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Wellcogen™ S. pneumoniae

REF ZL22/R30859001 .....30 Tests **EN**

1 INTENDED USE

Wellcogen™ S. pneumoniae is a rapid latex test for use in the qualitative detection of capsular antigen from Streptococcus pneumoniae (Pneumococcus), present in body fluids as a consequence of infection or in blood cultures.

NOTE: Tests performed directly on clinical specimens are intended for screening purposes and should augment, not replace, culture procedures. Results must be used in conjunction with other data; e.g. symptoms, results of other tests, clinical impressions etc.

2 SUMMARY

Pneumococci cause a wide variety of infections, including meningitis, otitis media and pneumonia. The infecting organisms possess capsules containing a type-specific polysaccharide, a quantity of which diffuses into body fluids such as cerebrospinal fluid (CSF), serum, and middle ear fluid and is excreted in the urine. The antigen in these body fluids can be detected by sensitive immunological methods including counterimmunoelectrophoresis and latex agglutination[6,8,11,13,14]. Latex agglutination may also be used to identify S. pneumoniae in blood cultures[9].

3 PRINCIPLE OF THE TEST

The Wellcogen S. pneumoniae reagent consists of polystyrene latex particles which have been coated with antibodies purified from an omnivalent serum which reacts with all the recognised serological types of pneumococci. These latex particles agglutinate in the presence of sufficient homologous antigen.

Some body fluid samples cause non-specific aggregation of latex particles, and a Control Latex preparation is provided in order to identify these samples.

4 SYMBOL DEFINITIONS

REF	Catalogue Number
IVD	In Vitro Diagnostic Medical Device
Σ	Contains sufficient for <n> tests
	Consult Instructions for Use
8°C 2°C	Temperature Limitation
LOT	Batch Code
	Use By
	Manufacturer
	Add water

5 KIT CONTENTS, PREPARATION FOR USE AND STORAGE

The Wellcogen S. pneumoniae kit includes sufficient reagents to perform Σ 30 tests.

See also **Precautions,** section **6**.

All components should be stored at 2 to 8°C under which condition they will retain their activity until the expiry date of the kit.

Before use, bring all reagents to room temperature (18 - 30°C) and mix. Return the unused reagents to the refrigerator after use.

Instructions for Use
Disposable Reaction Cards (1 pack)
Disposable Mixing Sticks (2 bundles)
Disposable Droppers (1 container)
Black rubber teat (1)

TEST LATEX

Test Latex

One dropper bottle (yellow cap) containing a 0.5% suspension of polystyrene latex particles in glycine saline buffer, pH 8.2, with 0.1% sodium azide and 0.05% Bronidox® as preservatives. The latex particles are coated with rabbit antibodies purified from an omnivalent S. pneumoniae antiserum.

CONTROL LATEX

Control Latex

One dropper bottle (dark blue cap) containing a 0.5% suspension of polystyrene latex particles in glycine saline buffer, pH 8.2, with 0.1% sodium azide and 0.05% Bronidox® as preservatives. The latex particles are coated with non-immune rabbit globulins.

The latex suspensions are provided ready for use and should be stored at 2 to 8°C in an upright position, until the expiry date of the kit. After prolonged storage some aggregation or drying of the latex may have occurred around the top of the bottle. Under these circumstances the bottle of latex should be shaken vigorously for a few seconds until resuspension is complete. DO NOT FREEZE.

CONTROL +

Polyvalent Positive Control

One bottle (blue cap) containing freeze-dried bacterial extracts including antigen from a representative strain of S. pneumoniae. Contains 0.01% bronopol before reconstitution and 0.004% when reconstituted.

Reconstitute using 3.6 ml of sterile distilled water. After the addition of water allow the bottle to stand for a few minutes and then swirl to mix. Store reconstituted antigen at 2 to 8°C for up to 6 months.

CONTROL −

Negative Control

One dropper bottle (white cap) containing Glycine saline buffer, pH 8.2, with 0.05% Bronidox® as preservative.

6 PRECAUTIONS

IVD

The reagents are for in vitro diagnostic use only.

For professional use only.

Please refer to the Safety Data Sheet (SDS) and product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

6.1 The Test and Control Latex contain 0.1% sodium azide. Azides can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small; nevertheless when disposing of azide-containing materials they should be flushed away with large volumes of water.

6.2 In accordance with the principles of Good Laboratory Practice it is strongly recommended that body fluids should be treated as

potentially infectious and handled with all necessary precautions.

