de la Maza LM, Pezzlo MT, Bittencourt CE, Peterson EM
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Staphylococcus, *Micrococcus*, and Other Catalase-Positive Cocci

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Members of the genera *Staphylococcus, Micrococcus*, *Kocuria*, and *Kytococcus* are characterized as being catalase-positive, Gram-positive cocci that occur in pairs and clusters. These organisms commonly colonize the surfaces of skin and mucosal membranes of mammals and birds. Members of the genus *Staphylococcus* are important human pathogens, whereas the other genera in this chapter play a lesser role in human infections and thus are discussed separately.

Traditionally, members of the genus *Staphylococcus* have been divided into those that are coagulase positive, i.e., *Staphylococcus aureus*, and those that are referred to as coagulase-negative staphylococci (CoNS), i.e., all others, based on their ability to clot rabbit plasma. The species of *Staphylococcus* most frequently associated with human infections is *S. aureus*, which is a major cause of morbidity and mortality. *S. aureus* can produce disease mediated by toxins or by direct invasion and destruction of tissues. *S. aureus* infections range from superficial skin infections to fatal systemic infections that can occur when the integrity of the skin is damaged, thus giving this pathogen access to sterile sites. Among the more common *S. aureus* infections are boils, folliculitis, cellulitis, and impetigo. Immunocompromised hosts are at particular risk of infection. Systemic infections include septicemia, which can result in the seeding of distant sites, producing osteomyelitis, pneumonia, and endocarditis. Toxigenic strains of *S. aureus* are capable of producing bullous impetigo, scalded-skin syndrome, and toxic shock syndrome. *S. aureus* is also a well-known contributor to food poisoning due to the elaboration of enterotoxins in foods such as potato salad, ice cream, and custards. Intense vomiting and diarrhea usually occur within 2 to 8 h after ingestion of food containing the toxin.

CoNS, in particular *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, and *Staphylococcus schleiferi*, play a role in human infection. In particular, *S. epidermidis* is recognized as a leading cause of health care-related infections, with immunocompromised hosts being at increased risk. Because CoNS are members of the normal skin and mucosal membrane microbiota, they are frequently considered a contaminant when isolated from clinical specimens and therefore may be overlooked as a cause of infection. This is compounded by the fact that their clinical presentation is subacute, unlike that of *S. aureus*. An important virulence property of CoNS is their ability to form a biofilm on the surface of indwelling or implanted devices, making them frequent agents of intravascular infections. *S. epidermidis* has also been implicated as a cause of endocarditis and is associated with right-side endocarditis in intravenous-drug users. *S. saprophyticus* is a leading cause of noncomplicated urinary tract infections in young, sexually active females, second only to *Escherichia coli* in this patient population. Of the more recently described CoNS human pathogens, *S. lugdunensis* and *S. schleiferi* have been implicated in serious infections, including endocarditis, septicemia, arthritis, and joint infections. *S. lugdunensis*, which more frequently colonizes skin and infects tissue

(causing, e.g., boils and abscesses) below the waist, at times can behave more like *S. aureus* than CoNS. This organism has been associated with aggressive infections, such as endocarditis, that have a high mortality rate; therefore, rapid recognition of this species is important for initiation of appropriate antimicrobial therapy. While other species of CoNS have been implicated in a variety of infections, they occur with less frequency.

An increasing problem with *S. aureus* and CoNS is resistance to antimicrobial agents, in particular methicillin. In the majority of methicillin-resistant *S. aureus* (MRSA) strains, this is due to production of an altered penicillin-binding protein, mainly PBP2a or PBP2c, encoded by the *mecA* or *mecC* gene, respectively, which is carried on a mobile genetic unit referred to as SCC*mec*. Overproduction of β-lactamase accounts for a smaller percentage of MRSA or MR-CoNS strains. In recent years, *S. aureus* strains with decreased susceptibility to vancomycin have been identified. These strains are referred to as vancomycin-intermediate *S. aureus* (VISA), as vancomycin-resistant *S. aureus* (VRSA) when the vancomycin MIC is ≥16 μg/ml, or, when their susceptibility to the glycopeptide class of antimicrobials as a whole is being addressed, as glycopeptide-intermediate *S. aureus* (GISA). Although only a few of these strains have been isolated, they pose a potential threat to effective treatment of serious *S. aureus* infections.

