SSI DIAGNOSTICA

Instructions For Use

IMMULEX™

ENGLISH

CONTENT

GENERAL INFORMATION

Quality Certificate

SSI Diagnostica is quality assured and certified in accordance with ISO 13485. Certificate of analysis can be downloaded from our website www.ssidiagnostica.com

Abbreviation list

E. faecalis	*Enterococcus faecalis*
E. faecium	*Enterococcus faecium*
GBS	Group B *Streptococcus*
PBS	Phosphate Buffered Saline
S. alactolyticus	*Streptococcus alactolyticus*
S. bovis	*Streptococcus bovis*
S. durans	*Streptococcus durans*
S. equinus	*Streptococcus equinus*
S. gallolyticus	*Streptococcus gallolyticus*
S. hazem	*Streptococcus hazem*
S. pneumoniae	*Streptococcus pneumoniae*

Intended use

The ImmuLex™ reagents are for *in vitro* diagnostic use only. The intended use for all ImmuLex™ reagents is bacterial serotyping. It is important to use pure culture isolates for the determination of bacterial antigens.

Description

Our ImmuLex™ reagents 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.
All products are sold as ready-to-use.

Principle

Before using the latex reagent, it is very important to bring the vial to room temperature and shake it. Use the reagent while the latex particles are in homogeneous solution.
When mixing the latex reagent with a homologue antigen, the latex particles will agglutinate and form large aggregates visual for the naked eye. The reaction is performed on a reaction card. Disposable reaction cards can be ordered separately (50 pcs., art no. 88464; 200 pcs., art no. 53285).

Negative controls

Pneumococcal and *H. influenzae* ImmuLex™ reagents:

Mix one drop of PBS pH 7.4 with the latex reagent on the reaction card. No agglutination should be observed.

Streptococcal ImmuLex™ reagents:

Mix one drop of physiological saline pH 7.4 with the latex reagent on the reaction card. No agglutination should be observed.

Storage and Shelf Life

Sodium azide has been added as a preservative to a final concentration of 0.0975%.

All ImmuLex™ products must be stored at 2-8°C in a dark place. Do not freeze (if the reagents accidentally have been frozen, they should not be used).

Expiry date is printed on the label.

Support

Additional information about the ImmuLex™ products and the serotyping guidelines are available at www.ssidiagnostica.com.

If you have any difficulties using the products, please contact SSI Diagnostica at info@ssidiagnostica.com.

LATEX AGGLUTINATION PROCEDURE

Do not perform more than 3 reactions simultaneously before reading the result.

1. Use a Todd-Hewitt broth culture showing visible growth or a culture from a 5-10% blood agar plate or a chocolate agar plate depending on the bacterium to be serotyped (see table 1). All cultures should be prepared by growing them overnight at 35-37°C.
2. Allow the latex reagent to reach room temperature before use.
3. Shake the latex reagent vial and use the reagent while the latex particles are in homogeneous solution. For each reaction, set of a drop of approx. 10 µL (squeeze the vial gently) of latex reagent onto the reaction card.
4. Prepare the bacterial culture according to table 1.

Table 1. Preparation of the bacterial culture

Bacterium	Preparation of the bacterial culture
Pneumococcus	Apply 10 µL PBS pH 7.4 on the reaction card next to the drop of latex reagent and suspend 1 colony from a 5-10% blood agar plate in the PBS*. Alternatively grow a Todd-Hewitt broth culture and apply approx. 10 µL bacterial suspension next to the drop of latex reagent.
S. agalactiae Group B	Apply 10 µL physiological saline pH 7.4 on the reaction card next to the drop of latex reagent and suspend 1 colony from a 5-10% blood agar plate in the physiological saline*. Alternatively grow a Todd-Hewitt broth culture and apply approx. 10 µL bacterial suspension next to the drop of latex reagent.
Streptococcus group A, B, C, D, F, and G	Suspend 1 µL inoculation loop of bacterium culture from a 5-10 % blood agar plate in 250 µL physiological saline pH 7.4 and add 10 µL of this bacterial suspension on the reaction card next to the latex reagent. If there is no reaction using bacterial suspension or if the reaction is indistinct, follow the procedure described on page 18.
H. influenzae	Suspend 1 colony from a chocolate agar plate in 200 µL PBS pH 7.4 and add 10 µL of this bacterial suspension on the reaction card next to the latex reagent*.

* It is very important not to use too much colony material since this might give false positive reactions.

