

Coagulase Test Protocol

Created: Thursday, 11 November 2010

Author • D. Sue Katz

Information **History**

The first report of coagulase activity was by Loeb in 1904 (16). He reported that staphylococci were capable of influencing the coagulation process of blood. In his experiments, he mixed small volumes of bouillon broth cultures of bacteria with 3 cubic centimeters of goose plasma. After incubation, he noted that "staphylococcus pyogenes aureus" (now known as *Staphylococcus aureus*) was capable of causing the blood to coagulate, usually within 5 hours. This version of the coagulase test is now referred to as the tube coagulase test. Nine species of bacteria were tested, and while some response was seen with several of the other species, *S. aureus* produced the strongest and swiftest response. A further experiment using rabbits determined that the organism was able to affect mammalian blood as well. He also observed that a sterilized culture of the organism was not able to coagulate blood. We now know that several other species of *Staphylococcus* are capable of giving a positive coagulase test (4). Shortly after that, in 1908, Much (18) described an apparently related phenomenon where *S. aureus* cells immediately clumped when mixed with plasma, which is now called the slide coagulase test or clumping test.

Loeb immediately recognized the clinical implications of the ability of the organism to clot plasma, "We may further conclude that under various pathological conditions the staphylococcus pyogenes aureus plays an important part in causing thrombosis and the formation of a fibrinous exudate" (16). Other researchers continued to examine and further characterize *S. aureus*. In their paper published in 1934, Chapman et al. (8) describe a series of experiments performed to correlate pigment production, hemolysis (ability to lyse red blood cells), tube coagulase test result, agglutination by antisera, and pathogenicity in rabbits. They determined that "coagulase is more consistently a property of '*Staph. aureus*.'"

In 1943, Cadness-Graves et al. (7) examined 442 strains of staphylococci and reported agreement between the slide and tube coagulase tests for 440 of the strains. Frobisher (13) mentioned the tests in the third edition of his textbook, in 1944, and in the fourth edition in 1949 (14), he gave a brief description of how to perform the tube coagulase test. The procedure he described is very similar to the procedure described below, with one major difference in that Frobisher's protocol used freshly prepared citrated or oxalated human plasma. By 1953, in

their clinical pathology manual, *Clinical Diagnosis by Laboratory Methods*, Todd, Sanford and Wells (24) noted that both coagulase tests are reliable methods of distinguishing between pathogenic strains and nonpathogenic strains, but the *ASM Manual of Microbiological Methods* was published in 1957 (1) without mention of coagulase. In the first edition of Bailey and Scott in 1962 (2), the tube coagulase test was the accepted method to identify a pathogenic *Staphylococcus*. Today, even though a variety of diagnostic tests are available to identify *S. aureus* in the clinical microbiology laboratory, the coagulase tests remain simple, reliable methods of identification. It is such a standard test that newly developed identification methods are evaluated by comparison with the tube coagulase test.

As it became more common to rely on coagulase as a test to identify *S. aureus*, researchers studied the characteristics of the coagulase reaction. Chapman, Berens and Stiles (9) and Turner and Schwartz (25) both examined the effect on coagulase of factors such as the species of organism donating plasma, the age of the inocula, the presence of anticoagulants, and the amount of salt. Researchers utilized a wide variety of plasmas to observe the activity of coagulase. For example, Chapman (10) felt that whole human blood worked best, while Orth, Chugg and Anderson (20) determined that human plasma was the best sera, while bovine, chicken, and pig were the worst. Yrios (27) found that heparinized pig plasma had results similar to commercial rabbit plasma. Ethylenediamine tetra-acetate (EDTA) was found to give superior results to citrated plasma (27). Bailey and Scott noted that, while various researchers were using plasma from a variety of species with differing results, consistent results were obtainable by using commercially available dehydrated rabbit plasma (2). Several chemicals are commonly used to prevent activation of the clotting cascade when blood is drawn. These include heparin, citrate, and ethylenediamine tetra-acetate. Heparin treated blood is no longer used for the coagulase test, and while citrate-treated blood can be used, it is possible that the organism being tested might utilize citrate metabolically, removing it from the solution and initiating the clotting cascade (12). For this reason, most plasma used for the coagulase test is treated with EDTA, which functions by chelating divalent cations such as Ca^{2+} which are required for enzymes to function.

