

## Comparison Of Tube Coagulase Test With Mannitol Fermentation Test For Diagnosis Of Staphylococcus Aureus

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**Abstracts:** Introduction: Staphylococcus is one of the most common bacteria routinely isolated in microbiological laboratories. Identification of staphylococcus at species level requires battery of tests. In developing countries identification at species level is usually done by coagulase test (slide & Tube). Coagulase test requires human or sheep plasma. Human & sheep plasma has the disadvantage of having low sensitivity & low specificity and risk of biohazard. Mannitol fermentation is an important tool for diagnosis of staphylococcus at species level and it also overcomes the disadvantage of coagulase test. The objective of this study was to compare sensitivity of mannitol fermentation test with coagulase test for identification of *S. aureus*. Material & Methods: Among all clinical samples received, 100 isolates of Staphylococcus were compared for coagulase test (slide & tube) & mannitol fermentation. Results: In our study, among 100 staphylococcal isolates, positive mannitol fermentation test reaction was seen in all 100 isolates while positive tube coagulase test was seen among 94 isolates. Conclusion: Sensitivity of Mannitol fermentation test is more as compared to coagulase test. Mannitol fermentation test can be used for diagnosis of *S. aureus* along with coagulase test to improve the sensitivity of coagulase test or it can also be used as single gold standard method for diagnosis of *S. aureus*. [Makwana G et al NJIRM 2012; 3(4) : 73-75]

**Key Words:** : Staphylococcus aureus, Mannitol fermentation, Coagulase test, Human plasma

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**Introduction:** *S. aureus* is the pathogenic species of genus staphylococcus. It is responsible for variety of infections in humans which range from simple localized skin infection to deep musculo- skeletal infection. It is also associated with respiratory tract infection, septicemia, endocarditis, meningitis & upper urinary tract infection. *S. aureus* produce toxins which is responsible for intoxication disease like toxic shock syndrome, food poisoning, & exfoliative skin disease.<sup>1</sup> Proper & prompt diagnosis of is necessary for management of such infections. Diagnosis of genus staphylococcus in microbiology laboratory is done by Gram staining, colony characteristics & catalase test. Diagnosis of *S. aureus* can be done by number of pathogenic tests. In developing countries with limited resources, *S. aureus* is diagnosed only by single test mainly Coagulase test<sup>2</sup>. For coagulase test, human or sheep plasma is required which has its own disadvantages of having low sensitivity & risk of biohazard. Another constant feature of *S. aureus* is mannitol fermentation, which differentiates *S. aureus* from nonpathogenic staphylococci. Though coagulase test is gold

standard test, mannitol fermentation test can be simultaneously used to increase the sensitivity of coagulase test or it can replace the coagulase test for diagnosis of *S. aureus*.

**Material and Methods:** During July 2011 to December 2011, among various samples received in bacteriology section of microbiology department at M.P. Shah medical college Jamnagar; 100 Staphylococcal isolates were confirmed by gram staining reaction, colony characteristics & Slide Coagulase test. Subsequently all 100 staphylococcal isolates were tested for Tube Coagulase Test and Mannitol fermentation test. Slide coagulase was done by mixing one drop of human plasma with staphylococcal colony over glass slide. It is rotated for 1 minute manually to see for clump production. For Tube coagulase test 0.5ml of human plasma was mixed with 0.1ml of staphylococcal suspension in a tube. Mixture is incubated in incubator for 2-4 hour. Coagulam is formed within 4 hr in case of *S. aureus* (fig:1). Human plasma was taken from Blood-Bank. Mannitol fermentation

was detected by inoculating staphylococcal suspension on Mannitol salt agar. Yellow color indicates mannitol fermentation (fig: 2). Pink color colony indicates no fermentation of mannitol.



**Figure: 1: Yellow color indicates mannitol fermentation**



**Figure: 2: Pink color colony indicates no fermentation of mannitol.**

**Result:** In present study 100 staphylococcal isolates were confirmed by gram staining, culture & slide coagulase test. All 100 slide coagulase positive staphylococcal isolates were tested for tube coagulase test & mannitol fermentation test. All isolates gave positive mannitol fermentation test (100%) & 94 isolate were positive for tube coagulase test (94%).