5. Mix the two drops with a mixing stick. Use a new stick for each reaction.
6. Spread to cover the area of the circle.
7. Rock the card slowly and watch for agglutination according to the guidelines (see page 8).

Pneumococcal ImmuLex™ reagents:
Rock the card for 5-10 seconds. Any agglutination after 10 seconds is not a positive reaction.

Streptococcal and *H. influenzae* ImmuLex™ reagents:
Rock the card for 30 seconds. Any agglutination after 30 seconds is not a positive reaction.

Positive and negative reactions are shown in figure 1.

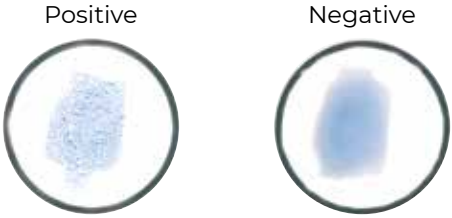

Figure 1. Illustration of a positive (left) and a negative (right) latex agglutination on a reaction card.

PNEUMOCOCCUS

IMMULEX™ PNEUMOCOCCAL POOL-GROUP-TYPE-FACTOR

Intended Use

The diagnostic ImmuLex™ pneumococcal antisera are intended to be used for serotyping of *S. pneumoniae*[1-5].

Description

Each ImmuLex™ pneumococcal Pool, Type, Group and Factor vial contains 1.5 mL (approx. 75 tests) ready-to-use reagent. The Factor antisera are only absorbed free of cross-reactions within the group.

Principle

The principle behind the latex agglutination is described on page 4.

Materials Required but not Provided

- Todd-Hewitt broth (Difco, Oxoid CM189, Sigma T-1438) or 5-10% blood agar plate
- PBS pH 7.4
- Pipette (droplet of approx. 10 µL)
- 1 µL inoculation loops
- Mixing sticks
- Disposable reaction cards (for order information see page 22)
- Incubator (35-37°C)

Procedure

Latex agglutination is performed as described on page 6-8.

NB: Rock the card for 5-10 seconds. Any agglutination after 10 seconds is not a positive reaction.

IMMULEX™ PNEUMOTEST KIT

Intended Use

The diagnostic ImmuLex™ Pneumotest Kit is intended to be used for serotyping of *S. pneumoniae* to the type level and for some to the group level[1-5].

Description

The kit contains 14 vials (Pool A - I and Pool P - T) each with 1.5 mL (approx. 75 tests) ready-to-use latex reagent plus 50 disposable reaction cards.

Principle

The principle behind the latex agglutination is described on page 4.

Materials Required but not Provided

- Todd-Hewitt broth (Difco, Oxoid CM189, Sigma T-1438) or 5-10% blood agar plate
- PBS pH 7.4
- Pipette (droplet of approx. 10 µL)
- 1 µL inoculation loops
- Mixing sticks
- Incubator (35-37°C)

Procedure

Latex agglutination is performed as described on page 6-8.
NB: Rock the card for 5-10 seconds. Any agglutination after 10 seconds is not a positive reaction.

Test the pneumococcus culture with latex Pool antisera A - I and proceed by testing with latex Pool antisera P - T (14 reactions).
The chessboard on the next page (table 2) guides you to the result. If the pneumococcus culture is a serotype within the 23-valent vaccine (marked in bold) one positive reaction is seen among Pool A - I and one positive reaction is seen among Pool P - T. The combination ex. Pool A and Pool P identifies that the tested pneumococcus is a serotype 1. If the result identifies a vaccine serogroup, then further serotyping is performed by using Factor antisera – see our website for availability.
If only one positive reaction is seen among Pool C - I, then the pneumococcus culture is a non-vaccine serotype.

Table 2. Typing of pneumococcus to the type level and for some to the group level by the Pneumotest Kit chessboard method.

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, 15)		13 28 (28F, 28A)
I						25 (25F, 25A) 38, 43, 44, 45, 46, 48

* Antisera needed in order to serotype the non-vaccine groups/types shown in this column are not included in the kit but can be purchased separately.

IMMULEX™ PNEUMOCOCCUS 7-10-13-VALENT KIT

Intended Use

The diagnostic ImmuLex™ Pneumococcus 7-10-13-valent Kit is a ready-to-use chessboard system for serotyping of *S. pneumoniae* serotypes included in the different vaccines for children. The following serotypes that can be identified are:

7-valent vaccine serotypes:	4, 6B, 9V, 14, 18C, 19F, 23F
10-valent vaccine serotypes:	1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F
13-valent vaccine serotypes:	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F

Description

The kit contains 7 vials (Pool I, II, III, IV, V, VI, VII) each with 1.5 mL (approx. 75 tests) ready-to-use latex reagent plus 25 disposable reaction cards.