While *Staphylococcus* species give similar results in the two coagulase tests, tube and slide, evidence began to accumulate that there were two different mechanisms of action. Duthie (11) found that heated and cooled plasma could not be used to form a clot in the tube test, although it retained activity in the slide clumping test. Duthie partially purified the clumping factor, the cell-bound protein which is now referred to as bound coagulase. This protein acts on fibrinogen to cause clumping of the bacterial cells. O'Connell et al. (19) identified bound coagulase as an adhesin of the microbial surface components recognizing adhesive matrix molecules (MSCRAMM) family. The secreted protein, staphylocoagulase, binds to prothrombin. Hemker, Bas and Muller (15) determined that, although this enzyme acts like thrombin, it is not. It has a different amino acid composition, it chromatographs differently, and it is not inhibited by heparin. Panizzi et al. (21) indicate that fibrinogen is

cleaved into fibrin by the action of staphylocoagulase-prothrombin. It is fibrin which forms the visible clot.

These discoveries verify that the slide and tube coagulase tests measure different reactions, and explain why some staphylococci give a negative slide test result and a positive tube test result. A positive slide test speeds up the identification of the organism in the clinical microbiology lab, but a negative reaction does not eliminate *S. aureus* from consideration. A negative tube test is required in order to eliminate *S. aureus* and that may take 24 hours. Latex agglutination kits, many of which utilize clumping or coagulase as one of the components of the test (3, 23, 26), have been marketed for quick identification of *S. aureus* and may be useful in identifying those rare strains of *S. aureus* which are coagulase negative (22).

Purpose

The coagulase test differentiates strains of *Staphylococcus aureus* from *S. epidermidis* and other coagulase-negative species. *S. aureus* strains are usually capable of coagulating EDTA-treated plasma in the tube test and will produce clumps of cells in the slide test.

Theory

The coagulase test can be performed using two different procedures. The slide test is simple, giving results within 10 seconds, but it can give false negatives. The tube test is the definitive test, however, it can take up to 24 hours to complete. For both tests, clumping or clots of any size indicate a positive response (5, 25). Likewise, the speed of clotting is not related to the virulence of the bacteria. While *S. aureus* is the most commonly isolated coagulase-positive organism, there are several other species of *Staphylococcus* which are positive for coagulase activity. *S. schleiferi* and *S. lugdunensis* may give positive results in the slide test for bound coagulase, and *S. schleiferi* and *S. intermedius* may give positive results in the tube coagulase test (6).

The slide test is performed by preparing a suspension of bacterial cells mixed into a drop of EDTA-treated rabbit plasma on a microscope slide. If bound coagulase is present on the bacterial cells, then the presence of plasma will cause the bacterial cells to clump. The clumping will occur because the clumping factor is an adhesin, a member of the MSCRAMM family of adhesion molecules, which causes the cells to bind to fibrinogen in the plasma. This will result in visible clumping of bacterial cells on the microscope slide. Figure 1 illustrates the visible clumping of cells on the microscope slide. Figure 2 is a comparison of how the cells in Figure 1 appeared when fixed, stained with methylene blue, and observed at 1,000X magnification in the light microscope.