6.3 When handling radiometric blood culture medium, the basic rules of radiation safety should be followed. These include:

a) Radioactive material should be stored in a designated area in an approved container.
b) Handling of radioactivity should take place in a designated area.
c) No mouth pipetting of radioactive material should be carried out.
d) No eating, drinking or smoking should take place in the designated area.
e) Hands should be washed thoroughly after using radioactive material.
f) The local Radiation Safety Officer should be consulted concerning disposal requirements.

6.4 Non-disposable apparatus should be sterilised by any appropriate procedure after use, although the preferred method is to autoclave for 15 minutes at 121°C. Disposables should be autoclaved or incinerated. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a standard bacterial disinfectant or 70% alcohol. Do NOT use sodium hypochlorite. Materials used to clean spills, including gloves, should be disposed of as biohazardous waste.

6.5 Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.

6.6 When used in accordance with the principles of Good Laboratory Practice, good standards of occupational hygiene and the instructions stated in these Instructions for Use, the reagents supplied are not considered to present a hazard to health.

ANALYTICAL PRECAUTIONS

6.7 Do not use the reagents beyond the stated expiry date.

6.8 Latex reagents should be brought to room temperature (18 to 30°C) before use. Latex reagents which show signs of aggregation or 'lumpiness' before use may have been frozen and must not be used.

6.9 It is important when using dropper bottles that they are held vertically and that the drop forms at the tip of the nozzle. If the nozzle becomes wet an incorrect volume will form around the end and not at the tip; if this occurs dry the nozzle before progressing.

6.10 The reagents provided with each kit are matched in performance and should not be used in conjunction with reagents from a kit having a different lot number.

6.11 Do not touch the reaction areas on the cards.

6.12 Mechanical rotators may be used in this assay. The following characteristics have been found to be satisfactory:

i) Orbital rotators (also known as dimensional rotators) operating at 25 rpm with approximate rotating angle of 9 to 10.5 degrees or operating at 18 rpm with a rotating angle of 16 to 17.5 degrees.

6.13 Avoid microbial contamination of reagents as this may lead to erroneous results.

7 SPECIMEN COLLECTION AND STORAGE

7.1 **Body fluid samples** (e.g. CSF, serum, urine) should be tested as soon after collection as possible. If the fluid cannot be tested immediately it may be stored overnight at 2 to 8°C, or for longer periods frozen at –15 to –25°C. If bacteriological analyses are required on the sample, these should be set up prior to performing the latex test, to avoid contaminating the sample.

7.2 **Blood cultures** may be sampled and tested after 18 to 24 hours incubation at 37°C and/or as soon as bacterial growth is observed.

8 TEST PROCEDURE

REQUIRED MATERIALS PROVIDED

See **Kit Contents**, section **5**.

MATERIALS REQUIRED BUT NOT PROVIDED

Boiling water bath.
Laboratory centrifuge or membrane filters (0.45 µm).
Rotator (optional – refer to **Precautions**, section **6**).

PREPARATION OF CLINICAL SPECIMENS

8.1 **Body fluid samples** must be heated[1,6] before testing by the Wellcogen procedure to minimise non-specific reactions. The following procedures are recommended:

a) For CSF and urine, heat the sample for 5 minutes in a boiling water bath. Cool the sample to room temperature (18 to 30°C) and clarify by centrifugation or membrane filtration (0.45µm) prior to testing. For maximum sensitivity urine samples may be concentrated up to 25-fold in a Minicon® B-15 concentrator. Clarify as above before testing.
b) For serum, add 3 volumes 0.1M disodium ethylenediaminetetra-acetate (EDTA) pH 7.4 per 1 volume serum, heat the sample for 5 minutes in a boiling water bath, cool to room temperature (18 to 30°C) and clarify as above[10]. A suitable EDTA solution (10 ml) is available (Code No. ZL29/R30164501).

8.2 **Blood cultures.** Centrifuge a 1 to 2 ml sample to pellet the red blood cells, for example at 1000 g for 5 to 10 minutes. Perform the latex test on the supernatant.

If a non-specific reaction occurs with a blood culture supernatant (see **Interpretation of Results,** section **10**), heat the sample in a boiling water bath for 5 minutes, cool to room temperature (18 to 30°C), clarify by centrifugation and repeat the test.

PROCEDURE

It is recommended that the section on **Precautions**, section **6**, is read carefully before performing the test.

NOTE: If there is only a limited volume of test sample available, it should be used with the Test Latex first and if a positive result is obtained the sample should be tested with the Control Latex. If sufficient sample is available, it should be tested against both the Test and Control Latexes simultaneously.

