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DrySpot Pneumo

REF DR0420M.....60 Tests **EN**

1. INTENDED USE

The Oxoid DrySpot™ Pneumo Test is a latex agglutination test for the detection of capsular antigen from *Streptococcus pneumoniae* to provide rapid identification of *Strep. pneumoniae* isolated from culture plates and blood culture.

2. PRINCIPLES OF THE TEST

Strep. pneumoniae is a primary cause of bacterial pneumonia, meningitis and otitis media. The anti-phagocytic properties of the polysaccharide capsule are the key to the organism's virulence[1].

The organism may harmlessly inhabit the upper respiratory tract but may also gain access to the lungs by aspiration where it may establish an acute pneumonia. In addition, this organism also accesses the blood stream and the meninges to cause acute, purulent life-threatening infections[2].

The Oxoid DrySpot Pneumo Test uses antibody sensitised blue latex particles dried onto cards covering most of the recognised serological types of pneumococci[3,4]. The latex will agglutinate in the presence of sufficient antigen to form visible clumps. This test provides a fast and simple screening procedure for *Strep. pneumoniae*.

3. COMPONENTS OF THE KIT (DR420M)

DrySpot Pneumo Test Reagent Cards.
Blue latex particles coated with rabbit antibodies specifically reactive with the recognised serological types of pneumococci and dried onto cards (Test Reaction Area).

Blue latex particles sensitised with non-reactive globulin (Control Reaction Area).

Two pouches each containing 10 cards and a moisture absorbent sachet. There are 3 Test Reaction Areas and 3 Control Reaction Areas on each card – 60 tests in total.

Positive Control Strips (10 sticks – pink spots).
Pink dyed inactivated antigenic extract of *Strep. pneumoniae*.

Negative Control Strips (10 sticks – green spots).
Green dyed inactivated extract of *Aerococcus viridans*.

Phosphate Buffered Saline (PBS).
pH 7.3 (±0.1). Contains 0.095% sodium azide as a preservative.

Mixing paddles.

Instructions For Use

2 x Clips for sealing pouches.

4. MATERIALS REQUIRED BUT NOT PROVIDED

Sterile microbiological loop.

Laboratory disinfectant e.g. Sodium hypochlorite solution >1.3% w/v.

Laboratory Centrifuge.

5. PRECAUTIONS

IVD This product is for *in vitro* diagnostic use only.

Specimen materials may contain pathogenic organisms, handle with appropriate precautions.

Sodium azide may react with lead and copper in plumbing to form highly explosive metal azides. If reagents containing sodium azide are disposed of in a sink, they should be flushed with plenty of water to prevent build up of metal azides.

6. STORAGE AND OPENING

The kit must be stored between 2°C and 25°C. If stored in a cold environment, allow pouches to reach room temperature before opening to prevent condensation of moisture on the cards. The DrySpot reagents will deteriorate and may give false results if they are allowed to absorb moisture.

Open the pouches by cutting with scissors just below the seal.

Once opened, remove the number of cards required for immediate testing (testing within the next 10 minutes) and re-seal the pouch immediately by clamping the open end of the bag between the two halves of the plastic clip provided.

If fewer tests are required, cut the reaction cards along the indicated lines and return the unused portions to the pouch. Do not return used portions because they will cause contamination of remaining cards in the pouch.

The Control Sticks are also provided in a moisture-impermeable pouch. Ensure that the same techniques are used to avoid moisture damage.

Under these conditions the reagents will retain their activity until the expiry date shown on the kit box.

7. CONTROL PROCEDURES

Add a 50 µl drop of saline to the small circle at the base of the test oval reaction area. Remove a Positive Control Strip from the pouch by tearing one off from the others on the strip, taking care to avoid touching the flexible end where the coloured spots are located. Re- seal the inner bag and pouch. Turn the stick over so that the coloured spots are at the bottom and place the stick on the card with the spots touching the liquid. Push down so that the end bends at the hinge and mix in a circular manner for 10 seconds to rehydrate the dried reagent. Continue to use the stick to mix the liquid into the appropriate DrySpot Pneumo Test Reagent until all the reagents are fully rehydrated and homogeneous. Rock the card and look for agglutination. This procedure should be repeated using a Negative Control Strip.

