SSI DIAGNOSTICA

Instructions for use

IMMULEX™ PNEUMOCOCCUS ANTISERA

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IMMULEX™ PNEUMOCOCCUS POOL, GROUP, TYPE AND FACTOR ANTISERA

For *in vitro* diagnostic use

Intended use

The SSI Diagnostica ImmuLex™ pneumococcus Pool, Group, Type and Factor antisera products are intended for visual qualitative confirmation, serogrouping and serotyping of *Streptococcus pneumoniae* (pneumococcus) by use of a rapid agglutination test.[1-5]

The products are for testing identified and confirmed, pure cultured isolates and strains of pneumococcus.

Description

The ImmuLex™ pneumococcus antisera products are provided in bottles. 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.

The ImmuLex™ pneumococcus antisera products are available as individual products in the following product groups: ImmuLex™ pneumococcus Pool, Group, Type and Factor.
Note: ImmuLex™ pneumococcus products are not available to fully determine all serotypes using a rapid agglutination test.

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).

The ImmuLex™ pneumococcus Pool, Group, Type and Factor antisera products are intended for visual qualitative confirmation, serogrouping and serotyping of pneumococcus.

Not all ImmuLex™ pneumococcus products to fully determine all serotypes are included in the product range. If ImmuLex™ products are not available, SSI Diagnostica offers antisera to determine 92 serotypes specifically, using the Neufeld test (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 bottle before use to ensure it is intact. Any damaged bottles should be discarded.

Materials provided

The ImmuLex™ pneumococcus Pool, Group, Type and Factor antisera products are provided in bottles. Each bottle contains 1.5 mL ready-to-use ImmuLex™ solution for approximately 75 tests.

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 (for ordering disposable reaction cards, see ssidiagnostica.com).

Storage and stability

The ImmuLex™ pneumococcus antisera products 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™ solution 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 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 bottles.

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 if 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

Before use of the ImmuLex™ solution, always 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.

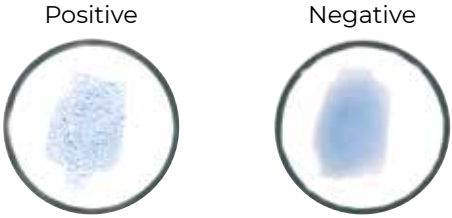

Figure 1. Positive and negative ImmuLex™ agglutination reactions on a reaction card. The positive reaction to the left shows ImmuLex™ solution agglutinating and forming large visible aggregates. The negative reaction to the right shows the ImmuLex™ solution with no agglutination.

