DrySpot Staphyetect Plus

1. INTENDED USE

DrySpot Staphyetect Plus™ is a latex slide agglutination test¹ for the differentiation of Staphylococcus aureus by detection of clumping factor, Protein A and certain polysaccharides found in methicillin-resistant S. aureus (MRSA) from those staphylococci that do not possess these properties.

2. PRINCIPLE OF THE TEST

Traditionally, differentiation between coagulase-positive and coagulase-negative staphylococci has been performed with the tube coagulase test that detects extracellular staphylocoagulase or the slide coagulase test that detects the clumping factor (bound coagulase) present on the bacterial cell surface. Several other differentiation tests are also available including the passive haemagglutination test (Oxoid Staphylase – DR595) and the DNase test.

It has been reported that approximately 97% of human strains of Staphylococcus aureus possess both bound coagulase and extracellular staphylococcal clumping factor. Protein A is found on the cell surface of about 95% of human strains of S. aureus and has the ability to bind the Fc portion of immunoglobulin G (IgG)².

It has been observed that certain methicillin-resistant strains of S. aureus (MRSA) may express undetectable levels of clumping factor and Protein A. It has also been shown, however, that these strains all possess capsular polysaccharide. The capsule can mask both Protein A and the clumping factor thereby preventing agglutination.

DrySpot Staphyetect Plus uses blue latex particles coated with both porcine fibrinogen and rabbit IgG including specific polyclonal antibodies raised against capsular polysaccharide of S. aureus³,⁴. The reagent is dried onto the reaction card. When the reagent is mixed with the sample, it forms a smooth blue suspension under normal lighting conditions. Do not use the test if reactions with the control organisms are incorrect.

3. COMPONENTS OF THE KIT

Blue latex particles coated with both porcine fibrinogen and rabbit IgG together with specific polyclonal antibodies raised against capsular polysaccharide of S. aureus (Test Reaction Area). Blue latex particles sensitised with non-reactive globulin (Control Reaction Area).

4. STORAGE AND OPENING

This kit must be stored between 2 and 25°C. If stored in a cold environment, allow the 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 exposed to high humidity.

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

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 to the pouch because they will cause contamination of remaining cards in the pouch.

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

5. PRECAUTIONS

This product is for in vitro diagnostic use only. Specimen material may contain pathogenic organisms. Handle with the appropriate precautions.

6. CONTROL PROCEDURES

On each occasion the kit is used, the following control procedures must be performed:

1. Positive Control: Use a known S. aureus strain such as ATCC® 25923 (Thermo Scientific Culti-Loops™ R4607010). Follow the method given in Test Procedure. Ensure that agglutination occurs within 20 seconds.

2. Negative Control: Use a known S. epidermidis strain such as ATCC® 12228 (Thermo Scientific Culti-Loops™ R4606500). Follow the method given in Test Procedure. Ensure that the reagent remains smooth and non-agglutinated for the entire 20 seconds of the test.

7. TEST METHOD

1. Use a sterile loop to pick up the equivalent of 0.5 cm² sized staphylococcal colonies (equivalent to 2–3 mm diameter of growth) from a culture media plate and smear onto the Control Reaction Area. Using the loop, spread the colony material into a thin film keeping the colony material clear of the dried latex reagents.

2. Dispense 1 drop of saline (0.85%) directly onto the thin film to form a smooth suspension and mix IMMEDIATELY.

3–6. As Standard Test Method.

7. READNG AND INTERPRETATION OF RESULTS

Positive Result

A result is positive if agglutination of the blue test latex particles occurs within 20 seconds. This presumptively identifies the strain as a S. aureus.

Negative Result

A result is negative if no agglutination occurs and a smooth blue suspension remains after 20 seconds in the test circle. This presumptively identifies the strain as non S. aureus.

Equivocal Result

Slight granularity of the test latex accompanied by no change in the appearance of the control latex should be interpreted as an equivocal result. Strains should be re-tested following subculture non-selective media.

Uninterpretable Result

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

Granular or Stringy Reactions

Occasionally granular or stringy reactions may be seen due to the particulate nature of the test material.

The use of fresh cultures grown overnight is recommended (18 to 36 hours incubation). The tendency of colonies to cause autoagglutinating reactions increases with incubation beyond the recommended 36 hour period.

8. IMPORTANT PROCEDURE NOTES

1. Add 1 drop (50 µl) of saline (0.85%) to the small rings (at the bottom of each oval) in both the test and control reaction areas, ensuring the agar does not mix with the dried latex reagents.

2. Use a sterile loop to pick up the equivalent of 0.5 cm² sized staphylococcal colonies (equivalent to 2–3 mm diameter of growth) from a culture media plate and smear onto the Test Reaction Area. Using the loop, spread the colony material into a thin film keeping the colony material clear of the dried latex reagents.

3. Pick up and rock the card for up to 20 seconds and observe for agglutination under normal lighting conditions. Do not use a magnifying glass.

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

5. The result is negative when there are no differences between clearing of the blue background using the Test and Control Reagents. Reactions occurring after 20 seconds should be ignored.

9. SPECIMEN COLLECTION AND PREPARATION

For details of specimen collection and treatment a standard test book should be consulted.

10. STANDARD TEST METHOD

1. Add 1 drop (50 µl) of saline (0.85%) to the small rings (at the bottom of each oval) in both the test and control reaction areas ensuring that the agar does not mix with the dried latex reagents.