Principle

The principle behind the latex agglutination is described on page 4.

Materials Required but not Provided

- Todd-Hewitt broth (Difco, Oxoid CM189, Sigma T-1438) or 5-10% blood agar plate
- PBS pH 7.4
- Pipette (droplet of approx. 10 µL)
- 1 µL inoculation loops
- Mixing sticks
- Incubator (35-37°C)

Procedure

Latex agglutination is performed as described on page 6-8.
NB: Rock the card for 5-10 seconds. Any agglutination after 10 seconds is not a positive reaction.

For interpretation of the typing results, see the chessboard in table 3.

Table 3. Typing of pneumococcus to the type level and for some to the group level by the Pneumococcus 7-10-13-Valent Kit chessboard method.

Pool	V	VI	VII
I	**1**	**18** (18F,18A,18B,18C)	**4**
II	**19** (19F,19A,19B,19C)	**6** (6A,6B,6C)	**3**
III	**7** (7F,7A,7B,7C)		**5**
IV	**14**	**23** (23F,23A,23B)	**9** (9A,9L,9N,9V)

If the result identifies a vaccine serogroup, then further serotyping is performed by using Factor antisera – see our website for availability.

STREPTOCOCCUS

IMMULEX™ STREPTOCOCCUS GROUP B TYPES
IMMULEX™ STREPTOCOCCUS GROUP B KIT

Intended Use
The diagnostic ImmuLex™ *Streptococcus* Group B Types and the diagnostic ImmuLex™ *Streptococcus* Group B Kit are intended to be used for serotyping of *S. agalactiae* Group B streptococci (GBS)[6,7].

Principle
The principle behind the latex agglutination is described on page 4.

Description
ImmuLex™ *Streptococcus* Group B Types can be purchased as individual vials each with 1.5 mL (approx. 75 tests).
The ImmuLex™ *Streptococcus* Group B Kit contains 10 vials (Type Ia to IX) each with 1.5 mL (approx. 75 tests) ready-to-use latex reagent plus 50 disposable reaction cards and mixing sticks.

Materials Required but not Provided
- 5-10% blood agar plate
- Physiological saline pH 7.4
- Pipette (droplet off approx. 10 µL)
- 1 µL inoculation loops
- Mixing sticks (included in the Kit)
- Incubator (35-37°C)

Procedure
Latex agglutination is performed as described on page 6-8.
NB: Rock the card for 30 seconds. Any agglutination after 30 seconds is not a positive reaction.

IMMULEX™ STREPTOCOCCUS GROUP KIT

Intended Use

The diagnostic ImmuLex™ *Streptococcus* Group Kit is intended to be used for streptococcal serotyping of group A, B, C, D, F, and G.

Principle

The ImmuLex™ *Streptococcus* Group Kit is a rapid latex agglutination test for streptococcal serotyping using a bacterial suspension (bacterium from a blood agar plate suspended in physiological saline) or a nitrous acid extraction of group specific antigens.
The principle behind the latex agglutination is described on page 4.

Description

The ImmuLex™ *Streptococcus* Group Kit consists of:

Latex reagents:
Six vials (Group A, B, C, D, F, and G) each with 1.5 mL (approx. 75 tests) ready-to-use reagent plus 50 disposable reaction cards and mixing sticks.

Extraction Reagent 1 (red cap):
Two vials each containing 2 mL of sodium nitrite solution. The reagent is classified as harmful and labelled:

⚠ Warning

H302: Harmful if swallowed.
P301+P312: If swallowed, call a POISON CENTER or doctor/physician if you feel sick.

Extraction Reagent 2 (yellow cap):
Two vials containing 2 mL each of an acid solution.

Neutralizing Reagent 3 (blue cap):
Two vials each containing 2 mL of a neutralizing solution (0.0975% sodium azide added as preservation).

Limitations

It is well known that *Enterococcus* species (group D antigen) and *Streptococcus* group D strains can cross-react in antiserum directed against other *Streptococcus* groups[8-11].

Materials Required but not Provided

- 5-10% blood agar plate
- Physiological saline pH 7.4
- Pipette (droplet off approx. 10 µL)
- 1 µL inoculation loops
- Gloves
- Tubes
- Fume hood
- Incubator (35-37°C)

Procedure

Latex agglutination is performed as described on page 6-8.
NB: Rock the card for 30 seconds. Any agglutination after 30 seconds is not a positive reaction.

