

Coagulase Test: Species Level Differentiation of Microorganisms

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SUMMARY

Biochemical tests are the most important and easy methods for identification of microorganisms especially in case of bacteria at genus or species level. This identification is mainly based on biochemical activities of microorganisms. The microorganisms show different protein and fat metabolism, carbohydrate metabolism, Enzyme production, compound utilization ability etc. These different biochemical activities help us to distinguish either genus or species level of microorganisms. Coagulase test is one of the biochemical tests used for species level identification of bacteria mainly for *Staphylococcus* spp. on the basis of coagulase enzyme production.

INTRODUCTION

A coagulase test is one of the biochemical tests most widely used to distinguish *Staphylococcus aureus* from other *Staphylococci* species like *S. epidermidis* and *S. saprophyticus* on the basis of the ability to produce the coagulase enzyme. This enzyme plays a virulence factor in some organisms as it interacts with the fibrinogen present on the host's cell surface. This enzyme also acts as a protective barrier of microorganisms, increasing their pathogenicity and resistance against the immune system of the host. Coagulase is of two types; free coagulase and bound coagulase, each of which is detected by different methods. The bound coagulase is called the clumping factor and is detected rapidly by a slide test. The free coagulase, in turn, is detected in the test tube as a result of the formation of a clot.

Objective

- To find free coagulase and bound coagulase produced by different organisms.
- To distinguish coagulase-positive *Staphylococci* from coagulase-negative *Staphylococci*.
- To identify and differentiate *S. aureus* from other *Staphylococcal* species.

Principle: Coagulase is an enzymatic protein that is a thermostable thrombin-like substance, which converts fibrinogen into fibrin resulting in clotting or clumping. In *S. aureus*, two different forms of coagulase are found; free coagulase and bound coagulase (Aryal, 2018).

Bound coagulase: It is otherwise called a clumping factor, is mainly present on bacterial cell walls and directly reacts with fibrinogen in the plasma. This reaction leads to alteration of fibrinogen and precipitation on the bacterial cells, causing clumping of plasma. The test for the clumping factor is rapid but requires several colonies, and also the factor might not be present in all *S. aureus* organisms.

Free coagulase: Another type of coagulase that is released by the organism is free coagulase. Free coagulase is different from bound coagulase in that the clotting mechanism of free coagulase requires the activation of a plasma coagulase-reacting factor (CRF). The free coagulase acts on plasma and forms a coagulase- CRF complex. The complex then reacts with fibrinogen to form the clotting of fibrin (Aryal, 2018).

Materials used

- Frozen plasma (preferably rabbit plasma) with EDTA. Human plasma is commonly not used for the test, as it is less sensitive and potentially infectious with human pathogenic viruses.
- 5% CaCl₂ (optional), Fresh culture of microorganism, Loops or sterile sticks, Glass or plastic tubes, Glass slides (Sapkota, 2020).

Procedure: Coagulase can be detected by two different methods; Tube test and Slide test.

Procedure for slide coagulase test

- It is for detection of bound coagulase.
- Take a drop of distilled water on each end of a slide or you can also use two separate slides.

- Emulsify a colony of the test organism (To avoid misidentifications, only perform this test on classic-looking white to yellow, creamy, opaque, haemolytic colonies of gram-positive cocci in clusters that are catalase positive.) in each of the drops to make two thick suspensions.

Note: Avoid taking colonies from mannitol salt agar culture because of not suitable for coagulase testing. The organism must first be cultured on nutrient agar or blood agar.

- Add just a loopful of plasma to one of the suspensions, and mix gently.
- Look for clumping of the organisms within 10 seconds, no plasma is added to the second suspension.
- This is used to differentiate any granular appearance of the organism from true coagulase clumping or agglutination (Tankeshwar, 2021).

Observation

- If 'positive', macroscopic clumping would be observed in the plasma within 10 seconds, with no clumping in the water drop.
- If 'negative', no clumping will be observed.

Procedure for Tube coagulase test

- It is for detection of free coagulase.
- Incubate the test organism (broth culture) overnight at 35°C.
- Add 0.2ml of incubated broth culture to 0.5ml of coagulase plasma ethylenediaminetetraacetate (EDTA) in a test tube.
- After gentle mixing, the test tubes were incubated at 35°C for an hour.
- The tube is finally observed for clot formation. If no clotting is observed, the tube should be examined at 30 minutes' intervals of up to 6 hours (Sperber&Tatini,1974).

Observation

- If 'positive', the plasma will coagulate, resulting in a clot.
- If 'negative', the plasma remains a liquid.