The **positive control** must show agglutination with the dried reagent within 60 seconds.

The **negative control** must show no agglutination within 60 seconds.

Do not use the test if reactions with the control reagents are incorrect.

8. IMPORTANT PROCEDURE NOTES

Do not touch the circles on the reaction cards as this may cause contamination and affect the reaction.

In a high humidity environment do not leave the pouches open for more than 2 minutes. Do not use the cards if there is evidence of moisture in the spots.

Do not place the drop of PBS directly onto the dry latex spots. Pouch clips can be retained for future use to allow multiple packs to be opened. Although suitable for room temperature storage the kit or pouches must not be stored near a heat source or where exposure to sunlight may cause increased temperatures.

9. SPECIMEN COLLECTION AND PREPARATION

A. Culture
For details of specimen collection and treatment a standard reference text should be consulted[5].

-Haemolytic, Gram-positive, catalase-negative colonies may be tested from the following culture media:

Blood Agar, Tryptone Soya Agar with 5% blood, Columbia Blood Agar, Columbia CNA Agar, Chocolate Blood Agar.

The use of fresh cultures grown overnight is recommended (18–36 hours incubation). The tendency of colonies to autoagglutinate increases with incubation beyond 36 hours.

B. Blood Cultures
Blood cultures may be sampled and tested after 18–24 hours incubation at 37°C and/or as soon as bacterial growth is observed. The presence of *Strep. pneumoniae* should be confirmed by performing a Gram stain.

10. TEST METHOD – CULTURE

1. Add 1 drop (50 µl) of PBS to the small ring (at the bottom of each oval) in both the Test and Control Reaction Areas ensuring that the liquid does not mix with the dried latex reagents.
2. Using a sterile loop (or one of the paddles provided) apply several suspect colonies from a culture medium plate in the Control Reaction Area. Emulsify colonies in the saline to obtain a slightly opalescent suspension. Ensure that the resulting suspension is smooth.
3. Using the loop or paddle provided, mix the suspension into the dried latex spots until completely suspended and spread to cover the Reaction Area. Discard the loop/paddle appropriately.
4. Using a separate loop or paddle provided, proceed in the same way with the Test Latex.
5. Pick up and rock the card for up to 60 seconds and look for agglutination under normal lighting conditions. Do not use a magnifying glass.
6. When the test is completed dispose of the reaction cards safely into disinfectant.

11. TEST METHOD – BLOOD CULTURES

1. Centrifuge the 1–2 ml sample to produce a pellet of red blood cells, for example at 1000 g for 5–10 minutes. Perform the latex test on the supernatant.
2. Dispense 1 drop of supernatant to the small ring (at the bottom of each oval) in both the Test and Control Reaction Areas ensuring that the liquid does not mix with the dried latex reagents.
3. Using a loop or paddle provided mix the supernatant into the dry control latex spot until completely suspended and spread to cover the Reaction Area. Discard the loop or paddle appropriately.
4. Using a separate loop or paddle, proceed in the same way with the Test Latex.
5. Pick up and rock the card for up to 2 minutes and look for agglutination under normal lighting conditions. Do not use a magnifying glass.
6. When the test is completed dispose of the reaction cards safely into disinfectant.
7. The presence of *Strep. pneumoniae* in blood culture samples, which are positive by latex agglutination, should be confirmed by conducting a Gram stain on the pellet.

12. READING AND INTERPRETATION OF RESULTS

Positive Result
A result is positive if agglutination of the latex particles is observed in the Test Reaction Area within 60 seconds for culture confirmation and within 2 minutes for blood cultures. This indicates the presence of *Strep. pneumoniae*.

