# Urea-Fibrinogen Slide Coagulase Test – A Simple Alternative Method for the Rapid Identification of Staphylococcus aureus

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*Slide Coagulase Test (SCT), Staphylococcus aureus, Tube Coagulase Test (TCT), Urea-Fibrinogen Slide Coagulase Test (UF-SCT).*

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**ABSTRACT**

**Introduction:** The accurate identification of *Staphylococcus aureus* clinical isolates requires a series of tests. Morphological features and slide coagulase test are two criteria on which *S. aureus* are identified. Resort to tube coagulase test is sought when results of slide coagulase test are equivocal or doubtful. Both coagulase tests detect the enzymes that convert fibrinogen into fibrin. Human, rabbit or sheep pooled plasma is used as substrate for both tests. Slide coagulase test is simpler and faster as compared to tube coagulase test. The plasma could be carrier of many human and animal pathogens like HIV, HBV, HCV etc. Storage of plasma for longer duration is fraught with chances of contamination. Improperly stored plasma can lead to false positive or negative results. Citrated plasma may be unsuitable for this test if contaminated with citrate utilizing bacteria. Considering the role of *S. aureus* as a common etiological agent in nosocomial and community infections, there is a need of implementing rapid, easy and cost-effective phenotypic test.

**Objectives:** Considering the disadvantages and risks associated with fresh plasma, this study aims to launch for safer, more reliable substitute with longer shelf life that may provide reliable results for prompt identification of *S. aureus* by slide coagulase test.

**Methods:** The present work evaluates slide coagulase test (SCT), and urea fibrinogen slide coagulase test (UF-SCT) for *S. aureus* detection considering Tube coagulase test (TCT) as the reference method. Sensitivity, specificity, positive predictive value and negative predictive values of SCT and UF-SCT were calculated using TCT as gold standard.

**Results:** A total of 150 staphylococcal isolates from different clinical specimens were selected for the evaluation of coagulase tests. All the specimens were subjected to SCT, UF-SCT and TCT. The UF-SCT showed better sensitivity (95.04%), specificity (100%), PPV (100%), and NPV (82.85%) with reference to TCT. UF-SCT showed similar sensitivity and specificity to SCT. None of the isolates were negative in UF-SCT

and positive in SCT. Since UF-SCT does not incorporate plasma directly and at the same time has a very good sensitivity and specificity, it is recommended that UF-SCT could replace SCT for identification of *S. aureus*.

**Conclusion:** The findings of present study shall encourage laboratories to use the urea-fibrinogen slide coagulase test routinely for the rapid identification of *S aureus*.

## INTRODUCTION

*Staphylococcus aureus* is a common etiological agent in nosocomial and community infections, therefore its correct identification is essential[1]. *Staphylococcus aureus* secretes coagulase enzymes which are not only virulence factors but also an important criterion for distinguishing it from coagulase negative staphylococci (CoNS). Several criteria like mannitol fermentation test, coagulase tests, agglutination test, DNAse etc are proposed for discrimination of *S. aureus* from other staphylococci[2,3]. However, these tests add to the cost and are not always available in developing countries.

In developing countries like Nepal, phenotypic tests are the mainstay in the diagnosis of staphylococcal infections, coagulase tests are usually accepted as confirmatory for *S. aureus*[4,5,6,7,8]. Coagulase tests are performed using the slide (SCT) or the tube (TCT) methods[9]. The gold standard for *S. aureus* identification is demonstration of free coagulase by tube coagulase test, a test of choice because of its high sensitivity and specificity[10,11]. Tube coagulase test may take as long as 24 hours. Therefore in resource limiting laboratories *S. aureus* is differentiated from CoNS mostly by slide coagulase test.

Although coagulase tests are invaluable for identification of *S. aureus*, few studies have evaluated their use in routine practice[12]. The issues associated with utilizing fresh plasma for the coagulase tests include; possible presence of viral agents (HIV, Hepatitis B and C etc), non availability of fresh plasma, and possible contamination of plasma leading to erroneous results. The plasma poses risk to laboratory workers and prior to use, it must be screened for safety, an expensive proposition. The shelf life of fresh plasma is limited and chances of contamination very high. Procurement of fresh uncontaminated plasma regularly is not always possible.