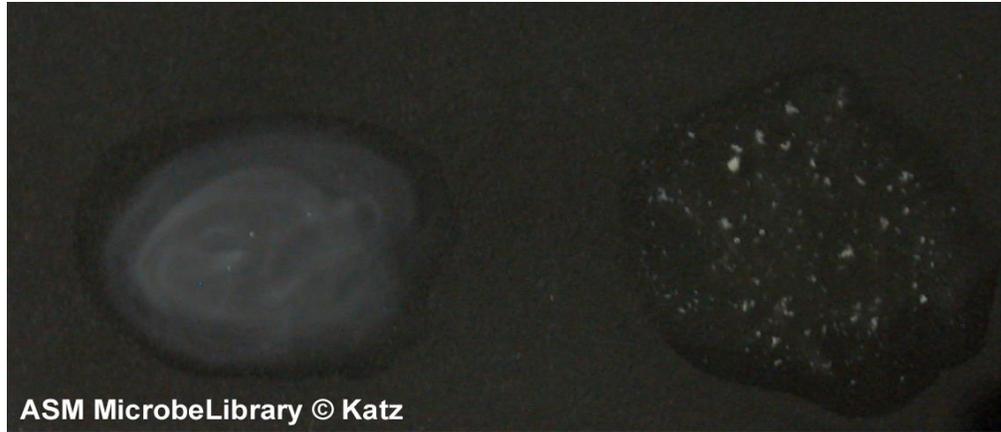
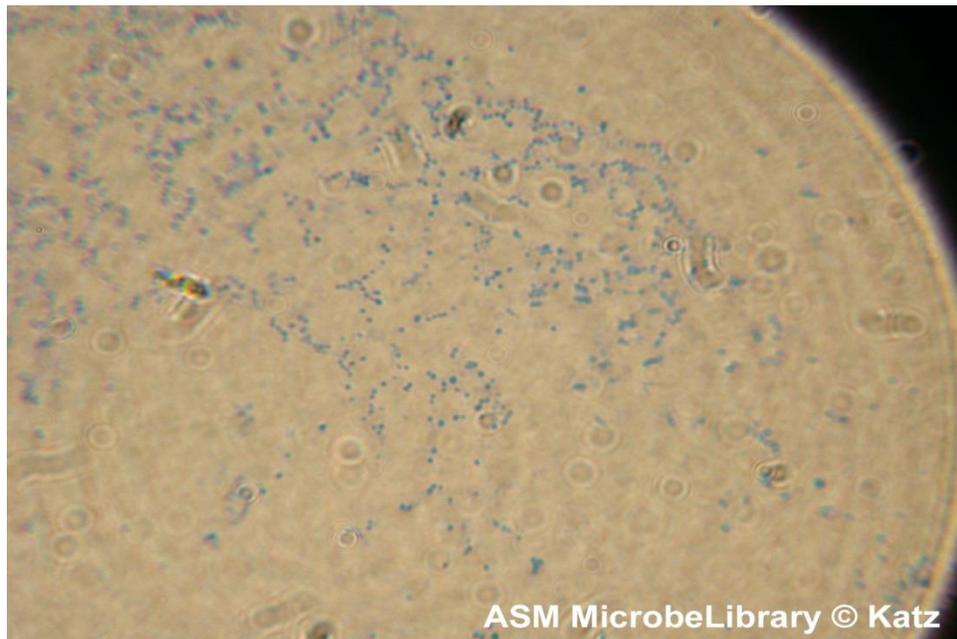
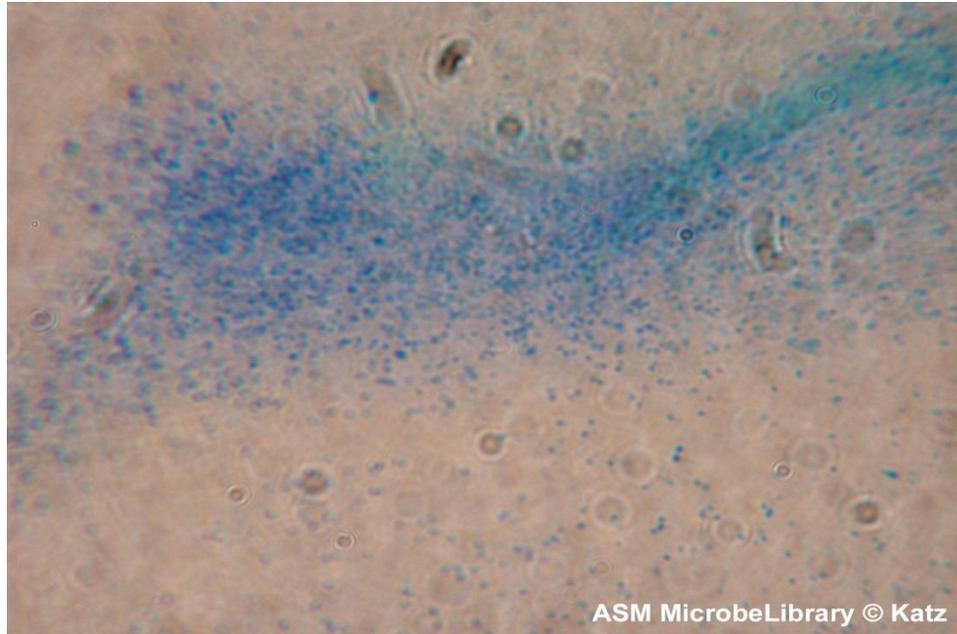


FIG. 1. Slide test. Coagulase-negative staphylococci are present on the left side of the slide, while coagulase-positive staphylococci are present on the right side of the slide.