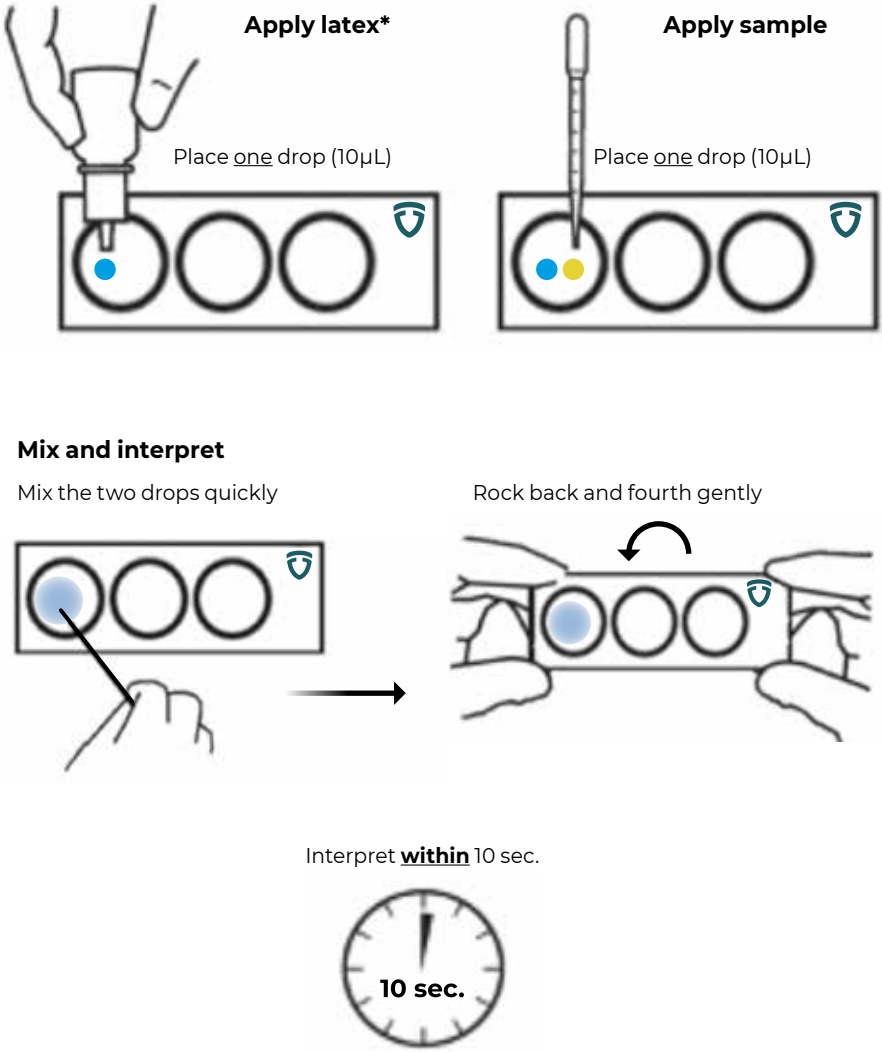

Figure 2. Quick guide to ImmuLex™

*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:

Interpretation of results

For serogroup and serotype determination of a pneumococcal isolate/strain, test the isolate with ImmuLex™ pneumococcus antisera products.

Overview, see details below

A. First, test the isolate in ImmuLex™ Pool antisera. When necessary, continue with B.
B. Test the isolate with ImmuLex™ Type and/or Factor antisera.

A. Test the isolate with pneumococcus ImmuLex™ Pool antisera.
   1. First, test the isolate in pneumococcus ImmuLex™ Pool A, B, C, D, E, F, G, H and I antisera.
   2. Proceed by testing the isolate in pneumococcus ImmuLex™ Pool P, Q, R, S and T antisera.
   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)[2]. 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 ImmuLex™ Pool B and Pool Q, the isolate is a serogroup 6 (see table 1).

B. Test the isolate with pneumococcus ImmuLex™ Type or Factor antisera.
   Note before using ImmuLex™ Factor antisera, the Pool or serogroup of the isolate must be known. Use only ImmuLex™ Factor sera within the serogroup for further testing of the isolate (see table 1). This is important because Factor antisera may cross-react to serotypes belonging to other serogroups.
   1. When the ImmuLex™ Pool or Group antisera provide a non-unique serotype result, test the isolate with the relevant ImmuLex™ Type or Factor antisera of the Pool or Group (see tables 1 and 2).
   2. A non-unique result is e.g. an isolate positive in ImmuLex™ Pool B and Pool Q, that contains both serotype 6A, 6B, 6C and 6D (see table 1). Test the isolate in ImmuLex™ Factor 6b, 6c, and 6d. Is the isolate positive with Factor 6b it is serotype 6A (see table 2). Use a negative control with saline instead of antiserum to compare with.

To determine the exact serotype if ImmuLex™ products are not available, SSI Diagnostica offers antisera for Neufeld test to determine 92 serotypes specifically (see ssidiagnostica.com and the section “Limitations”).

Chessboard scheme for identification of pneumococcus serogroups/serotypes

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)
G						29, 34 35 (35F, 35A, 35B, 35C) 42 47 (47F, 47A)
H	**14**	**23** (**23F**, 23A, 23B)		**15** (15F, 15A, **15B**, 15C)		13 28 (28F, 28A)
I						25 (25F, 25A) 38, 43, 44, 45, 46, 48

Table 1. Chessboard scheme for identification of pneumococcus serogroups/serotypes[2].

Note: ImmuLex™ pneumococcus products are not available for all Type and Factor antisera.

23-valent vaccine types are indicated by boldface. ( ) states types within the group.

Key to pneumococcal factor sera

Sero type	Reactions in factor antisera				Antigenic form
	6b	6c	6d		
6A	+	-	-		6a, 6b
6B	-	+	-		6a, 6c
6C	-	-	+		6a, 6d
6D	-	+	+		6a, 6c, 6d
	7b	7c	7e	7f	
7F	+	-	-	-	7a, 7b
7A	(+)	+	-	-	7a, 7b, 7c
7B	-	-	+	-	7a, 7d, 7e, 7h
7C	-	-	-	+	7a, 7d, 7f, 7g, 7h
	9b	9d	9e	9g	
9A	-	+	-	-	9a, 9c, 9d
9L	+	-	-	-	9a, 9b, 9c, 9f
9N	+	-	+	-	9a, 9b, 9e
9V	-	+	-	+	9a, 9c, 9d, 9g
	10b	10d	10f		
10F	+	-	-		10a, 10b
10A	-	+	-		10a, 10c, 10d
10B	+	+	-		10a, 10b, 10c, 10d, 10e
10C	+	-	+		10a, 10b, 10c, 10f
	11b	11c	11f	11g	
11F	+	-	-	+	11a , 11b, 11e, 11g
11A	-	+	-	-	11a, 11c, 11d, 11e
11B	+	-	+	+	11a, 11b, 11f, 11g
11C	+	+	+	-	11a, 11b, 11c, 11d, 11f
11D	+	+	-	-	11a, 11b, 11c, 11e
	12b	12c	12e		
12F	+	-	-		12a, 12b, 12d
12A	-	+	-		12a, 12c, 12d
12B	+	+	+		12a, 12b, 12c, 12e
	15b	15c	15e	15h	
15F	+	+	-	-	15a, 15b, 15c, 15f
15A	-	+	-	-	15a, 15c, 15d, 15g
15B	+	-	+	+	15a, 15b, 15d, 15e, 15h
15C	-	-	+	-	15a, 15d, 15e
	16b	16c			
16F	+	-			16a, 16b, 11d
16A	-	+			16a, 16c
	17b	17c			
17F	+	-			17a, 17b
17A	-	+			17a, 17c