Step 1	Process the sample as described under **Preparation of Clinical Specimens**.	
Step 2	Shake the latex reagents.	
Step 3	For each test sample place 1 drop of **Test Latex** in one circle on a Reaction Card, and 1 drop of **Control Latex** in a separate circle. Ensure that the dropper bottles are held vertically to dispense an accurate drop. (See **Precautions**, section **6**).	**1 drop**
Step 4	Using a Disposable Dropper, dispense 1 drop (approximately 40 µl) of **Test Sample** next to each drop of latex.	**1 drop**
Step 5	**Mix** the contents of each circle with a Mixing Stick and spread to cover the complete area of the circle. Use a separate stick for each circle and discard it for safe disposal after use.	
Step 6	**Rock** the card slowly and **observe** for agglutination for 3 minutes, holding the card at normal reading distance (25 to 35 cm) from the eyes. Do not use a magnifying lens. Mechanical rotation (3 minutes) may be used (See **Precautions**, section **6**). The patterns obtained are clear cut and can be recognised under all normal lighting conditions.	**3 mins**
Step 7	Discard the used Reaction Card for safe disposal.	

9 QUALITY CONTROL

The following procedures should be carried out initially with each shipment of test kits and with each run of test samples. In practice, a run may be defined as a testing period of up to 24 hours.

Any departure from the expected results indicates there may be a problem with the reagents, which must be resolved before further use with clinical samples.

VISUAL INSPECTION

The latex suspensions should always be inspected for aggregation as they are dropped onto the test card and if there is evidence of clumping before addition of the test sample, the suspension must not be used. After prolonged storage, some aggregation or drying may have occurred around the top of the bottle. If this is observed, the bottle should be shaken vigorously for a few seconds until resuspension is complete.

POSITIVE CONTROL PROCEDURE

The reactivity of the test can be confirmed by adding Polyvalent Positive Control to a reaction circle in which the test sample has not agglutinated the Test Latex after 3 minutes rotation.

Step 1	Use a Disposable Dropper to add 1 drop of Positive Control to the circle containing Test Latex and specimen.	**1 drop**
Step 2	Mix using a Mixing Stick and discard it for safe disposal.	
Step 3	Rock the card manually, or by a rotator for a further 3 minutes. After this time, definite agglutination should be visible in the Test Latex.	**3 mins**
Step 4	Discard the used Reaction Card for safe disposal.	

NEGATIVE CONTROL PROCEDURE

If at least one test sample within a run gives a negative result with Test and Control Latexes (or Test Latex only where no Control Latex has been used), this constitutes a valid negative control for the reagents and no further testing is necessary.

If a test sample gives agglutination with the Test Latex and no agglutination with the Control Latex then the Test Latex should be tested either with the Negative Control or uninoculated blood culture medium, as appropriate (see below).

Step 1	Place 1 drop of Test Latex in one circle on a Reaction Card.	**1 drop**
Step 2	Dispense 1 drop of Negative Control or uninoculated blood culture medium next to the Test Latex.	**1 drop**
Step 3	Mix using a Mixing Stick and discard it for safe disposal.	
Step 4	Rock the card manually or by a rotator for a further 3 minutes. After this time, there should be no significant agglutination in the Test Latex.	**3 mins**
Step 5	Discard the used Reaction Card for safe disposal.	

For tests with body fluid samples, the Negative Control provided with the kit should be used.

For tests with blood cultures a sample of uninoculated blood culture medium from the same source as the specimen should be used as a negative control. Note: testing uninoculated media is important as false-positives can occur with some formulations of blood culture media.

NOTE: Previously assayed positive and negative samples, aliquoted and stored at –15 to –25°C or below, may be used as positive and negative controls respectively, if desired. The Positive Control can also be used in place of the test sample.

10 RESULTS

READING OF RESULTS

A **positive** reaction is indicated by the development of an agglutinated pattern within 3 minutes of mixing the latex with the test sample, showing clearly visible clumping of the latex particles (Figure 1).

The speed of appearance and quality of agglutination depend on the strength of the antigen, varying from large clumps which appear within a few seconds of mixing, to small clumps which develop rather slowly.

Figure 1

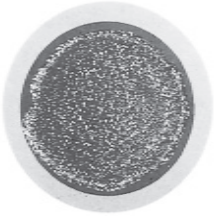

Figure 2

In a **negative** reaction the latex does not agglutinate and the milky appearance remains substantially unchanged throughout the test (Figure 2). Note, however, that faint traces of granularity may be detected in negative patterns, depending on the visual acuity of the operator.

