181-186, 2005.
3. Reynolds, R., Journal of Antimicrobial Chemotherapy 62 (suppl. 2): ii15-ii28, 2008.
4. Sanz, J.C. et al., Journal of Clinical Microbiology, 48(2):593-595, 2010.
5. Kuch, A. et al., Journal of Clinical Microbiology, 52(7):2647-2649, 2014.
6. 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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