DIAGNOSTIC CHALLENGES IN DETECTING COAGULASE-NEGATIVE *STAPHYLOCOCCUS AUREUS* BY COAGULASE TESTING

Tien Tran Thi Cam[1], Hanh Thu Quang Le[1], Bac V.G Nguyen[2*]

ABSTRACT

Background: *Staphylococcus aureus* is a major human pathogen whose identification in clinical laboratories often relies on the detection of coagulase activity. While rabbit plasma has long been considered the gold standard for the tube coagulase test, discrepancies in test results between plasma sources have been reported, raising concerns about diagnostic accuracy.

Methods: A total of 64 clinical isolates of *S. aureus* from human skin, along with the reference strain ATCC 25923, were tested for coagulase activity using human and rabbit plasma. Tube coagulase tests were performed at 4 and 24 hours, and slide coagulase tests were conducted in parallel. Positive results were defined by visible clot formation in the tube tests or clumping within 30 seconds in the slide tests.

Results: With human plasma, 32.8% of isolates were coagulase-positive at 4 hours, increasing to 51.6% after 24 hours. In contrast, only 4.7% of isolates tested positive with rabbit plasma at both 4 and 24 hours. Slide coagulase testing with human plasma demonstrated 100% positivity, whereas the same assay with rabbit plasma yielded only 10.9% positivity.

Conclusion: Coagulase detection in *S. aureus* is strongly influenced by the plasma source and incubation time. Sole reliance on rabbit plasma may result in significant underdiagnosis, particularly for human-adapted strains. Incorporating multiple plasma sources, extending incubation, and employing confirmatory methods can enhance diagnostic accuracy and prevent misclassification of coagulase-negative *S. aureus*.

Keywords: Staphylococcus aureus, *coagulase test, human plasma, rabbit plasma, diagnostic accuracy, false-negative*

[1] *Faculty of Medical Laboratory Techniques, Nguyen Tat Thanh University, Ho Chi Minh City*
[2] *School of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City*
Responsible person: Bac V.G Nguyen
Email: nguyenvugiangbac@ump.edu.vn
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I. INTRODUCTION

Staphylococcus aureus is a Gram-positive, facultatively anaerobic bacterium that is a major cause of both community-acquired and healthcare-associated infections. It is implicated in a wide spectrum of diseases, ranging from superficial skin infections and food poisoning to life-threatening conditions such as sepsis, endocarditis, and pneumonia (1, 2). Rapid and accurate identification of *S. aureus* is therefore essential for timely clinical intervention and appropriate antimicrobial therapy.

Among the various diagnostic methods available, the detection of coagulase activity remains a cornerstone for differentiating *S. aureus* from other staphylococcal species, particularly the coagulase-negative staphylococci (CoNS) (3). Coagulase is an enzyme that catalyzes the conversion of fibrinogen to fibrin, leading to clot formation-a process closely associated with the pathogenicity of *S. aureus* (4). The coagulase test can be performed in two main formats: the slide test, which detects bound coagulase (clumping factor), and the tube test, which identifies free coagulase in the culture supernatant (5).

The serum coagulase test, typically performed in a test tube using rabbit plasma, is considered the gold standard for detecting free coagulase due to its high specificity and reliability (6). However, several studies have

reported the existence of *S. aureus* strains that fail to produce detectable coagulase, posing challenges for routine identification (7, 8). Such coagulase-negative *S. aureus* isolates have been observed when using both human and animal plasma, raising concerns about potential misidentification in clinical settings (9, 10).

Given this context, the present study aims to report and characterize *S. aureus* isolates that do not produce detectable coagulase when tested with both human and rabbit serum, thereby highlighting diagnostic limitations and proposing considerations for accurate laboratory identification.

II. MATERIALS AND METHODS

Bacterial Preparation

Sixty-four clinical isolates of *Staphylococcus aureus* recovered from human skin, along with the reference strain ATCC 25923, were obtained from previously preserved glycerol stocks. The isolates were subcultured on Tryptic Soy Agar (TSA) plates and incubated at 37 °C for 18 hours to generate actively growing cultures. After incubation, bacteria were suspended in 0.9% NaCl solution and adjusted to a final concentration of 1×10^8 CFU/mL by measuring and calibrating the optical density at 600 nm (OD_{600}) with a spectrophotometer. This standardization step ensured uniformity and reproducibility across all downstream experiments.

Tube Coagulase test

The coagulase test was performed using 1 mL of human plasma or rabbit plasma as the testing medium. A previously activated bacterial suspension was inoculated into the plasma and incubated at 35–37°C. Clot formation was assessed at two time points: 4 hours and 24 hours post-incubation. Each test was conducted in parallel with a positive control strain, *Staphylococcus aureus* ATCC 25923 and a negative control, physiological saline solution. After incubation, the tubes were gently tilted to observe for clotting. A positive result was defined as visible coagulation of the plasma, indicating coagulase activity. In contrast, a negative result showed no clot formation, implying the absence of coagulase activity.