2. Use a sterile loop to pick up the equivalent of 0.5 cm² sized staphylococcal colonies (equivalent to 2–3 mm diameter of growth) from a culture media plate and smear onto the Test Reaction Area. Using the loop, spread the colony material into a thin film keeping the colony material clear of the dried latex reagents.

3. Pick up and rock the card for up to 20 seconds and observe for agglutination under normal lighting conditions. Do not use a magnifying glass.

4. The result is positive if agglutination of the blue test latex particles occurs within 20 seconds. This presumptively identifies the strain as a S. aureus.

5. The result is negative if no agglutination occurs and a smooth blue suspension remains after 20 seconds in the test circle. This presumptively identifies the strain as non S. aureus.

6. The result is equivocal if there is a change in the appearance of the control latex but not of the test latex.

7. The result is uninterpretable if the Control Reaction shows agglutination. This indicates that the culture causes autoagglutination.

Granular or Stringy Reactions

Occasionally granular or stringy reactions may be seen due to the particulate nature of the test material.

The use of fresh cultures grown overnight is recommended (18 to 36 hours incubation). The tendency of colonies to cause autoagglutinating reactions increases with incubation beyond the recommended 36 hour period.

11. TEST METHOD FOR OXACILLIN RESISTANCE SCREENING AGAR

1. Use a sterile loop to pick up the equivalent of 0.5 cm² sized staphylococcal colonies (equivalent to 2–3 mm diameter of growth) from a culture media plate and smear onto the Control Reaction Area. Using the loop, spread the colony material into a thin film keeping the colony material clear of the dried latex reagents.

2. Dispense 1 drop of saline (0.85%) directly onto the thin film to form a smooth suspension and mix IMMEDIATELY.

3–6. As Standard Test Method.

12. READING AND INTERPRETATION OF RESULTS

Positive Result

A result is positive if agglutination of the blue test latex particles occurs within 20 seconds. This presumptively identifies the strain as a S. aureus.

Negative Result

A result is negative if no agglutination occurs and a smooth blue suspension remains after 20 seconds in the test circle. This presumptively identifies the strain as non S. aureus.

Equivocal Result

Slight granularity of the test latex accompanied by no change in the appearance of the control latex should be interpreted as an equivocal result. Strains should be re-tested following subculture non-selective media.

Uninterpretable Result

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

Granular or Stringy Reactions

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

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

2. The result is negative when there are no differences between clearing of the blue background using the Test and Control Reagents. Reactions occurring after 20 seconds should be ignored.

13. LIMITATIONS OF THE PROCEDURE

1. The tendency of isolated colonies to cause autoagglutination increases with incubation times beyond the recommended 36 hour period.

2. The antibody used in Staphyetect Plus has been optimised to avoid potential cross-reactions with shared antigens from coagulase-positive staphylococci. It should be noted that this has been shown to reduce sensitivity to some type 18 MRSA strains⁵.

3. Some species of staphylococci other than S. aureus notably S. hyicus, S. intermedius, S. lugdunensis, S. xylosus, S. schleiferi and S. chromogenes may give positive results in coagulase tests and/or rapid latex procedures. If necessary the species may be identified by biochemical test procedures e.g. using a test for PYRase activity (Oxoid O.B.I.S. PYR ID5080M). S. aureus and S. hyicus will be PYRase negative and all the other strains named above will be positive⁶,⁷. S. hyicus and S. intermedius are rarely encountered in the clinical laboratory.
4. Staphyloccoci isolated from urine specimens which give a weak positive result with Staphytyct Plus may be Staphylococcus saprophyticus. Further identification of such isolates may be conducted using biochemical tests and novobiocin sensitivity (S. saprophyticus is resistant to novobiocin).

5. Some streptococci and possibly other organisms possessing immunoglobulin or plasma binding factors may react in the latex test and some species such as Escherichia coli are able to agglutinate latex particles non-specifically. To overcome these non-specific results a Gram-stain should be performed so only typical staphylococci are tested.

14. PERFORMANCE CHARACTERISTICS

The performance characteristics of OXOID DrySpot Staphytyct Plus have been determined using data from the studies detailed below. It is important to note, however, that S. aureus is known to show considerable antigenic variation with respect to different geographical locations.

Clinical Study

OXOID DrySpot Staphytyct Plus was evaluated at a large Australian teaching hospital. A total of 300 isolates was tested using tube coagulase as the gold standard method. In the following data analysis, results from strains known to be cross reactors and results from autoagglutinating strains have been omitted (n=284). The relative sensitivity was 100% and the relative specificity was 96.9%.

Industrial Study

OXOID DrySpot Staphytyct Plus was evaluated in food laboratories in a multi-centre study in the United Kingdom. A total of 621 samples was evaluated, these were colonies taken from Baird-Parker Agar, which had been inoculated with food or environmental material. The gold standard method was tube coagulase. In the following data analysis, results from strains known to be cross reactors and results from autoagglutinating strains have been omitted (n=603). The relative sensitivity was 98.4% and the relative specificity was 96.3%.

15. REFERENCES


10. Data on file at OXOID Ltd.


REFERENCE

Catalogue Number: DR0100M..................120 Tests

In vitro diagnostic medical device

Consult instruction for use

Temperature limitation

Batch code

Use by

Contains sufficient for <N> tests

Manufacturer

OXOID Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, England.