Testing for MRSA can be difficult due to heteroresistance, in which the resistance is expressed to a different extent among subpopulations. In susceptibility tests performed by disk diffusion or tests to determine a MIC, cefoxitin has been shown to have greater sensitivity for detecting MRSA than oxacillin, also a common antibiotic used to test for MRSA. Molecular assays to directly detect the *mecA* gene, as well as rapid assay formats employing monoclonal antibodies to the altered PBP2a protein, have been used to circumvent the problems of *in vitro* susceptibility testing for MRSA. In addition, due to the importance of rapidly identifying cultures positive for *S. aureus*, in particular MRSA, primers and probes for *S. aureus* and MRSA have been incorporated into a number of nucleic acid-based panels and individual nucleic acid amplification assays. Depending on the assay, these tests can be performed directly from clinical specimens or on blood cultures positive for Gram-positive cocci in pairs and clusters. In addition, screening for MRSA from nares cultures can be done by nucleic acid amplification or use of selective chromogenic agars. Identifying VISA strains by standard susceptibility methods remains a challenge; however, VRSA strains with higher vancomycin MICs can be identified using broth dilution, select automated systems, and screening agar incorporating 6 μg/ml of vancomycin.

Upon incubation in air at 35°C for 24 to 48 h, staphylococci grow rapidly on a variety of media, with colonies that range from 1 to 3 mm in diameter. On blood agar, staphylococci produce white to cream, opaque colonies. *S. aureus* colonies typically are cream in color but occasionally have a yellow or golden pigment, a phenotypic characteristic that led to the species name. *S. aureus* can be beta-hemolytic, and it is not uncommon to see both large and small colonies in the same culture, a phenotypic characteristic shared by several heteroresistant MRSA strains. CoNS, especially *S. epidermidis*, produce white colonies; however, other CoNS strains and species can have colonies with a slight cream pigment. In general, CoNS strains are nonhemolytic; however, some produce a small zone of beta-hemolysis on blood agar.

Since *S. aureus* is frequently isolated in mixed cultures, selective and differential media are used to facilitate the detection of these organisms in clinical material, particularly in nasal swabs, which are used to screen for carriage of this bacterium. Mannitol salt agar is an example of this, where the high concentration of salt (7.5%) inhibits many other organisms. Mannitol, along with the phenol red indicator in the medium, facilitates the discrimination of *S. aureus*, which can ferment mannitol, from most CoNS. However, since other organisms can grow on this medium and strains of CoNS can also ferment mannitol, additional testing is required. As mentioned above, chromogenic media selective and differential for MRSA are more commonly used for screening nasal cultures.

In addition to their distinctive Gram stain morphology (Gram-positive cocci in pairs and clusters), a common characteristic of these organisms is that they are catalase positive. The coagulase test, which measures the ability to clot plasma by converting fibrinogen to fibrin, is useful in distinguishing *S. aureus* from other bacteria that appear similar. A suspension of the organism to be identified is inoculated into rabbit plasma containing EDTA and incubated at 35°C for 4 h. The tube is tilted gently, and the presence or absence of clot formation is noted. If the test is negative at 4 h, the suspension is incubated for up to 24 h. The 4-h reading is important because some strains

produce fibrinolysin, which can dissolve a clot upon prolonged incubation, causing a false-negative result. Some strains of MRSA produce a very weak coagulase reaction, resulting in a negative reading. Bound coagulase (clumping factor) can be detected by a slide agglutination test, in which a suspension of the organism is emulsified on a slide with a drop of rabbit plasma. If bound coagulase is present, the organisms agglutinate. For correct interpretation of this test, a control in which saline is used instead of plasma is needed to check for autoagglutination. Of the CoNS, *S. lugdunensis* and *S. schleiferi* can also test positive for bound coagulase but can be differentiated from *S. aureus* by a negative tube coagulation test. Alternatively, commercially available tests can be used that are based on latex particles that have been coated with plasma, immunoglobulin, or (in some versions of this test) antibodies to the more common polysaccharide antigens. The plasma detects bound clumping factor, while the immunoglobulin binds protein A and the antibody to the polysaccharide antigens binds serotype antigens present on the surface of *S. aureus*. Some strains of MRSA, however, may be negative by this method because of low levels of bound coagulase and protein A, and false-positive reactions can occur due to the presence of the polysaccharide antigens present on some CoNS isolates.