The advantages of solutions of fibrinogen instead of plasma for carrying out the slide coagulase test were pointed out by Berger[13]. Its use avoided false positives due to naturally occurring staphylococcal agglutinins in rabbit and human plasma, in addition to the fact that these solutions retained their activity considerably longer. Spencer published protocol on preparation and storage for solutions of crude fibrinogen for the slide coagulase test and found that 10% urea had a sufficiently strong antibacterial action to suppress almost all accidental contamination of the solution[14]. This study re-evaluated the role of UF-SCT method with slight modification for the rapid identification of *S. aureus* and compared its performance with SCT and TCT.

The results of this study shall encourage laboratories to use the UF-SCT method routinely for the rapid identification of *S. aureus.* It has a special interest for those laboratories which have no ready access to an animal house, or where procuring fresh plasma regularly may not be feasible.

## METHODS

### Study setting

This study was conducted in the Clinical Microbiology Laboratory of the Department of Microbiology, Manipal College of Medical Sciences, from April through October, 2014. This laboratory based study used stored *S. aureus* clinical isolates from blood, cerebral spinal fluid, urine, sputum, respiratory secretions, anterior nares, pus, and wound swabs of different outpatients and inpatients.

### Phenotypic identification of *Staphylococcus aureus*

One hundred and fifty (N=150) specimens were inoculated onto 5% sheep blood agar and incubated at $37^0$C overnight. Staphylococci were identified by colony characteristics, cell morphology and arrangement, and catalase test[10]. All the strains were subjected to the SCT, UF-SCT and TCT in order to evaluate the performance of the SCT and UF-SCT for identification of *S. aureus*, using TCT as the gold standard.

**Slide coagulase test**

Smooth suspensions in sterile saline from test colony were prepared at two sites marked control and test on a slide. A small amount of fresh human plasma was added to the test side by a wire loop. The slide was rocked gently and observed for clumping within one minute. The test was considered positive when control did not show any clumping but clumping was observed on test side[15].

**Urea-fibrinogen slide coagulase test**

Smooth suspensions in sterile saline from test colony were prepared at two sites marked control and test on a slide. The methodology was optimized by three different methods of applying UF solution to the smooth suspension; i) a drop of urea-fibrinogen (UF) solution, ii) a loopful of UF solution and iii) just touching the UF solution with a sterile straight wire and rub it in the smooth suspension of colony. All three methods of applying UF-solution for UF-SCT were equally effective giving clumps within 5 - 10 seconds with positive controls and no clumps with negative controls. Thus, in order to save the reagents as well as to make the method effective the third method of applying UF solution with straight wire was followed for further tests.

The UF solution was prepared and stored as suggested by Spencer[14]. Briefly, human citrated plasma was mixed with saturated ammonium sulphate solution in 4 : 1 ratio. Following thorough mixing, it was allowed to stand for 10 minutes and centrifuged at 3000 rpm for 20 minutes. The supernatant was poured off and the container was inverted on filter paper to allow it to drain for a few minutes. The precipitate was then taken up in 10% aqueous urea solution to a final volume of 5 ml. Thus prepared UF solution was stored at $4^0$C till use.

**Tube coagulase test**

For tube coagulase tests, colonies of test isolates were re-suspended in 2 ml of diluted citrated human plasma (plasma: saline, 1 : 5) in sterile glass test-tubes. Since citrate is utilized by enterococci[16], pure colonies of Gram positive, catalase positive staphylococci were selected. Positive control tubes with citrated plasma and coagulase producing clinical isolate of *S. aureus* (which efficiently coagulates citrated plasma) were included. To rule out citrate utilization by other microorganisms, control TCTs containing citrated plasma with CoNS were included. In addition, reagent control tubes containing citrated plasma alone (with no cultures inoculated) were included. The tubes were incubated at 37°C and observed for clot from one to four hours or, if clotting did not occur, the tubes were incubated at room temperature for an additional 18 hours[9]. Tubes were studied without agitation in order not to disrupt partially formed clots.

**Quality Control**

To minimize cross contamination, standard micro-biological procedures were strictly followed. Positive and negative controls were always included in the test sets. Confirmed clinical isolate of *S. aureus* and CoNS were used as positive and negative control respectively.

**Statistical analysis**

The data were analyzed using a 2 × 2 contingency table for diagnostic specificity and sensitivity. Diagnostic sensitivities and specificities were calculated as follows:

Sensitivity (%) = [True positive/ (True Positive + False Negative)] × 100

Specificity (%) = [True Negative/ (False Positive + True Negative)] × 100

The positive predictive value (PPV) (%) = [True Positive/ (True Positive + False Positive)] × 100

The negative predictive value (NPV) (%) = [True Negative/ (True Negative + False Negative)] × 100.

