IVD

Streptococcus pneumoniae Antisera

INTENDED USE

Serotyping of *Streptococcus pneumoniae*.

SAMMARY AND EXPLANATION

Streptococcus pneumoniae is the most common cause of community-acquired bacterial pneumonia (CAP), and otitis media, and the second most common cause of meningitis in infants and children, which may lead to learning disabilities and hearing impairment. Mortality occurs in approximately 10-15% of hospitalized adults with invasive disease. While a licensed 23-valent-polysaccharide vaccine is now available, widespread overuse of antibiotics has contributed to the emergence of drug resistant strains.

S. pneumoniae is serotyped based on the antigenicity of the capsular polysaccharide. This product contains antibodies specific to the capsular antigens of *S. pneumoniae*. The sera are prepared by hyper-immunizing healthy rabbits with reference strains (ATCC) of. *S. pneumoniae*, heated at 56°C for 30 minutes, and removing cross agglutinins by absorption and aseptic filtration.

PRINCIPLE

When the reagent is mixed with *Streptococcus pneumoniae* cells, which have antigens corresponding to the reagent, an antigen-antibody reaction occurs to produce agglutination. The reaction is macroscopically observed to determine each serotype.

PRODUCT

Streptococcus pneumoniae Antisera are produced from rabbits and contain 0.08 w/v% sodium azide as a preservative. The following sera are provided in 2 mL vials with pipette and are ready to use.

Set : Polyvalent antisera 8 vials
Monovalent antisera 39 vials
Each serotype is listed as follows;

Polyvalent	Monovalent
1	1 2 3 4 5
2	6 8 9 10
3	11 12 14 15 16
4	17 18 21 22
5	20 29 31 33 34 35 47
6	23 25 28 41 46
7	27 32 36 38 39
8	7 19 24 40

PRECAUTION FOR USE AND HANDLING

1. General precautions

1) For in vitro diagnostic use only.
2) Only bacteriological trained laboratory staff should handle the reagents.
3) Reagents should only be used for the intended use.
4) Reagents should be used according to the described procedures.
5) Antigenic factors of *Streptococcus pneumoniae* are shared widely with other bacterium. It is important to identify the specimen is as *Streptococcus pneumoniae* by biochemical properties.

2. Handling precautions

1) All specimens, samples and containers coming into contact with samples should be treated as infectious substance.
2) If reagent comes into contact with skin, eyes, or mouth wash immediately with copious amounts of water, seek medical attention if necessary.
3) Do not freeze the reagents or use past the expiration date as this may result in poor reagent performance.
4) Reagents should be allowed to stand at 15°C-25°C for at least 30 minutes before use.
5) Used containers should not be used for other purposes.
6) Sera with different lot numbers should not be mixed.
7) Special precautions should be taken to ensure that the reagent caps are not exchanged.
8) Avoid microbial contamination of opened reagent bottles. Do not use reagents if they are contaminated or cloudy.

3. Precautions for disposal

1) The reagent contains 0.08 w/v% sodium azide. Sodium azide may react with lead or copper to form explosive heavy metal azides. The reagent should be disposed with copious amounts of water.
2) All specimens, spills, inoculated products and equipment used in this test should be treated by one of the following methods.
   [1] Soaking in 0.1 w/v% hypochlorite for 1 hour or more.
   [2] Autoclave at 121°C for 20 minutes or more.

TEST PROCEDURE

1. Material required but not provided

Physiological saline, pipettes, micropipettes and tips, fluorescent light, bacteriological loops, glass slides, glass pencil, blood agar plate, 37°C incubator

2. Preparation of reagents

Ready to use.

3. Specimens

Pure culture of *Streptococcus pneumoniae* identified by biochemical properties should be tested. If the specimen consists of multiple strains or is contaminated , it may not show correct results.