Negative Result
A negative result is obtained if no agglutination is observed in the Test Reaction Area and a smooth blue suspension remains after 60 seconds for culture confirmation and 2 minutes for blood cultures.

Reactions occurring after this time should be ignored.

Uninterpretable Result
The test is uninterpretable if the Control Reagent shows agglutination. This indicates that the culture causes autoagglutination.

Granular or Stringy Reactions
Occasionally granular or stringy reactions may be observed due to the particulate nature of the test material. When such reactions are observed they should be interpreted using the following criteria:

The result is **positive** when, using the Test Reagent, greater clearing of the blue background is observed compared with the reaction of the Control Reagent.

The result is **negative** when there is no difference between clearing of the blue background using the Test and Control Reagents.

13. LIMITATIONS

The Mixing Paddles provided are not sterile. They may be sterilised by autoclaving if required.

The DrySpot Pneumo Test provides a presumptive result. Confirm positive results using biochemical tests.

A positive test from blood culture depends on the presence of a detectable level of antigen in the blood culture medium. Tests performed directly on clinical specimens are intended for screening purposes and should not replace culture procedures.

False negative reactions may also occur if an insufficient number of colonies are used for testing. In this case, the isolate should be sub- cultured and tested when there is sufficient growth.

If a pneumococcal strain does not possess a capsulate antigen it cannot be identified using immunological techniques.

False positive reactions may be found with certain strains of Group C streptococci and with *Streptococcus mitis*, *Streptococcus mitori*, *Streptococcus sanguis* and *Streptococcus oralis*[6]. In addition several examples of serological cross-reactions between pneumococci and Gram-negative bacteria have been observed e.g. *E. coli, Klebsiella spp.* and *H. influenzae*[7,8].

14. PERFORMANCE CHARACTERISTICS

Culture Samples

The DrySpot Pneumo kit was evaluated at the National Reference Centre for Streptococcus and Diptheria in the UK[9]. Columbia Agar with 5% horse blood was inoculated with 216 strains (144 *Strep. pneumoniae* and 72 non-*Strep. pneumoniae*). After incubation the pure cultures were tested with the DrySpot Pneumo kit and another commercially available latex agglutination kit.

The sensitivity of the DrySpot Pneumo kit was 97.9% and the specificity was 93.2%. Thirteen non-*Strep. pneumoniae* strains gave an uninterpretable result (agglutination of the control latex), with DrySpot Pneumo and 16 gave an uninterpretable result in the competitor latex kit. Of the four non-*Strep. pneumoniae* strains that gave false positive results with DrySpot Pneumo, three were organisms known to demonstrate serological cross reactions with *Strep. pneumoniae*[6]. The performance of DrySpot Pneumo was equivalent to or better than that of the other kit tested.

Blood Culture Samples

The DrySpot Pneumo kit was also evaluated for the detection of *Strep. pneumoniae* from blood cultures at Oxoid Ltd[9]. The evaluation consisted of inoculating Oxoid Signal™ blood culture medium and two other commercially available blood culture media with 37 *Strep. pneumoniae* and 35 non-*Strep. pneumoniae* strains. After incubation the cultures were tested with the DrySpot Pneumo kit and with two other commercially available latex agglutination kits.

With Signal blood culture medium, the sensitivity of the DrySpot Pneumo kit was 97.3% and the specificity was 96.8%. The results with the other blood culture media were comparable. Four non-*Strep. pneumoniae* strains gave uninterpretable results with all three kits in all three blood culture media.

The performance of the DrySpot Pneumo kit was equivalent to or better than that of the other kits tested.

15. REFERENCES

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9. Data on file, Oxoid.

Symbol Legend

REF	Catalogue Number
IVD	*In Vitro* Diagnostic Medical Device
	Consult Instructions for Use
	Temperature Limitation
LOT	Batch Code
	Use By
	Manufacturer

CE

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For technical assistance please contact your local distributor