**RESULTS**

Total of 150 staphylococcus isolates were included in the study based on their morphology and catalase tests. Among 150 isolates, 76.66% (115/150) were positive by all three tests and 4.00% (6/150) were positive by TCT but negative by UF-SCT and SCT whereas the remaining 19.33% (29/150) were negative by all three tests (Table 1).

**Table 1:** Comparative results of SCT, UF-SCT and TCT

| SCT | UF-SCT | TCT | Number (%) |
|---|---|---|---|
| Negative | Negative | Positive | 6 (4.00%) |
| Negative | Positive | Positive | 0 |
| Negative | Positive | Negative | 0 |
| Positive | Negative | Negative | 0 |
| Negative | Negative | Negative | 29 (19.33%) |
| Positive | Positive | Positive | 115 (76.66%) |
| | **Total** | | **150 (100%)** |

Performance of different test methods for detection of *S.*

*aureus* were analysed for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) considering TCT as gold standard (Table 2 and 3).

Among 150 isolates studied by SCT and TCT, 115 were positive by both, while 6 were positive by TCT but negative by SCT. Both tests revealed 29 isolates to be negative, but no isolates were negative by TCT and positive by SCT. With reference to TCT, the SCT had sensitivity 95.04%, specificity 100%, and predictive value of positive test 100%, predictive value of negative test 82.85%, percentage of false negative 4.95% and percentage of false positive 0% respectively (Table 2).

**Table 2:** Comparison of SCT with reference to TCT

| | Test results | TCT +ve | TCT -ve | Sensitivity | Specificity | PV+ | PV- | False -ve | False +ve | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| SCT | +ve | 115 | 0 | 95.04% | 100% | 100% | 82.85% | 4.95% | 0% | <0.05 |
| | -ve | 6 | 29 | | | | | | | |

Among 150 isolates studied by UF-SCT and TCT, 115 were positive by both, while six were positive by TCT but negative by UF-SCT. Both tests revealed 29 isolates to be negative, but none were negative by TCT and positive by UF-SCT. With reference to TCT, the UF-SCT had sensitivity 95.04%, specificity 100%, and predictive value of positive test 100%, predictive value of negative test 82.85%, percentage of false negative 4.95% and percentage of false positive 0% respectively (Table 3).

**Table 3:** Comparison of UF-SCT with reference to TCT

| | Test and results | TCT +ve | TCT -ve | Sensitivity | Specificity | PV+ | PV- | False -ve | False +ve | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| UF-SCT | +ve | 115 | 0 | 95.04% | 100% | 100% | 82.85% | 4.95% | 0% | <0.05 |
| | -ve | 6 | 29 | | | | | | | |

Out of 130 isolates subjected to UF-SCT and SCT for the coagulase test, 115 isolates were positive by both tests and none were found to be positive only by SCT or UF-SCT, while 15 were negative by both tests. While comparing the UF-SCT with SCT as a standard, the sensitivity of UF-SCT was 100%, specificity was 100%, predictive value of positive test was 100%, predictive value of negative test was 100%, percentage of false negative was 0%, and percentage of false positive 0% respectively. No isolate was found to be positive by SCT and negative by UF-SCT (Table 4).

**Table 4:** Comparison of UF-SCT with reference to SCT

| | Test and results | SCT +ve | SCT -ve | Sensitivity | Specificity | PV+ | PV- | False -ve | False +ve |
|---|---|---|---|---|---|---|---|---|---|
| UF-SCT | + ve | 115 | 0 | 100% | 100% | 100% | 100% | 0% | 0% |
| | - ve | 0 | 15 | | | | | | |

## DISCUSSION

Coagulase testing is the single most reliable method for identifying *S. aureus*[9]. Coagulase production can be detected using either the SCT or the TCT. Slide Coagulase Test detects bound coagulase (also called "clumping factor")[9], which reacts directly with fibrinogen in plasma, causing rapid bacterial cell clumping. Negative isolates following SCT require confirmation with the superior TCT, since strains deficient in clumping factor may produce free coagulase. Tube coagulase detects secreted, extracellular, free coagulase that reacts with a substance in plasma called "Coagulase-Reacting Factor (CRF)" to form a complex, which in turn reacts with fibrinogen to form fibrin clot[9]. Tube coagulase test (TCT) is considered gold standard for demonstration of coagulase enzyme of *S. aureus*. This study evaluated the performance of TCT, SCT and UF-SCT, the phenotypic methods commonly practiced for the identification of *S. aureus*. The role of the UF-SCT method for the rapid identification of *S. aureus* was reevaluated and compared with SCT and TCT.