**Discussion:** In developing countries, phenotypic tests are the mainstay in the diagnosis of staphylococcal infections, in which coagulase tests

are usually confirmatory for *S. Aureus*<sup>3,4</sup>. Coagulase testing is performed using the slide coagulase (SCT) or the tube coagulase (TCT) methods<sup>5</sup>. Although these tests efficiently identify *S. aureus*, their performances vary from setting to setting and need improvement<sup>5,6</sup>. In many settings, the use of the TCT is curtailed by reliance on human plasma, since the recommended plasmas from rabbit, horse are either expensive or if locally available, are of poor quality<sup>7</sup>. Human plasma is reported to give discordant results as they are usually obtained from blood banks as outdated material, it contains variable amounts of CRF (Coagulase-Reacting Factor) and anti-staphylococcal antibodies<sup>5</sup>. This type of plasma is not recommended for coagulase tests<sup>5</sup>. The study by David P.Katee & colleagues shows the sensitivity of human plasma 91%<sup>2</sup>. In our study the sensitivity of human plasma was 94 %.

**Table:1- sensitivity of Tube coagulase test & Mannitol fermentation test**

N=100, staphylococcus confirmed by gram stain, culture & slide coagulase test.	positive tube coagulase result	positive Mannitol fermentation test
Sensitivity of test	94 (94%)	100 (100%)

Other factors which make human plasma inappropriate for the coagulase tests include; a high burden of viral infections (such as HIV, Hepatitis B and C) in resource limited settings that can render the plasma risky to laboratory workers and prior to use, it must be screened for safety. Whereas Mannitol salt agar was developed for the presumptive isolation of *S. aureus* in a single step, which is convenient for diagnostic laboratories. This study reveals that there is no single test (including the tube coagulase test) that can guarantee reliable results for the identification of *S. aureus*. The rapid identification of *S. aureus* is important so that the appropriate antibiotic therapy can be initiated, and this bacterial species

must be differentiated from coagulase-negative staphylococci, which frequently appear as contaminants or as a separate cause of bacteremia and urinary tract infections. Several methods for the rapid identification of *S. aureus* have been evaluated. These methods include coagulase tests, a panel of commercial agglutination tests, a hybridization test for rRNA and an enzymatic test for the detection of thermostable nuclease<sup>8</sup>. However, improved diagnostic sensitivity and specificity of the tube coagulase test were achieved upon simultaneous testing of the isolates with mannitol salt agar. In order to improve the identification of *S. aureus* in resource limited settings, sequel testing of the isolates with Mannitol salt agar & coagulase is proposed.

**Conclusion:** Coagulase test, though gold standard method for diagnosis of *S. aureus*, it has disadvantage of low sensitivity & risk of biohazard. Mannitol fermentation is one of the constant characteristic of *S. aureus* & Mannitol fermentation test has 100% sensitivity. So mannitol fermentation test can be used simultaneously with coagulase test to improve the sensitivity of coagulase test or mannitol fermentation test can replace the coagulase test in diagnosis of *S. aureus*.

#### References:

1. Ananthnarayan and paniker 's textbook of microbiology, 8<sup>th</sup> edition,2009, staphylococcus : 197- 199
2. Kateete DP, Kimani CN, Katabazi FA et al. Identification of Staphylococcus aureus: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. *Annals of Clinical Microbiology and Antimicrobials* 2010, 9:23. doi:10.1186/1476-0711-9-23.
3. Mugalu JNM, Kiguli S, Kaddu-Mulindwa DH: Aetiology, risk factors and immediate outcome of bacteriologically confirmed neonatal septicaemia in Mulago hospital, Uganda. *Afr Health Sc* 2006;6(2):120-126.
4. Sperber WH, Tatini SR. Interpretation of the tube coagulase test for identification of Staphylococcus aureus. [Appl Microbiology](#). 1975;29(4):502-5.
5. Koneman EWAS, Janda WM, Schreckenberger PC et al. *The Gram positive cocci: Staphylococci and related organims. In Color Atlas and Textbook of Diagnostic Microbiology*. 5th edition. Edited by Koneman EW. Philadelphia: Lippincott-Raven; 1997:551-576.
6. Bello CSS, Qahtani A. Pitfalls in the routine diagnosis of Staphylococcus aureus. *African Journal of Biotechnology* 2005;4(1): 83-86.
7. McDonald CL, Chapin K: Rapid identification of *Staphylococcus aureus* from blood culture bottles by a classic 2-hour tube coagulase test. *Journal of Clinical Microbiology* 1995;33(1):50–52.
8. Martineau F, Picard FJ, Roy PH, Ouellette M, Bergeron MG: Species-specific and ubiquitous-DNA-based assays for rapid identification of Staphylococcus aureus. *Journal of Clinical Microbiolog* 1998;36(3):618-623.