Strains of *S. aureus* that produce a weak coagulase reaction can be further tested by the DNase test or a thermostable-endonuclease test. *S. aureus* and *S. schleiferi* possess enzymes that can degrade DNA, a DNase and a thermostable endonuclease. Both tests use the same basic medium containing agar that incorporates DNA and the metachromatic dye toluidine blue O. A heavy suspension of organisms is spotted onto the plate; after 24 h of incubation at 35°C, a pink haze appears around the colony, in contrast to the azure blue of the medium. In tests for the thermostable endonuclease, a suspension of the organism is boiled before being placed on the DNA plate.

CoNS can be identified to the species level based on their susceptibility profiles in response to selected agents, most notably novobiocin, as well as key biochemicals. A variety of commercial systems combine several biochemical tests to allow differentiation among the CoNS. While most of the CoNS of clinical importance are novobiocin susceptible, *S. saprophyticus* is novobiocin resistant. Other tests that can be used to differentiate among the species are those for phosphatase activity, production of acetoin, polymyxin susceptibility, pyrrolidonyl arylamidase activity, and acid production from carbohydrates.

While biochemical tests are still commonly used to identify strains of *Staphylococcus* to the species level, matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry is rapidly replacing traditional biochemical algorithms.

Several species that once were in the genus *Micrococcus* and that have been reported to play a role in human infections have been reclassified into the genera *Kocuria* and *Kytococcus*. Despite the reclassification, collectively these organisms are often referred to as micrococci. Members of the micrococci have a higher G+C content than the staphylococci. They are also common inhabitants of the skin but have a fairly low pathogenic potential. However, infections with these organisms have occurred in immunocompromised hosts. *Micrococcus luteus* and related organisms have been implicated in a variety of infections, including meningitis, central nervous system shunt infections, endocarditis, and septic arthritis.

Micrococci, in addition to forming pairs and clusters, can appear as tetrads. Like the staphylococci, they can be easily grown in the laboratory and can be recovered from a variety of media. However, in comparison to staphylococci, they are slower growing, with smaller colonies present after 24 h of incubation at 35°C. In addition, depending on the species, the colony color can range from cream to yellow (*M. luteus*) or rose red. As with CoNS, a variety of commercial systems that incorporate several tests, including urease, acid production from carbohydrates, esculin, and gelatin, have been employed to aid in the differentiation of this group. Bacitracin, lysostaphin, and furazolidone have been used to aid in differentiating staphylococci from micrococci. In general, staphylococci are resistant to bacitracin (0.04-U disk), in contrast to micrococci, which are susceptible, while the opposite is found with furazolidone (100-μg disk) and lysostaphin (200-μg disk), where micrococci are resistant. MALDI-TOF has aided in the identification of micrococci, and with more strains being added to present databases, this method is rapidly becoming the method of choice for identification of micrococci.

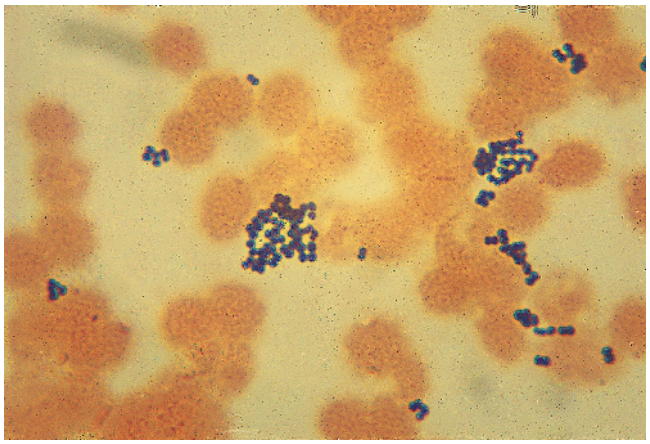


Figure 1-1 Gram stain of *Staphylococcus aureus*. A Gram stain of a positive blood culture shows Gram-positive cocci in grape-like clusters. On subculture to solid medium, *S. aureus* was isolated.