Sensitivity and specificity were calculated to evaluate the performance of individual test in detecting *S. aureus*. The findings of this study showed that SCT had sensitivity 95.04%, specificity 100%, and predictive value of positive test 100%, predictive value of negative test 82.85%, percentage of false negative 4.95% and percentage of false positive 0% respectively with reference to TCT. Here, SCT and UF-SCT showed slightly lower sensitivity by failing to detect 6 (4%) of *S. aureus* strains in comparison to TCT. This data is in agreement with previous findings in which sensitivity of free coagulase test was higher than bound coagulase test[17,18,19,20]. The tube coagulase test showed very good sensitivity (98.7%), specificity (98.1%), PPV (99.5%) and NPV (94.4%) than slide coagulase test and Slidex Staph Plus[17]. Van *et al*[18] have reported similar findings with 98.2% sensitivity and 98.9% specificity of Slidex Staph Plus test. Tube coagulase has demonstrated the highest sensitivity (98.7%) and specificity (98.1%). Similarly in another study Luijendijk *et al*[19] have evaluated free-coagulase test (Bacto coagulase plasma;

Difco Laboratories, Detroit, Mich.), bound coagulase test, and the Pastorex Staph Plus (Sanofi Diagnostics Pasteur, SA, Marnes-La-Coquette, France) for the detection of *S. aureus.* They found 98.0% sensitivity with free-coagulase test and 99.0% with bound coagulase test and 100.0% with Pastorex Staph Plus.

In current study SCT and UF-SCT were evaluated considering the TCT as gold standard for the identification of *S. aureus*. Overall, of the individual tests studied, UF-SCT test was found to be an ideal rapid test to detect *S. aureus* with 95.04% sensitivity, 100% specificity, 100% PPV, 82.84% NPV, 4.95% false negativity and 0% false positivity in comparison to TCT as gold standard. The SCT and UF-SCT had the similar sensitivity, specificity for the detection of *S. aureus* (95.04% sensitivity and 100% specificity). The slightly lower sensitivity of UF-SCT may partly be due to the non-specific detection of other coagulase positive staphylococci, such as *Staphylococcus schleiferi* subspecies coagulans, *Staphylococcus delphini*, *Staphylococcus intermedius* and *Staphylococcus hyicus*.

The accurate identification of *S. aureus* clinical isolates requires a battery of tests. *S. aureus* infections are more frequent than those by other bacteria, particularly in settings with high HIV/AIDS prevalence[20,21,22]. Potential risk to laboratory workers while using whole plasma for the coagulase test and the need to screen plasma for infectious agents in resource limited settings, adds extra efforts and cost. Since SCT and UF-SCT have similar sensitivity and specificity with reference to TCT, UF-SCT could be safer, less expensive and more suitable alternative to SCT.

Use of UF-SCT avoided false positives due to naturally occurring staphylococcal agglutinins in rabbit and human plasma, in addition to the fact that UF solution retains its activity considerably longer[13]. The 10% urea solution used to prepare UF solution has a sufficiently strong antibacterial action to suppress almost all accidental contamination of the solution[14]. Since tube coagulase test provides results only after 4 - 24 hr and is burdensome while UF-SCT test is rapid and easy to perform, this disadvantage of tube coagulase is certainly outstripped by its better efficacy. The slide coagulase test should be complimented by tube coagulase test when required. It is recommended that UF-SCT could be an alternative to SCT in clinical microbiology laboratory. The urea fibrinogen solutions appears to be an interesting application, and has a special interest for those laboratories which have no ready access to an animal house, or where sufficient amounts of fresh plasma may not be available or where screening of fresh plasma for pathogens is not practicable.

## CONCLUSIONS

This study evaluated the performance of laboratory tests used routinely for the identification of *S. aureus*. Since use of fresh, unscreened, human plasma is inappropriate and risky it should be avoided. A very good alternative UF-SCT method is recommended.

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### Conflict of interest

The authors declare that they have no financial or non-financial potential conflicts of interest.

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