A



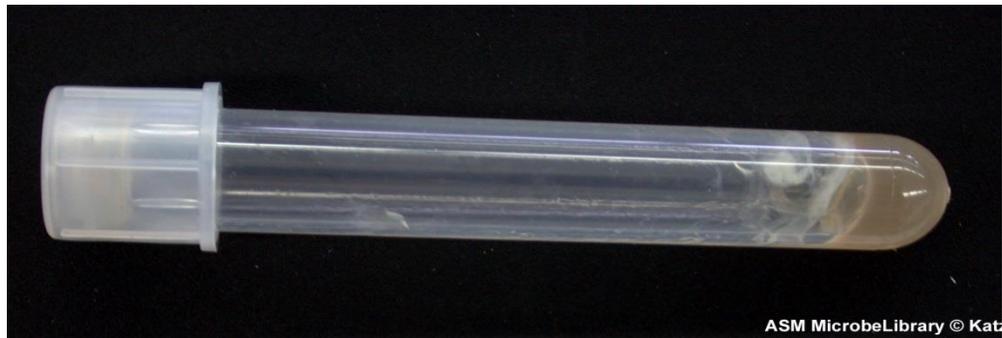
B

FIG. 2. Cells on the slide coagulase test slides were dried, heat fixed, and stained with methylene blue. (A) Coagulase-negative staphylococci. Note the individual cells and lack of large clumps of cells. (B) Coagulase-positive staphylococci. While some individual cells are observed, the majority of cells are gathered into a clump that is large enough to be visible to the naked eye. Photographs are at 1,000X magnification.

The tube coagulase test is performed by mixing bacterial cells into a larger volume of plasma in a small test tube. As the bacteria multiply in the plasma, they secrete staphylocoagulase. Staphylocoagulase initiates blood coagulation by activating prothrombin (15). Staphylocoagulase adheres to fibrinogen, forming a complex that cleaves fibrinogen into fibrin, bypassing the blood clotting cascade and directly causing a clot of fibrin to form. Formation of a clot will be noted within 24 hours for a positive response. Figure 3 shows a negative reaction and a positive reaction.



A



B

FIG. 3. (A) A negative tube coagulase test reaction indicating coagulase-negative cells. (B) A positive tube coagulase test reaction indicating coagulase-positive cells.

RECIPE

Both forms of the coagulase test require fresh bacterial cultures (24 hours or younger), grown in noninhibitory media. Acceptable noninhibitory media include blood agar, trypticase soy agar, and chocolate agar. Phenylethylalcohol and mannitol salt agars are considered inhibitory media. Agar media with antibiotics are also considered inhibitory.

Commercially available coagulase plasma is obtained from a supplier as lyophilized citrated or EDTA-treated rabbit plasma, and rehydrated with sterile distilled water immediately prior to use. As mentioned above, EDTA treatment is preferred. The amount of water used to rehydrate will be indicated by the manufacturer. The rehydrated plasma should be stored at 4°C if it will be used within 1 week. Otherwise it should be aliquotted and frozen at -20°C. Frozen aliquots should be stored in a non-frost-free freezer, since cycles of thaw and refreeze inactivate the plasma. Examples of suppliers include: Pel-Freez Biologicals (item 31241-1), Becton-Dickson (item 240826); Gentaur (item RB02211), and EMD Chemicals (item 1.13306). While human plasma was often used historically, the use of fresh, citrated or EDTA-treated human plasma is not recommended because of the risk of infection.