4. Method

A) Cultivation of specimen

Inoculated test strains on a blood agar plate and incubate at 37°C for 18 to 24 hours under aerobic conditions. Because of *S. pnumoniae* cell easily cause autolysis, serotyping must be done in a same day after cultivation.

B) Agglutination test

1) Place a drop on Polyvalent serum and physiological saline(30 μ L) another section of the partitioned slide.
2) Place a small but visible amount of bacterial cells on the area above each drop of serum or physiological saline, and mix well using the loop.
3) Tilt the glass slide back and forth for one minute and observe for agglutination.
4) Check whether spontaneous agglutination occurs with the physiological saline.
5) When Polyvalent antiserum gives a positive result, place a drop of each monovalent antiserum on a glass slide, and repeat the procedures above to determine the result.

INTERPRETATION OF RESULTS

Agglutination can be grossly observed using an indirect light source over a darkened background. It should be confirmed that no agglutination is found in the control (reaction with test antigen

and physiological saline). Only strong agglutination observed within 1 minute of the reaction with each serum should be regarded as positive. Delayed or weak agglutination is regarded as negative (refer to the table below interpretation)

Results of polyvalent sera	Determination and additional tests
Some polyvalent sera test positive.	Serotyping is performed using monovalent sera consisting of the polyvalent sera, which tested positive.
Multiple polyvalent sera test positive.	Serotyping is performed using monovalent sera consisting of the polyvalent sera, which tested positive.

Results of monovalent sera	Determination
One monovalent sera tests positive.	The name of the monovalent serum, which tested positive is interpreted as the serotype of the specimen.
Multiple monovalent sera test positive or all monovalent sera test negative.	Determination is suspended.

Precautions in interpretation

1. When tested stain showed multi-positive reaction, confirm purity of the test specimen. It the tested strain is pure and shows multi-positive result, the strain may possess complex antigen.
2. Regarding types 29, 35, and 47, these serotypes share common antigenic factors and show positive reactions with these antisera. the interpretation of these serotypes is done according to the table below.

		Antiserum		
		Type 29	Type 35	Type 47
Type of bacterium	Type 29	+	-	-
	Type 35	(+)	+	-
	Type 47	-	+	+

PERFORMANCE CHARACTERISTICS

1. Sensitivity test

When a drop of the product is reacted on a glass slide with a reference strain of a known serotype, granular agglutination is observed.

2. Specificity test

When the product is tested according to the similar manner to the sensitivity test, agglutination is observed in the reaction with a reference strain of corresponding serotype, but not observed in the reaction with a reference strain of a different serotype(Types 29, 35, and 47 show cross-reactivity).

STORAGE AND SHELF LIFE

Storage: 2°C-10°C.
Shelf life: Up to the expiry date on the label.

PACKAGE

Streptococcus pneumoniae Antisera: 2 mL serum vial with pipette.

- Set: 47 vials/set
  Each serum is individually available.

REFERENCES

1. Oguri, T.: *Streptococcus Pneumoniae,* Clinical and Microbe **23**, 685 (1996).
2. Fukumi, H. *et al.*: *The Clinical Study of Streptococcus Pneumoniae,* The Journal of Infectious Disease Study **58**, 39 (1984).

Please feel free to contact us at the following with your questions or comments :
TEL : +81-3-3669-9421 FAX : +81-3-3669-9390

Symbols

LOT	Batch code
	Use by
IVD	In Vitro Diagnostic Medical Device
	Temperature limitation (Store at)
REF	Catalogue number
	Consult Instruction for use
Cont.	Contents of kit

CE

DENKA SEIKEN CO., LTD.
3-4-2 Nihonbashikayaba-cho, Chuo-ku, Tokyo, Japan
TEL : +81-3-3669-9421 FAX : +81-3-3669-9390

DENKA SEIKEN UK Ltd.
2 Coronation Lane
Oakthorpe, Swadlincote,
Derbyshire DE12 7QY
United Kingdom
TEL : +44 1530 270010 FAX : +44 1530 272009

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