Slide Coagulase test

Three separate drops of 20 µL physiological saline were placed on a clean, grease-free glass slide. A loop of the isolated colony were emulsified in each drops to make three thick suspensions. Add 20 µL rabbit plasma to the first suspensions, 20 µL human plasma to the second ones and 20 µL physiological saline to the third ones. All mixtures were gently agitated by rotating the slide in a figure-eight motion. The development of visible granulation or clumping in the plasma-containing suspension within 30 seconds, accompanied by a smooth appearance in the saline control, was interpreted as a positive slide coagulase test.

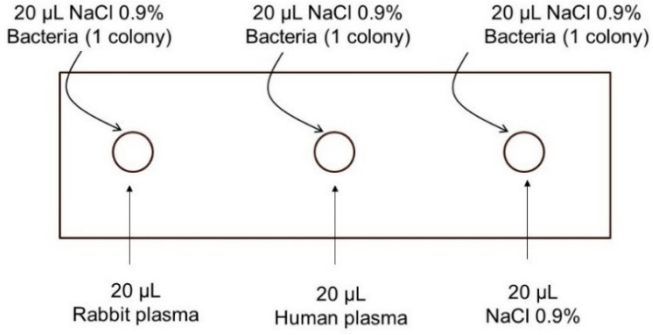

Figure 1. ***Diagram of coagulase slide test***

III. RESULTS

Table 1. ***Distribution of Coagulase postive and negative strains of S. aureus within clinical samples***

	Positive	Negative
Coagulase 4h (human plasma)	21 (32.8%)	43 (67.2%)
Coagulase 24h (human plasma)	33 (51.6%)	31 (48.4%)
Coagulase 4h (rabbit plasma)	3 (4.7%)	61 (95.3%)
Coagulase 24h (rabbit plasma)	3 (4.7%)	61 (9.3%)
Slide test (human plasma)	64 (100%)	0 (0%)
Slide test (rabbit plasma)	7 (10.9%)	57 (89.1%)

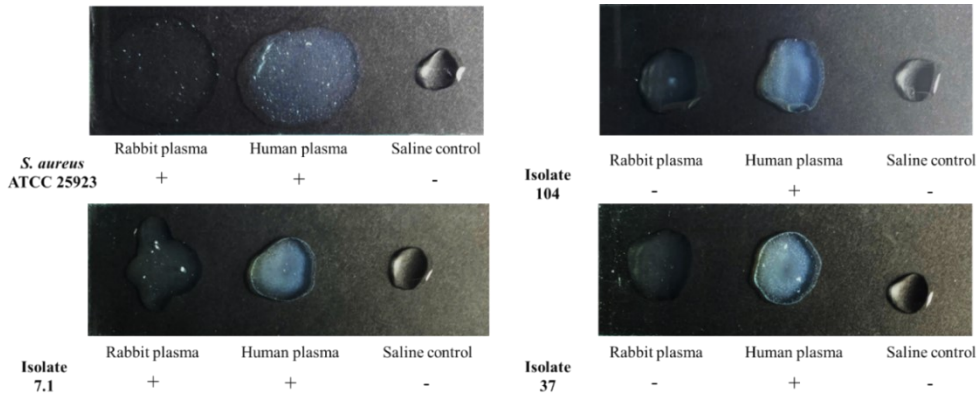

Figure 2. ***Slide coagulase test results of S. aureus ATCC 25923 and some isolates***

