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Staphytect Plus

REF DR0850M..... Σ 100
DR0850B..... Σ 500

EN

1. INTENDED USE

Staphytect Plus™ is a latex slide agglutination test[1] 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 either 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 staphylocoagulase.

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[3,4,5]. It has been shown however that these strains all possess capsular polysaccharide[6]. The capsule can mask both Protein A and the clumping factor thereby preventing agglutination. Staphytect Plus uses blue latex particles coated with porcine fibrinogen and rabbit IgG including specific polyclonal antibodies raised against capsular polysaccharides of *S. aureus*[7,8]. When the reagent is mixed on a card with colonies of *S. aureus*, rapid agglutination occurs through the reaction between (i) fibrinogen and clumping factor, (ii) Fc portion of IgG and Protein A, (iii) specific IgG and capsular polysaccharide. Agglutination may also occur with other species which possess clumping factor or Protein A such as *Staphylococcus hyicus* and *Staphylococcus intermedius*. If neither clumping factor, Protein A nor specific capsular polysaccharides are present, agglutination will not occur and the result will be regarded as negative. The most frequent coagulase and Protein A negative isolates of staphylococci are *Staphylococcus epidermidis*.

3. COMPONENTS OF THE KIT (DR850M)

DR0851M Staphytect Plus Test Reagent (5.6 ml)

A suspension of synthetic blue latex particles coated with both porcine fibrinogen and rabbit IgG together with specific polyclonal antibodies raised against capsular polysaccharide of *S. aureus*. Each bottle contains sufficient reagent for 100 tests.

DR0852M Staphytect Plus Control Reagent (5.6 ml)

A suspension of unsensitised synthetic blue latex particles. Each bottle contains sufficient reagents for 100 tests.

DR0500G Reaction Cards.

There are 35 disposable reaction cards provided in the kit.

Instructions for Use.

4. MATERIALS REQUIRED BUT NOT PROVIDED

Timer

Microbiological Loop

A suitable laboratory disinfectant

Positive Control: *S. aureus* strain such as ATCC 25923

Negative Control: *S. epidermidis* strain such as ATCC 12228.

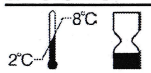
5. PRECAUTIONS

IVD This product is for *in vitro* diagnostic use only. Do not freeze.

Reagents contain 0.095% sodium azide as a preservative. Sodium azide is toxic and may react with lead or copper plumbing to produce metal azides which are explosive by contact detonation. To prevent azide accumulation in plumbing flush with copious amounts of water immediately after waste disposal.

Specimen materials may contain pathogenic organisms handle with appropriate precautions.

6. STORAGE



This kit must be stored at 2–8°C. Do not freeze. Under these conditions the reagents will retain their activity until the expiry date shown on the kit box.

7. CONTROL PROCEDURES

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

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

7.2. Negative Control: Use a known *S. epidermidis* strain such as ATCC12228 (Culti-Loop™ 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.

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

Important Procedure Notes

Do not allow the test latex to become contaminated by letting the dropper tip touch the specimen on the reaction card.

Ensure that the caps are securely fitted after each use to prevent contamination and drying out of the reagent. After use return the kit to the refrigerator ensuring that the bottle is stored in an upright position. Ensure that sufficient growth is removed from the culture plate; an insufficient quantity may give rise to false negative reactions.

8. SPECIMEN COLLECTION AND PREPARATION

For details of specimen collection and treatment a standard text book should be consulted[9]

Gram positive, catalase positive colonies may be tested from any of the following culture media:

Blood Agar
Nutrient Agar
Tryptone Soya Agar
Tryptone Soya Agar with 5% blood
Mannitol Salt Agar
Columbia Blood Agar
Columbia CNA Agar
Mueller-Hinton Agar with 5% blood
Baird-Parker Agar
CLED Medium
Iso.Sensitest Agar
Iso.Sensitest Agar with 5% blood
Oxacillin Resistance Screening Agar (ORSA).

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.

9. STANDARD TEST METHOD

9.1. Bring the latex reagents to room temperature. Make sure that the latex reagent is mixed by vigorous shaking and expel any latex from the dropper pipette for complete mixing.

9.2. Dispense 1 drop of test latex onto one of the circles on the reaction card and 1 drop of control latex onto another circle.

9.3. Using a loop, pick up and smear the equivalent of 5 average-sized suspect Staphylococcal colonies (equivalent to 2–3 mm diameter of growth) onto a circle from a culture media plate and mix this in the Control Latex reagent. Spread to cover the circle. Discard the loop appropriately.

9.4. Using a separate loop proceed in the same way with the Test Latex.

9.5. 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.

9.6. When the test is completed dispose of the reaction cards safely into disinfectant.

9.7. Test Method for Oxacillin Resistance Screening Agar

9.7.1 As Standard Test Method.

9.7.2 Using a loop, pick up and smear the equivalent of 5 average-sized suspect Staphylococcal colonies (equivalent to 2–3 mm diameter of growth) from a culture media plate onto a circle. Using the loop, spread the colony material into a thin film.

9.7.3 Dispense 1 drop of Control Latex directly onto the thin film and mix IMMEDIATELY.

9.7.4–6. As Standard Test Method.

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

Equivocal Result

Slight graininess 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 onto non-selective media.

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 reactionsmay 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:

The result is **positive** when using the test reagent greater clearing of the blue background is observed when compared with the reaction of the control reagent. The result is **negative** when there are no differences between the clearing of the blue background using the Test and Control Reagents.

Reactions occurring after 20 seconds should be ignored.

11. LIMITATIONS OF THE PROCEDURE

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

11.2. The antibody used in Staphytect Plus has been optimised to avoid potential cross-reactions with shared antigens from coagulase negative staphylococci.

It should be noted that this has been shown to reduce sensitivity to some type 18 MRSA strains[10].

11.3. Some species of Staphylococci otherthan *S. aureus* notably *S. hyicus, S. intermedius, S. lugdunensis, S. xylosus, S. schleiferi* and *S. haemolyticus*[11,12,13,14], 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 ID0580M), *S. aureus* and *S. hyicus* will be PYRase negative and all the other strains named above will be positive[15,16,17]. *S. hyicus* and *S. intermedius* are rarely encountered in the clinical laboratory.

11.4. Staphylococci isolated from urine specimens which give a weak positive[18] result with Staphytect 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).

11.5. 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[19,20] non-specifically. To overcome these non-specific results a Gram-stain should be performed to ensure only typical Staphylococci are tested.

12. PERFORMANCE CHARACTERISTICS

The performance characteristics of Oxoid Staphytect 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 Staphytect Plus was evaluated at a large French hospital. A total of 299 isolates was tested using tube coagulase as the gold standard method. In the following data analysis, results from strains known to be cross reactors[11,12,13,14] and results from autoagglutinating strains have been omitted (n=283). The relative sensitivity was 96.5% and the relative specificity was 97.6%.

Industrial study

Oxoid Staphytect 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[11,12,13,14] and results from autoagglutinating strains have been omitted (n=604). The relative sensitivity was 97.6% and the relative specificity was 97.1%.

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14. SYMBOL DEFINITIONS

REF	Catalogue Number
IVD	*In Vitro* Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
Σ N	Contains sufficient for <N> tests
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured by

CE

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For technical assistance please contact your local distributor.