PROTOCOL

The slide test is performed first to detect bound coagulase, since it is a very quick procedure, and only a negative strain is further tested in the tube coagulase test. If a positive result is obtained, the organism is reported as coagulase positive, presumptively *S. aureus*. The tube test is the definitive version of the coagulase test, but it can take up to 24 hours to complete. Before these tests are used in the teaching laboratory, please review the safety considerations below. *S. aureus* should be used in a biosafety level 2 laboratory, and then, only by students with sufficient experience to use the organism safely. The appropriate safety

equipment should be available for the students.

Slide Coagulase Test

Demonstrating bound coagulase, otherwise known as clumping factor

Materials

Microscope slide
Overnight plate culture of the organism to be tested, grown on noninhibitory agar
Control organisms, usually include *S. epidermidis* (negative control) and
S. aureus (positive control)
Water or physiological saline
Nonsterile dropper
Reconstituted coagulase plasma
Sterile dropper
Method of transferring bacteria from plate (such as a wire loop and flame,
sterile wooden applicator stick, or sterile disposable loop)
Marking pen or wax pencil
Discard container for contaminated materials

Procedure

1. Draw two circles on the slide using the marking pen. Draw the circles as far apart as possible so there will be room to keep the suspensions separate and to mix each of the suspensions. Identify where the water (or saline) and plasma will be placed. If control strains will be utilized, additional slides will be required to test them using the procedure outlined below.
2. With the nonsterile dropper, place a small drop of water or saline into the circle on the appropriate end of the slide. The saline or water is a control to verify that the strain does not autoagglutinate and clump with itself instead of being easily emulsified.
3. Using the sterile dropper, place a small drop of reconstituted plasma into the circle on the opposite end of the slide.
4. With the loop or applicator stick, collect cells from one colony and emulsify the cells in the water (or saline) and then in the drop of plasma. The order is important to prevent carryover of small amounts of plasma into the control drop. If using a disposable loop or applicator stick, carefully place it into the discard container. If using a reusable loop, sterilize the loop before proceeding.
5. Watch for clumping within 10 seconds of adding the bacterial cells to the plasma. The clumping will become more visible if the slide is rocked gently in a figure 8 motion. The control drop, saline or water, should show no clumping of bacterial cells. Three results are possible.

If the strain shows clumping in the saline control and is difficult to emulsify, it exhibits autoagglutination, and no results can be obtained

from the slide test. This strain must be tested in the tube test.

If the strain shows clumping only when emulsified in the plasma and there is no autoagglutination, this is a positive slide test. This strain is not further tested in the tube test.

If the strain shows no clumping in either saline or plasma, it has tested negative on the slide coagulase test and should be further tested using the tube coagulase test.

The positive control species should show clumping only when emulsified in the plasma and the negative control species should not show clumping in either water (saline) or plasma.

6. When the test is complete, place the slides in the discard container for contaminated materials.

Tube Coagulase Test

Demonstrating staphylocoagulase production

Materials

Small sterile test tubes (for example, 12 X 75-mm snap-capped tubes)

Sterile wooden applicator stick or transfer loop

Discard container for contaminated materials

Test tube rack

Culture of organism to be tested, must be fresh (less than 24 hours) on

noninhibitory media

Control organisms, usually include *S. epidermidis* (negative control) and

S. aureus (positive control)

Watch or timer

Incubator set at approximately 37°C

Pipettor (to transfer 0.5 ml volume)

Sterile tips

Marking pen or wax pencil

Reconstituted coagulase plasma

Procedure

1. Label the test tube with the organism to be tested.
2. Using the pipettor, aseptically transfer 0.5 ml of the reconstituted plasma into the test tube.
3. Select two or three isolated colonies of the bacteria to be tested and collect them using the sterile loop or applicator stick.
4. Emulsify the bacteria in the 0.5 ml of plasma and place in the incubator. Discard the disposable loop or applicator stick into the discard container or sterilize the reusable loop before proceeding.

5. Note the time when the test began. At intervals over the next 4 hours, observe the culture, looking for evidence of a clot. Any clot formation is a positive result.
6. If no clot is observed by the end of 4 hours, then the test may be continued with an overnight incubation at room temperature and a final observation at 24 hours.
7. The positive control organism should exhibit clotting by the end 24 hours, while the negative control organism should not.
8. When the test is completed, dispose of the contaminated materials appropriately.