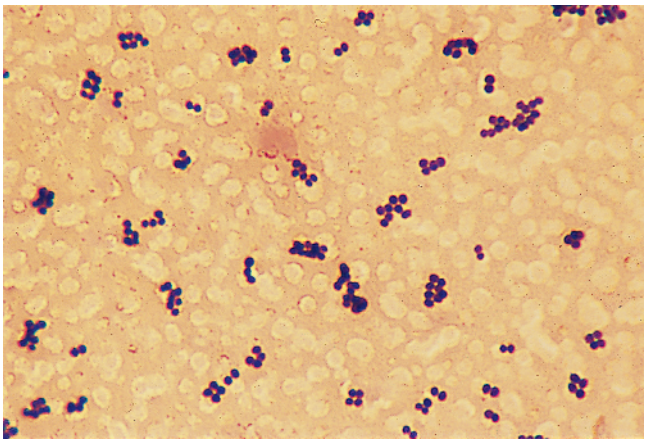


Figure 1-2 Gram stain of *Micrococcus luteus*. *M. luteus* is a Gram-positive coccus that, like *S. aureus*, can appear in pairs and clusters. However, it also tends to form tetrads, as depicted in this Gram stain.

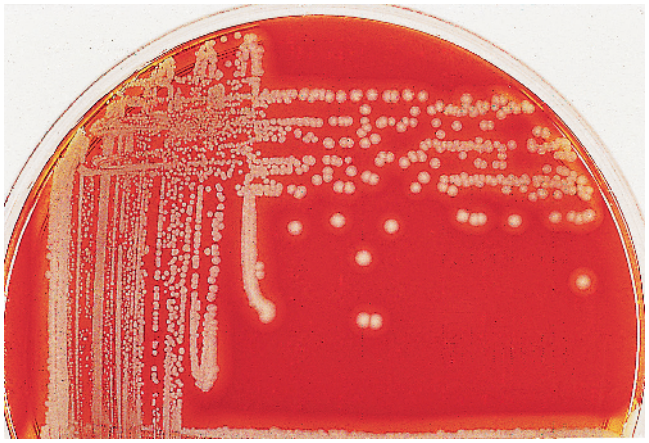


Figure 1-3 *Staphylococcus aureus* on blood agar. Shown in this image is a culture of *S. aureus* grown overnight at 35°C on blood agar. The colonies are cream colored and opaque and have a smooth, entire edge. A zone of beta-hemolysis surrounds the colony.

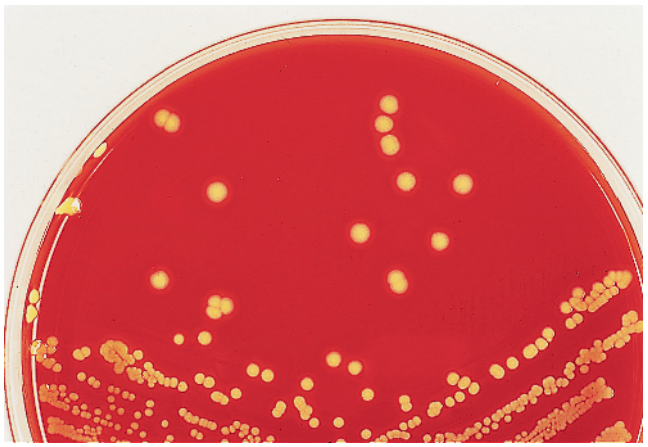


Figure 1-4 Golden pigment of *Staphylococcus aureus*. *S. aureus* is capable of producing the golden pigment that led to its species name. In practice, strains with this degree of pigment are not frequently isolated from clinical specimens. The isolate shown was incubated overnight on blood agar at 35°C and then left at room temperature for an additional day. When left at room temperature or refrigerated following incubation, isolates tend to develop more intense pigment.