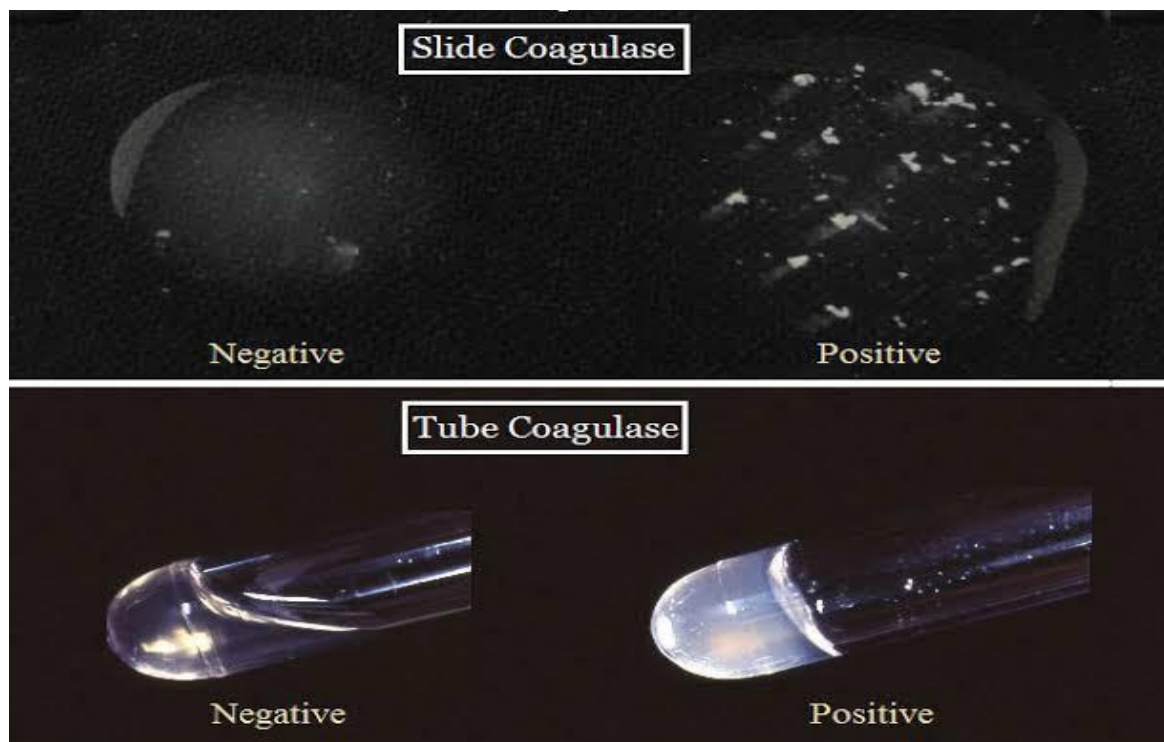


Figure 1: Formation of clumping and clotting in slide and tube coagulase test respectively

(Source: <http://laboratorytests.org/coagulase-test/>)

Result interpretation

Slide Test: A positive test is the demonstration of the agglutination of the bacterial cells after the plasma is added whereas a negative test is demonstrated by the lack of agglutination.

Tube Test: A positive test meets one of the following criteria:

- Complete clot formation or any degree of clot formation before 24 hours.
- No clot formation after the addition of 1 or 2 drops of 5% CaCl₂ to a tube without a clot at 24 hours.
- Examples are: *Staphylococcus aureus*, *S. pseudintermedius*, *S. intermedius*, *S. schleiferi*, *S. hyicus*

A negative test meets one of the following criteria:

- A lack of clot formation at 24 h at 25°C.
- No clot after 24 hours at 35°C, but after the addition of 1 or 2 drops of 5% CaCl₂ to the tube, a clot forms.
- Some of the examples are: *Staphylococcus epidermidis*, *S. saprophyticus*, *S. warneri*, *S. hominis*, *S. caprae*

Limitations

- Methicillin-resistant *S. aureus* can be deficient in bound coagulase, which results in a negative slide test.
- Citrated blood should not be used as false positive results can occur.
- Coagulase testing cannot be performed from growth on mannitol salt agar.

CONCLUSION

Coagulase production is one of the most reliable criteria for identification of microorganisms like staphylococcus spp. Coagulase test can replace the standard gold test for detection of staphylococcus spp. This test is useful for differentiating potential pathogenic staphylococci from other catalase positive cocci. This test is inexpensive and easy to perform by anybody.

REFERENCES

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