

**KCMC Biotechnology
Laboratory, Microbiology**

**STANDARD
OPERATING
PROCEDURE**

**Effective Date
10 May 2006**

**SOP-Number
MIC.027**

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**Date
10 May 2006**

Title: COAGULASE TEST

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Approvals/Date:

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This SOP has been read and understood by:

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Annual Review	
By	Date

Title: COAGULASE TEST

Document History:

Version Number	Reason for Changes	Date

Copies distributed to:

Name	Date

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PURPOSE

To differentiate *Staphylococcus aureus* (coagulase positive) from the coagulase negative species e.g. *S. epidermidis*, *S. saprophyticus*, *S. intermedius*.

PRINCIPLE

There are 2 forms of coagulase: one bound to the cell wall (bound coagulase) and one liberated by the cell (free coagulase). The slide coagulase test determines the presence of bound coagulase, and the tube test detects both bound and free coagulase. The exact mechanism by which the enzyme coagulase coagulates plasma is not known. It is generally accepted that a plasma factor is involved and that it resembles prothrombin.

SCOPE

This Standard Operating Procedure applies to the testing for coagulase by technical staff in the microbiology laboratory that have been trained and are competent in performing this procedure.

MATERIALS/STORAGE

Coagulase plasma (rabbit plasma)

Preparation:

1. Reconstitute vial by adding 1.5 ml of sterile water.
2. Dispense 0.5 ml for tube coagulase, 100 ul for slide coagulase into 13 x 100 tubes, labeled "COAG PLASMA", lot# of plasma, date of preparation and expiration date
3. Freeze at -20° C. Thaw at room temperature each day of use. Do not refreeze, discard at the end of the day.

18-24 hr culture of test organism

Glass slide

Saline

QUALITY CONTROL

Frequency – Each shipment/lot prior to testing.

Control organisms/Acceptable Results:

Staphylococcus aureus - Positive

Coagulase negative staphylococcus - Negative

Corrective actions for Unacceptable Results:

1. Subculture new control strain and retest.
2. If test is still unsatisfactory, do not use plasma for patient testing.
3. Arrange for replacement of product.

Documentation:

1. Record QC results on COAGULASE QC sheet.

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2. Document all corrective actions on unacceptable results on QC DEVIATION FORM and submit to supervisor for review.
3. Supervisor will review QC monthly.

PROCEDURE

Slide coagulase test:

1. In a drop of saline or distilled water on a slide make a heavy suspension of the staphylococcus to be tested.
2. Observe for autoagglutination (if auto agglutination occurs, slide test cannot be performed, set up tube coagulase test).
3. Add a loopful of fresh coagulase plasma.
4. Mix with saline suspension while observing for appearance of clumping. Specific clumping should occur immediately). This is a positive test.

Interpretation Slide Coagulase:

Clumping/agglutination of bacteria - Positive

NO clumping/agglutination of bacteria - Negative -

Tube coagulase test

1. Make a heavy suspension of the staphylococcus to be tested in 2 ml of broth (e.g. Brain Heart Infusion, Trypticase Soy Broth).
2. Add 0.4 ml of coagulase plasma.
3. Incubate at 37° C. Observe at half-hour intervals for appearance of coagulum by gently tilting tube (avoid agitation).
4. Incubate overnight if negative after 4 hours.

Interpretation Tube Coagulase Test:

Positive - formation of clot

Negative - No clot formed

RECORDING RESULTS

Enter results on POSITIVE CULTURE form.

NOTES

1. Not all slide coagulase positive strains are *S. aureus* and 10-15% of *S. aureus* are coagulase negative using the slide test.
2. Other *Staphylococcus* spp. produce bound coagulase/clumping factor so will be positive in the slide test: *S. lugdenensis*, *S. intermedius*, *S. schleiferi*. Others produce free coagulase and will be tube coagulase positive: *S. intermedius*, *S. hycius*.

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3. An 18-24 hour culture must be used. Weak coagulase activity may be overlooked if a culture is too old; false negatives occur when very early growth is used.
4. When reading the tube test, do not shake or agitate tube, when checking for coagulum formation. A false negative result may occur by breakdown of the coagulum in the early coagulation stage which will not reform upon further incubation.
5. Some *S. aureus* strains produce a large amount of fibrinolysin which may result in the absence of coagulum due to early lysis. The tube test must be checked for clot formation every 30 minutes especially during the first hour of incubation.
6. Coagulase is formed only by *S. aureus* but other organisms are capable of clot formation in citrated plasma, through a different mechanism. It is essential, therefore that the organism being tested be known to be staphylococcus and that a pure culture of this organism be used.
7. The slide coagulase test is a screening test. If any doubt exists about its results, or if the reaction is not the expected one, (i.e. cream or golden, hemolytic staph with negative test) a tube coagulase test must be performed.
8. Any mucoid staphylococcus must be tested by the tube coagulase method as mucoid strains of *S. aureus* will be slide coagulase negative, but tube coagulase positive.

REFERENCE

Bannerman, TL. 2003. Staphylococcus, Micrococcus and other catalase positive cocci that grow aerobically. In: Manual of Clinical Microbiology. 8th Ed. ASM Press, Washington, DC.

APPENDIX A – Coagulase QC sheet

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APPENDIX A

**KCMC Biotechnology Laboratory
Microbiology**

**Quality Control
COAGULASE TEST**

CONTROLS/ACCEPTABLE RESULTS:

Staphylococcus aureus - Positive

Coagulase Negative staphylococcus (CNS)- Negative

YEAR: _____

QC DATE	QC BY	LOT#/EXP DATE	<i>S. aureus</i> (+)	CNS (-)	A/NA*

*A/NA = Acceptable/Not acceptable

DOCUMENT ALL CORRECTIVE ACTION ON QC DEVIATION FORM

Supervisor Review :							
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COAGULASE QC/QC SHEETS