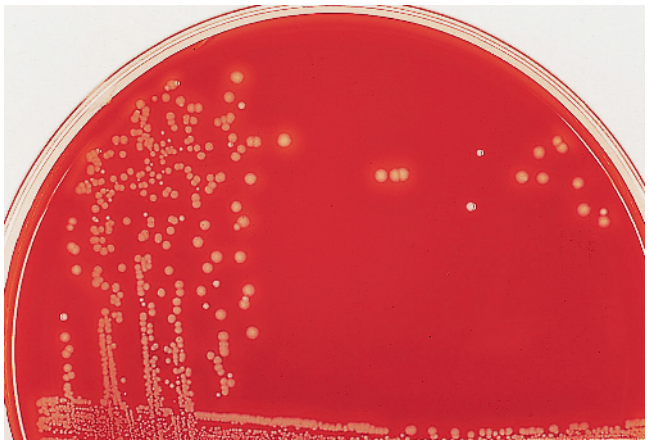


Figure 1-5 Size variation of *Staphylococcus aureus* colonies. It is not uncommon for strains of *S. aureus*, especially MRSA strains, to produce colonies that are heterogeneous in size and the degree of hemolysis. Colonies shown were grown on blood agar for 24 h at 35°C.

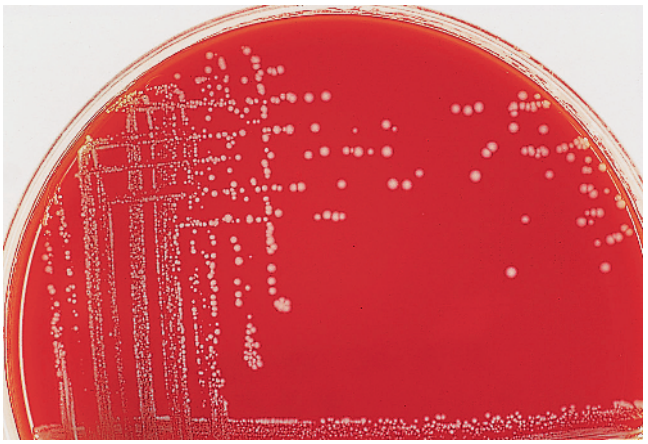


Figure 1-6 *Staphylococcus epidermidis* on blood agar. *S. epidermidis*, in contrast to both *S. aureus* and other CoNS, produces a white colony with little or no pigment. The isolate shown here was grown on blood agar for 24 h at 35°C. This strain of *S. epidermidis* also exhibits some variation in colony size.

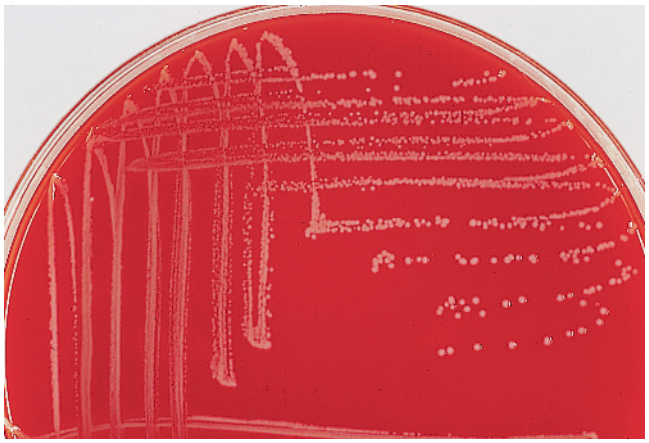


Figure 1-7 *Staphylococcus lugdunensis* on blood agar. Colonies of *S. lugdunensis* on blood agar resemble *S. epidermidis* colonies; however, they tend to be cream colored, in contrast to the typical white colonies of *S. epidermidis* (Fig. 1-6).