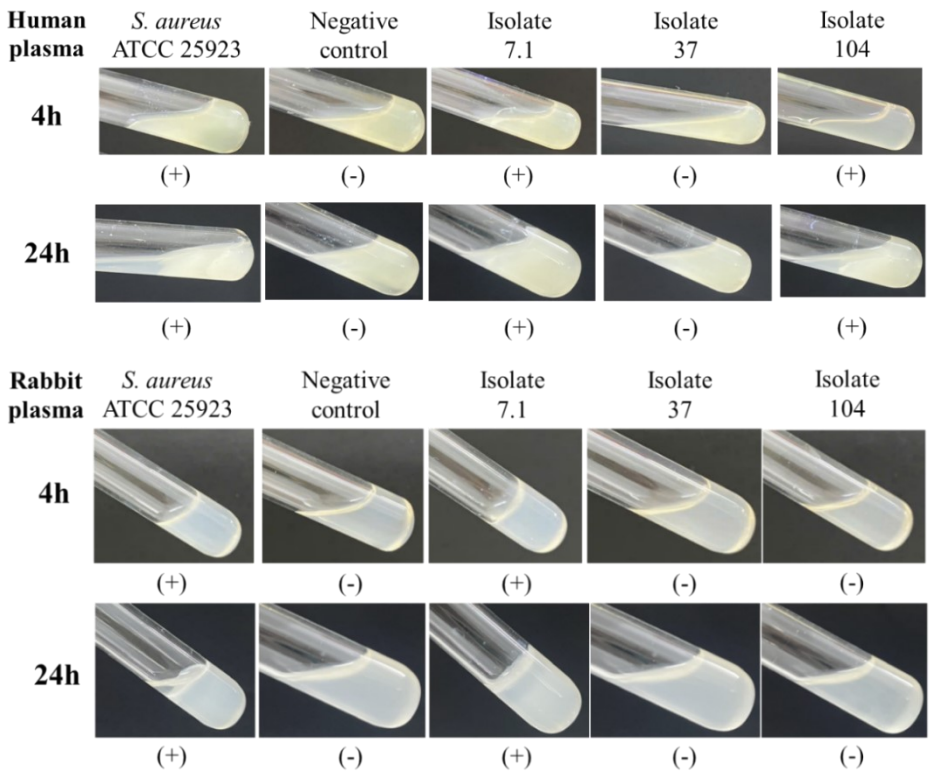

Figure 3. ***Tube coagulase test results of S. aureus ATCC 25923 and some isolates***

The distribution of coagulase-positive and coagulase-negative *S. aureus* isolates under different testing conditions is presented in Table 1. In the tube coagulase test using human plasma, 21 isolates (32.8%) were positive after 4 hours of incubation, increasing to 33 isolates (51.6%) after 24 hours. In contrast, when rabbit plasma was used, only 3 isolates (4.7%) were positive at both 4 and 24 hours, with the majority (95.3%) remaining negative throughout. The slide coagulase test performed with human plasma demonstrated positivity in all isolates (100%), whereas the same assay with rabbit plasma yielded only 10.9% positivity. These results indicate a marked variability in coagulase detection depending on both the plasma source and the testing method, with rabbit plasma consistently showing substantially lower sensitivity compared to human plasma.

IV. DISCUSSION

The present study highlights significant variability in coagulase detection in *Staphylococcus aureus* depending on the plasma source and testing method. Using human plasma in the tube coagulase test, more than half of the isolates (51.6%) were detected as coagulase-positive after 24 hours, whereas rabbit plasma yielded only 4.7% positivity at both 4 and 24 hours. These results indicate that rabbit plasma, despite being widely used as the gold standard, may underestimate the prevalence of coagulase-positive *S. aureus* strains, particularly those adapted to human hosts.

Our findings are consistent with earlier studies reporting that coagulase detection is influenced by host-specific plasma factors such as fibrinogen structure and coagulase-binding components (6, 4). Several authors have also described *S. aureus* strains that fail to express detectable coagulase in rabbit plasma but remain positive in human plasma, suggesting that strain-specific variability may contribute to false-negative results when rabbit plasma is used exclusively (7, 10). This phenomenon underscores the importance of considering plasma origin in diagnostic workflows.

When comparing tube and slide coagulase tests, the slide test with human plasma demonstrated 100% positivity, which is notably higher than the tube test results at both 4 and 24 hours. This discrepancy reflects the different biological targets of the two assays: the slide test detects bound coagulase (clumping factor) on the bacterial cell surface, whereas the tube test identifies free coagulase secreted into the medium (5). Previous reports have suggested that the slide test is more rapid and sensitive, but less specific, as autoagglutination or cross-reactions may occasionally yield false positives (3). In contrast, the tube test is generally considered more specific but may require extended incubation to detect weak coagulase producers (2). The results of our study reinforce this distinction, showing that while the slide test rapidly identifies *S. aureus*, the tube test provides a more stringent assessment of coagulase activity.

From a clinical perspective, the underdetection of *S. aureus* when using rabbit plasma alone has important implications. Misclassification of true *S. aureus* isolates as coagulase-negative may lead to inappropriate reporting as coagulase-negative staphylococci (CoNS), which are often regarded as less pathogenic. This could result in delayed or suboptimal treatment, particularly in severe infections such as bacteremia, endocarditis, or postoperative wound infections where timely recognition of *S. aureus* is critical (1). Furthermore,

coagulase-negative *S. aureus* strains have been reported to retain virulence factors and antimicrobial resistance genes similar to their coagulase-positive counterparts (7, 10). Therefore, false-negative results not only compromise diagnostic accuracy but also pose a risk to patient management and infection control. Our findings support the recommendation that clinical laboratories adopt combined or complementary testing strategies to minimize diagnostic errors and improve clinical outcomes.

V. CONCLUSION

This study demonstrates that coagulase detection in *Staphylococcus aureus* varies significantly with the choice of plasma source and the duration of incubation. Human plasma detected a substantially higher proportion of coagulase-positive isolates compared to rabbit plasma, while slide testing with human plasma yielded uniformly positive results. These findings suggest that exclusive reliance on rabbit plasma for the tube coagulase test may lead to false-negative outcomes and misclassification of clinically relevant *S. aureus*. To enhance diagnostic accuracy, laboratories should consider using both human and rabbit plasma, extend incubation periods where necessary, and complement phenotypic testing with molecular or proteomic confirmation. Such an integrated approach is essential to ensure reliable identification, guide effective clinical management, and strengthen infection control measures.

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