Follow the procedure below if there is no reaction using bacterial suspension or if the reaction is indistinct.

Nitrous acid extraction of bacterial antigen:

1. Warning: The combination of reagent 1, 2, and 3 produces hazardous fumes. Step 3-6 in the below procedure is recommended to be performed under a fume hood and wearing gloves.
2. Add one drop of reagent 1 (red cap) to a tube.
3. Suspend a 1 µL inoculation loop of bacterial culture from a 5-10% blood agar plate in the tube containing reagent 1.
4. Add one drop of reagent 2 (yellow cap) to the tube and mix.
5. Wait min. 10 minutes (max. 60 minutes). Add one drop of reagent 3 (blue cap) to the tube and mix.
6. Follow the latex procedure on page 6 mixing the bacterial extract with the latex reagents.

NB: Rock the card for 30 seconds. Any agglutination after 30 seconds is not a positive reaction.

Interpretation

A positive result in one of the latex reagents identifies the group.

Example:

- If there is only a positive reaction in the group B latex reagent, the strain is a *Streptococcus* group B.
- As the group antigen D is known to cross-react with other *Streptococcus* groups, there may be more than one positive reaction with the latex reagents.
- If, however, there is a positive reaction in the group D latex reagent, the strain is a *Streptococcus* group D or an *Enterococcus* species (which also have the group D antigen).
- The group D latex reagent is developed in order to identify the following species: *S. alactolyticus*, *S. bovis*, *S. durans*, *S. hazem*, *S. gallolyticus*, *S. equinus*, *E. faecium*, and *E. faecalis*.

H. INFLUENZAE

IMMULEX™ H. INFLUENZAE POOL-TYPE

Intended Use

The diagnostic ImmuLex™ *H. influenzae* products are intended to be used for serotyping of *H. influenzae* serotype a, b, c, d, e and f.

Description

Each ImmuLex™ *H. influenzae* vial contains 1.5 mL ready-to-use latex reagent (approx. 75 tests). A polyvalent pool containing all 6 serotypes is also available.

Principle

The principle behind the latex agglutination is described on page 4.

Materials Required but not Provided

- Chocolate agar plate
- PBS pH 7.4
- Pipette (defined volumes off approx. 10 µL and 200 µL)
- Mixing sticks
- 1 µL inoculation loops
- Disposable reaction cards (for order information see page 22)
- Incubator (35-37°C)

Procedure

Latex agglutination is performed as described on page 6-8.

NB: Rock the card for 30 seconds. Any agglutination after 30 seconds is not a positive reaction.

Interpretation

Figure 2 shows three reactions. From left to right:
A positive reaction and two negative reactions. Although the last negative reaction shows some granules, this is not a true positive reaction.

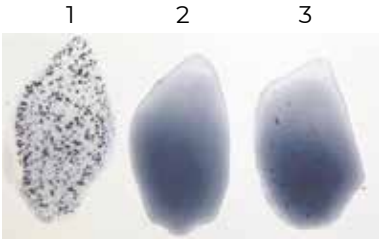

Figure 2. Interpretation of a positive and negative result.
1) positive result, 2) negative result and 3) negative result.

Performance

Two studies were conducted at Statens Serum Institute, Copenhagen, Denmark. The first study consisted of 120 *H. influenzae* strains (43 serotype b and 77 other serotypes or non-capsulated strains), all of which were tested in ImmuLex™ *H. influenzae* serotype b. The results showed that all 43 serotype b strains were detected by ImmuLex™ *H. influenzae* serotype b, and there were no cross-reactions to the other *H. influenzae* serotypes.

A second study tested 51 capsulated *H. influenzae* serotypes (a (n=10), b (n=6), c (n=4), d (n=4), e (n=15), f (n=12)) in all seven ImmuLex™ *H. influenzae* products. The results showed 100% specificity and 100% sensitivity. As expected, 22 non-capsulated *H. influenzae* strains were negative in all seven ImmuLex™ *H. influenzae* products.

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Information and ordering

SSI Diagnostica A/S

Herredsvejen 2

3400 Hillerød

Denmark

Tel.: +45 4829 9100

info@ssidiagnostica.com

www.ssidiagnostica.com

shop.ssidiagnostica.com

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Notes

SSI Diagnostica A/S
Herredsvejen 2
3400 Hillerød
Denmark

ssidiagnostica.com

EXPERIENCE MATTERS