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the [ASM Curriculum Recommendations: Introductory Course in Microbiology](#) and the [Guidelines for Biosafety in Teaching Laboratories](#).

COMMENTS AND TIPS

Comments and tips are from discussion at the ASM Conference for Undergraduate Educators 2010.

Positive and negative controls should be utilized for the slide and tube tests. For a positive control organism, *S. aureus* ATCC 12600 is recommended. For a negative control organism, *S. epidermidis* ATCC 14990 is recommended (17).

To keep expenses at a minimum, it is possible to do one positive and one negative control for the class as a whole, instead of having each student or group perform them.

Clumping in the slide test might not be easily observed by holding the slide on top of the workbench. It will be more visible when observed over a dark surface.

If the student has allowed enough time for the drop of plasma to dry on the slide, then there may be a false-positive response. Encourage the students to be aware of this possibility before they start.

Some microbiologists prefer to characterize the degree of clotting present in the tube test. Turner and Schwartz (25) used a four-point scale. 1⁺ have small unorganized clots, 2⁺ have a small organized clot, 3⁺ feature a large organized clot, and 4⁺ show the entire contents of the tube coagulated and unable to be displaced when the tube is inverted. A negative showed no evidence of fibrin formation.

While primarily used to differentiate species within the



genus *Staphylococcus*, several other clinically important organisms can be differentiated from similar organisms by this test (17). *Peptostreptococcus indolicus* is positive while other *Peptostreptococcus* species are negative. *Erysipelothrix rhusiopathiae* is positive while *Listeria* and *Corynebacterium* spp. are negative.

REFERENCES

1. **ASM Committee on Bacteriological Testing.** 1957. Manual of microbiological methods. McGraw-Hill Book Company, New York, NY.
2. **Bailey, W. R., and E. G. Scott.** 1962. Diagnostic microbiology. C. V. Mosby Company, St. Louis, MO.
3. **Baker, J. S., M. A. Borman, and D. H. Boudreau.** 1985. Evaluation of various rapid agglutination methods for the identification of *Staphylococcus aureus*. **21**:726–729.
4. **Bascomb, S., and M. Manafi.** 1998. Use of enzyme tests in characterization and identification of aerobic and facultatively anaerobic gram-positive cocci. Clin. Microbiol. Rev. **11**:318–340.
5. **Blair, J. E.** 1939. The pathogenic staphylococci. Bacteriol. Rev. **3**:97–146.
6. **Brown, D. F. J., D. I. Edwards, P. M. Hawkey, D. Morrison, G. L. Ridgway, K. J. Towner, and M. W. D. Wren.** 2005. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). J. Antimicrob. Chemother. **56**:1000–1018.
7. **Cadness-Graves, B., R. E. O. Williams, G. J. Harper, and A. A. Miles.** 1943. Slide test for coagulase positive staphylococci. Lancet **1**:736–738.
8. **Chapman, G. H., C. Berens, A. Peters, and L. Curcio.** 1934. Coagulase and hemolysin tests as measures of the pathogenicity of staphylococci. J. Bacteriol. **28**:343–363.
9. **Chapman, G. H., C. Berens, and M. H. Stiles.** 1941. The coagulation of plasma by staphylococci. J. Bacteriol. **41**:431–440.
10. **Chapman, G. H.** 1944. The comparative value of human plasma and human whole blood for testing the coagulating power of staphylococci. J. Bacteriol. **47**:211.
11. **Duthie, E. S.** 1954. Evidence for two forms of staphylococcal coagulase. J. Gen. Microbiol. **10**:427–436.
12. **Finegold, S. M., W. J. Martin, and E. G. Scott.** 1978. Bailey and Scott's diagnostic microbiology, 5th ed. C. V. Mosby Company, Saint Louis, MO.
13. **Frobisher, M., Jr.** 1944. Fundamentals of bacteriology, 3rd ed. W. B. Saunders Company, Philadelphia, PA.
14. **Frobisher, M., Jr.** 1949. Fundamentals of bacteriology, 4th ed. W. B. Saunders Company, Philadelphia, PA.
15. **Hemker, H. C., B. M. Bas, and A. D. Muller.** 1975. Activation of a pro-enzyme by a stoichiometric reaction with another protein. The reaction between prothrombin and staphylocoagulase. Biochim. Biophys. Acta **379**:180–188.
16. **Loeb, L.** 1903. The influence of certain bacteria on the coagulation of the blood. J. Med. Res. **10**(3):407–419. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2105975/>.