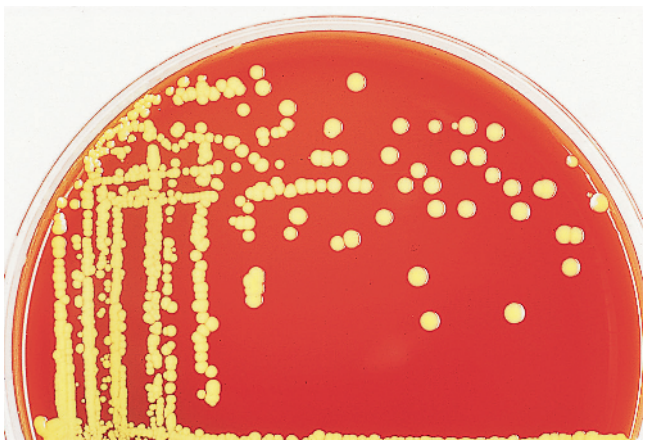


Figure 1-8 *Micrococcus luteus* on blood agar. A distinguishing feature of *M. luteus* is the vivid yellow colonies it produces. The isolate shown here was grown on blood agar for 72 h at 35°C. In general, *Micrococcus* is slower growing than *Staphylococcus*.

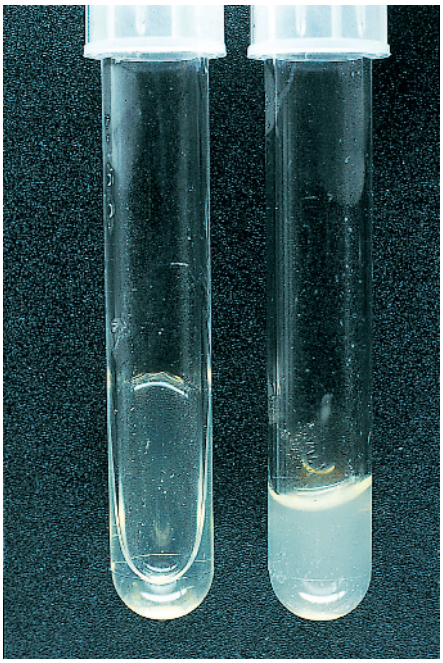


Figure 1-9 Coagulase test. A common method used to distinguish *S. aureus* from other *Staphylococcus* spp. is the tube coagulase test shown here. *S. aureus* is positive, and CoNS are negative. Colonies of the isolate to be identified were emulsified in 0.5 ml of rabbit plasma. The tube was incubated at 35°C for 4 h and tipped gently to look for clot formation. The tube on the left is negative, with the plasma remaining liquid, while the tube on the right is positive, as evidenced by the clot formation. Tubes giving negative results at 4 h should be incubated for up to 24 h.

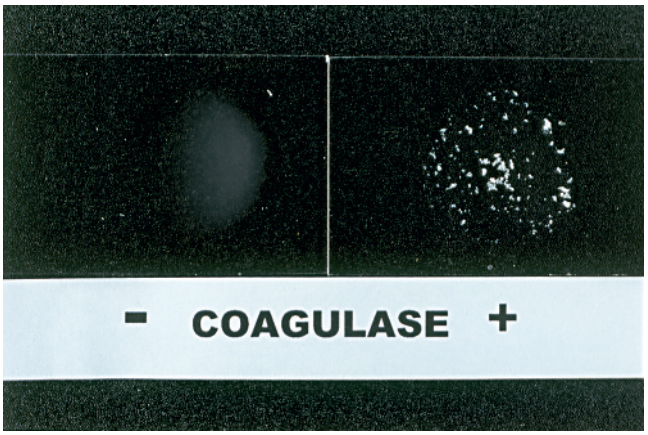


Figure 1-10 Slide coagulase test. The slide coagulase test is a rapid assay that tests for clumping factor on the surface of the organism. The test is performed by emulsifying the organism to be identified in both saline, which serves as a control for autoagglutination (left), and rabbit plasma (right). Agglutination of the organisms only in plasma is a positive result. *S. aureus* (right) is positive by this test as shown in this figure, as are strains of *S. lugdunensis* and *S. schleiferi*.