Sero type	Reactions in factor antisera					Antigenic form
	18c	18d	18e	18f		
18F	+	-	+	+		18a, 18b, 18c, 18e, 18f
18A	-	+	-	-		18a, 18b, 18d
18B	-	-	+	-		18a, 18b, 18e, 18g
18C	+	-	+	-		18a, 18b, 18c, 18e
	19b	19c	19f	7h		
19F	+	-	-	-		19a, 19b, 19d
19A	-	+	-	-		19a, 19c, 19d
19B	-	-	-	+		19a, 19c, 19e, 7h
19C	-	-	+	+		19a, 19c, 19f, 7h
	22b	22c				
22F	+	-				22a, 22b
22A	-	+				22a, 22c
	23b	23c	23d			
23F	+	-	-			23a, 23b, 18b
23A	-	+	-			23a, 23c, 15a
23B	-	-	+			23a, 23b, 23d
	24c	24d	24e			
24F	-	+	-			24a, 24b, 24d, 7h
24A	+	+	-			24a, 24c, 24d
24B	-	-	+			24a, 24b, 24e, 7h
	25b	25c				
25F	+	-				25a, 25b
25A	+	+				25a, 25c, 38a
	28b	28c				
28F	+	-				28a, 28b, 16b, 23d
28A	-	+				28a, 28c, 23d
	32a	32b				
32F	+	-				32a, 27b
32A	+	+				32a, 32b, 27b
	33b	33e	33f	6a	20b	
33F	+	-	-	-	-	33a, 33b, 33d
33A	+	-	-	-	+	33a, 33b, 33d, 20b
33B	-	-	+	-	-	33a, 33c, 33d, 33f
33C	-	+	(+)	-	-	33a, 33c, 33e
33D	-	-	+	+	-	33a, 33c, 33d, 33f, 6a

Sero type	Reactions in factor antisera					Antigenic form
	35a	35b	35c	29b	42a	
35F	+	+	-	-	-	35a, 35b, 34b
35A	+	-	+	-	-	35a, 35c, 20b
35B	+	-	+	+	-	35a, 35c, 29b
35C	+	-	+	-	+	35a, 35c, 20b, 42a

Sero type	Reactions in factor antisera		Antigenic form
	41a	41b	
41F	+	+	41a, 41b
41A	+	-	41a
	47a	43b	
47F	+	-	47a, 35a, 35b
47A	+	+	47a, 43b

Table 2. Key to pneumococcus Factor sera. + : positive reaction, – : negative reaction, (+) : positive reaction with some but not all isolates/strains.

Note: The ImmuLex™ product range does not include all pneumococcus factor antisera. See ssidiagnostica.com

In accordance with the international and Danish nomenclature of pneumococcal serotypes all serotypes are written with capital letters, e.g. serotype 18F, 18A, 18B and 18C, whereas Factor antisera to determine the serotypes within serogroup 18 are written with non-capital letters, e.g. Factor 18c, 18d, 18e and 18f antisera.

23-valent vaccine types are indicated by **boldface**.

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 products from SSI Diagnostica.

- The ImmuLex™ pneumococcus antisera products are intended for the serogrouping and serotyping of pure cultures of capsulated pneumococci only.

- The ImmuLex™ 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

ImmuLex™ pneumococcus antisera products, overall results		
	Percent (number positive/ actual positive)	95% confidence interval
Sensitivity	100% (150/ 150)	98%-100%
Specificity	100% (214/ 214)	99%-100%
Repeatability	100% (546/ 546)	99%-100%

Table 3, Sensitivity, specificity and repeatability for ImmuLex™ antisera products

Reproducibility

The reproducibility of the ImmuLex™ pneumococcus antisera products is 100% (confidence interval 99%-100%). Therefore, the ImmuLex™ pneumococcus antisera products 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

IVD CE

REF

For the list of products and composition, see our website:
https://www.ssidiagnostica.com/ImmuLex-pneumococcus-antisera/

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Information and ordering

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Revision history

Indication of 23 valent vaccine types in the tables 1 and 2.
Change of logo of notified body

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