



ANI Biotech

ANI TM *S. aureus* TEST

FOR RAPID IDENTIFICATION OF *S. aureus*

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A. TEST PRINCIPLE

Coagulase positive staphylococci produce extracellular coagulase enzyme, which activates prothrombin thereby initiating clot formation in the plasma. Free coagulase is traditionally identified by the tube coagulase test.

The coloured latex particles of the ANI *S. aureus* TEST are coated with fibrinogen and immunoglobulin G (IgG). The reagent is dried on a test card. The test identifies cell-associated coagulase (clumping factor), which normally reacts with fibrinogen to cause rapid aggregation (clumping) on the latex particles.

The cell walls of pathogenic strains of *S. aureus* almost always contain protein A, which in turn reacts with the Fc-component of the IgG molecules on the latex particles, which also causes aggregation of the reagent. Both the bound coagulase (clumping factor) and protein A are important, mutually independent, factors used in the identification of *S. aureus* bacteria.

B. TEST COMPONENTS

- The ANI *S. aureus* TEST contains 15 test cards packed in three aluminium foil cartons. Each card contains eight dried reagent spots.
- 10 ml phosphate buffered saline (PBS) for the preparation of bacterial suspensions (ANI *S. aureus* TEST-buffer).
- 10 cards of 12 ANI sampling / mixing sticks.

C. TEST PROCEDURE

Open the test package by tearing from the point of perforation or using scissors, leaving sufficient packag material to enable reclosure by folding. Place the test card on the working surface. Add one drop (appr. 40 µl) of PBS to a reagent dot on the card. Gather 1-3 gram-positive and catalase-positive colonies of typical size, shape and colour from a suitable agar medium (sheep's blood agar, Baird-Parker agar or some other suitable medium) and the bacterial mass is smeared onto the test card **next to the reagent spot** as a thin layer. If the colonies are small it is recommended that 4-6 colonies are used for each smear.

Using the ANI sampling/mixing stick, **the suspended blue reagent dot and the bacterial mass are mixed** until a homogeneous bacterial suspension is formed. Next, **the bacterial suspension and the latex reagent spot are mixed together** until the latex reagent is **suspended completely** in the mixture. The test card is then inclined and rotated so that the suspensions are mixed evenly in the test circles. Signs of agglutination are examined for 30 seconds from mixing of the bacterial suspension and the latex suspension.

D. INTERPRETATION OF THE RESULT

The test result is considered positive if an obvious clumping or agglutination occurs in the test circle within 30 seconds.

Some *S. aureus* strains react very rapidly and strongly with the latex reagent. In such cases the positive result may be observed as soon as the reagent is mixed with the bacterial suspension.

E. CHECKING THE FUNCTIONING OF THE TEST

The functioning of the test can be verified by cultivating a known *S. aureus* strain on a suitable nutrient agar medium and investigating the agglutination reaction obtained with this strain.

When pure buffer, or a suspension of other bacteria (e.g. *S. saprophyticus* or *S. epidermidis*) in the buffer, is used in the test the reagent should remain as a homogeneous suspension (negative control) after mixing for at least 30 seconds.

A false result may be obtained if any other buffer than the ANI *S. aureus* TEST-buffer is used for suspension of the bacterial cells.

F. STORAGE

Test packs can be stored unopened at +2 . . . +8°C for one year. The packs are opened by cutting with scissors along the marked end. Opened packages should be carefully protected from the effects of moisture, e.g. by resealing with masking tape.

G. WARNING

General bacteriological procedures and precautions should be followed when mixing the test components.

All *S. aureus* strains and many more of the bacterial strains being investigated are potential pathogens. Used test cards and sampling / mixing sticks must therefore be treated with due caution and inactivated before disposal.

ANI sampling / mixing sticks are not sterile. Slight contamination of the colonies on the agar medium is therefore possible when bacterial mass is taken from the colonies with the sticks. This should be taken into account in any further bacteriological investigations.

The dried latex reagents contain small amounts of toxic sodium azide used as preservative. All used test materials should therefore be disposed with due caution.

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