17. **McFaddin, J. F.** 1999. Biochemical tests for the identification of medical bacteria, 3rd ed. Lippincot Williams & Wilkins, Baltimore, MD.
18. **Much, H.** 1908. Ober eine vorstufe des fibrinfermentes in kulturen von Staphylokokkus aureus. Biochemische Zeitschrift (now published as Eur. J. Biochem.) **14**:143–155.
19. **O’Connell, D. P., T. Nanavaty, D. McDevitt, S. Gurusiddappa, M. Höök, and T. J. Foster.** 1998. The fibrinogen-binding MSCRAMM (clumping factor) of *Staphylococcus aureus* has a Ca²⁺-dependent inhibitory site. J. Biol. Chem. **273**:6821–6829.
20. **Orth, D. S., L. R. Chugg, and A. W. Anderson.** 1971. Comparison of animal sera for suitability in coagulase testing. Appl. Microbiol. **21**:420–425.
21. **Panizzi, P., R. Friedrich, P. Fuentes-Prior, W. Bode, and P. E. Bock.** 2004. The staphylocoagulase family of zymogen activator and adhesion proteins. Cell. Mol. Life Sci. **61**:2793–2798.
22. **Personne, P., M. Bes, G. Lina, F. Vandenesch, Y. Brun, and J. Etienne.** 1997. Comparative performances of six agglutination kits assessed by using typical and atypical strains of *Staphylococcus aureus*. J. Clin. Microbiol. **35**:1138–1140.
23. **Spears, D. J., T. R. Olma, and G. L. Gilbert.** 1998. Evaluation of four methods for rapid identification of *Staphylococcus aureus* from blood cultures. J. Clin. Microbiol. **36**:1032–1034.
24. **Todd, J. C., A. H. Sanford, and B. B. Wells.** 1953. Clinical diagnosis by laboratory methods, 12th ed. W. B. Saunders Company, Philadelphia, PA.
25. **Turner, F. J., and B. S. Schwartz.** 1958. The use of a lyophilized human plasma standardized for blood coagulation factors in the coagulase and fibrinolytic tests. J. Lab. Clin. Med. **52**:888–894.
26. **van Griethuysen, A., M. Bes, J. Etienne, R. Zbinden, and J. Kluytmans.** 2001. International multicenter evaluation of latex agglutination tests for identification of *Staphylococcus aureus*. J. Clin. Microbiol. **39**:86–89.
27. **Yrios, J. W.** 1977. Comparison of rabbit and pig plasma in the tube coagulase test. J. Clin. Microbiol. **5**:221–224.

REVIEWERS

This resource was peer reviewed at the ASM Conference for Undergraduate Educators 2010.

Participating reviewers:

Susan Barbaro
Rivier College, Nashua, NH

Carolyn Bouma
West Texas A&M University, Canyon, TX

Benita Brink
Adams State College, Alamosa, CO

Rebecca Buxton
University of Utah, Salt Lake City, UT

Anne Hanson
University of Maine, Orono, ME

Diane Hartman
Baylor University, Waco, TX

Jan Hudzicki
University of Kansas Medical Center, Kansas City, KS

Archana Lal
Independence Community College, Independence, KS

Min-Ken Liao
Furman University, Greenville, SC

Gail P. Lillis
Grossmont Community College, El Cajon, CA

Paula Mister
Johns Hopkins University, Baltimore, MD

Roger Peffer
Montana State University—Great Falls, Great Falls, MT

Cheryl Power
University of Melbourne, Melbourne, Victoria, Australia

Karen Reiner
Andrews University, Berrien Springs, MI

Andrea Rediske
Valencia Community College, Orlando, FL

Melissa Schreiber
University of Central Florida, Orlando, FL

Patricia Shields
University of Maryland, College Park, College Park, MD

Diane Spicer
Bastyr University, Kenmore, WA

Erica Suchman
Colorado State University, Ft. Collins, CO

Igor Zaitsev
Borough of Manhattan Community College, New York, NY