INTERPRETATION OF RESULTS

Positive Result

Clear agglutination of the Test Latex accompanied by a lack of agglutination of the Control Latex indicates the presence of pneumococcal antigen in the body fluid or blood culture supernatant.

Negative Result

Lack of agglutination in both reagents means that no pneumococcal antigen is detectable in the test sample – it does not eliminate the possibility of pneumococcal infection, and if symptoms persist it may be desirable to perform the test on subsequent or alternative specimens, or after concentration of the urine specimen.

Non-interpretable Result

Visible agglutination of the Control Latex, whether stronger or weaker than the Test Latex, indicates a non-specific reaction. In most cases, non-specific reactions with body fluids may be eliminated by heating and clarifying the sample (see **Preparation of Clinical Specimens**, section **8**). If a non-specific reaction occurs with a blood culture supernatant, heat the sample in a boiling water bath for 5 minutes, cool to room temperature (18 to 30°C), clarify by centrifugation and repeat the test.

11 PERFORMANCE LIMITATIONS

11.1 A positive result in the test depends on the presence of a detectable level of antigen in the body fluid or blood culture medium.

11.2 A few examples have been reported of unrelated bacteria which possess common antigens and, as with any immunological test system, the possibility of cross reactions occurring in the latex test can not be ruled out[2,3,4,7,12]. Note that there will be batch to batch variation in the level of antigen which can be detected.

12 EXPECTED RESULTS

Samples containing a detectable level of S. pneumoniae capsular antigen will give an agglutination reaction with the Test Latex.

13 PERFORMANCE CHARACTERISTICS

Clinical studies were carried out in hospital laboratories[5] using body fluid samples (fresh and stored frozen) and supernatants from aerobic and anaerobic blood cultures. Both traditional and radiometric cultural techniques were used in the blood culture studies. Stored body fluid samples were not heat treated as described under **Preparation of Clinical Specimens**, section **8**. Extensive laboratory testing has shown no significant loss of antigen after heating by this procedure.

SENSITIVITY

The sensitivity of Wellcogen S. pneumoniae was established from tests on samples found to be culture positive for the homologous organism or for which there was other evidence of infection (clinical diagnosis plus a positive result in another antigen test). Table 1 shows the numbers of each type of specimen tested together with the number of positive results obtained. The sensitivity of Wellcogen S. pneumoniae was 88% (45/51) for CSF samples and 96% (109/113) for blood culture samples.

SPECIFICITY

The specificity of Wellcogen S. pneumoniae was evaluated using 483 CSF (fresh and frozen), 13 serum, 320 urine and 1512 blood culture samples from patients with bacterial or aseptic meningitis, pneumonia and other unrelated conditions.

The organisms isolated from the infected body fluid samples were Haemophilus influenzae type b, Neisseria meningitidis groups A, B, C, Y, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, beta-haemolytic streptococcus groups A and B and Mycobacterium tuberculosis.

Two of the 483 control CSF samples tested gave a positive reaction with Wellcogen S. pneumoniae, Enterobacter aerogenes was isolated from one sample and a coliform bacterium from the other. Positive results were obtained with 7 of the 1512 control blood cultures tested. The bacteria isolated from these 7 cultures were: Strep. viridans (4 cultures), Strep. sanguis, Staph. epidermidis plus Enterococcus (mixed culture) and Pseudomonas (Table 1).

The specificity of Wellcogen S. pneumoniae in tests on all the body fluids studied was 99.8% (814/816) and in tests on blood cultures was 99.5% (1505/1512). Nine non-specific reactions were observed with blood culture supernatants and all but one of these were removed by heat treatment of the sample as described under **Preparation of Clinical Specimens**, section **8**.

Table 1 Results of clinical studies on Wellcogen S. pneumoniae

	Sensitivity[a]		Specificity[b]	
Sample	No. tested	No. positive	No. tested	No. positive
CSF	51	45	483[c]	2[d]
Serum	6	6	13	0
Urine	105	46	320[e]	0
Blood Culture	113	109	1512	7[f]

[a] S. pneumoniae isolated/indicated (clinical diagnosis and/or other antigen test positive).

[b] Bacteria other than S. pneumoniae/no growth.

[c] One additional CSF sample gave a non-specific reaction.

[d] Enterobacter aerogenes; coliform bacterium.

[e] Three additional urine samples gave non-specific reactions.

[f] Pseudomonas; Strep. sanguis; Staph. epidermidis, plus Enterococcus; Strep. viridans from 4 samples.

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