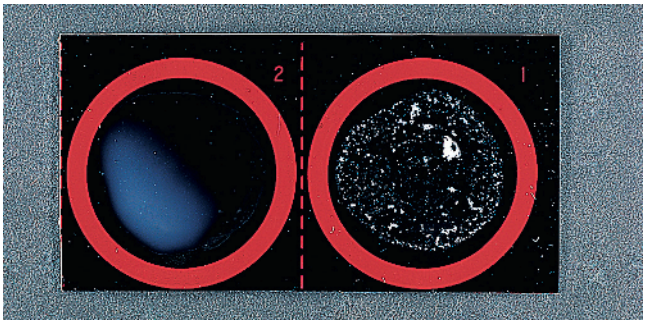


Figure 1-11 Latex test for the identification of *Staphylococcus aureus*. In the test depicted here, latex particles have been coated with antibody that can recognize bound coagulase as well as immunoglobulin that will bind to protein A present on the surface of most strains of *S. aureus*. *S. epidermidis* (left), which serves as a negative control, and the isolate to be identified (right) were emulsified with coated latex beads. The isolate shown here was identified as *S. aureus*. As with the slide coagulase test, some strains of MRSA may be negative and some strains of CoNS, namely, *S. lugdunensis* and *S. schleiferi* strains, may be positive.

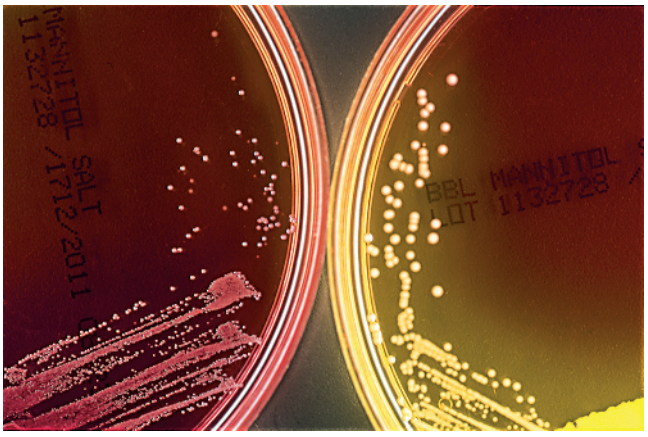


Figure 1-12 Mannitol salt agar. Mannitol salt agar is a selective and differential medium used for the isolation and presumptive identification of *S. aureus*. The high salt concentration inhibits the growth of many organisms that inhabit skin and mucosal membranes. The phenol red indicator incorporated into the medium detects acid production (yellow) resulting from the fermentation of mannitol. Here, CoNS (left) and *S. aureus* (right) were inoculated on the agar and then were incubated overnight.

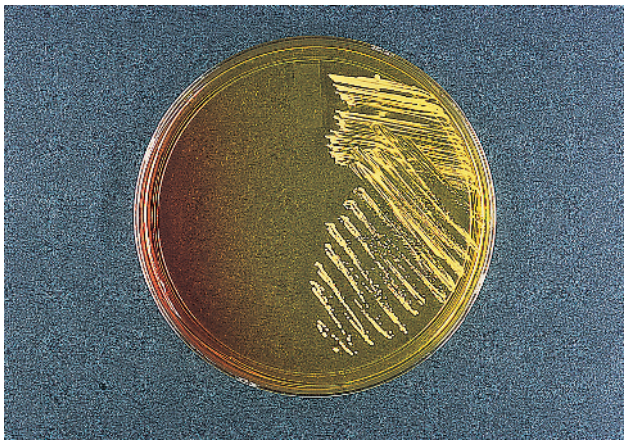


Figure 1-13 Mannitol salt agar containing oxacillin. Mannitol salt agar with oxacillin can be used to screen for the presence of MRSA in nasal specimens, since the 7.5% salt and 6 µg of oxacillin in this medium inhibit most other organisms that normally colonize the nares. MRSA turns the medium yellow as a result of the fermentation of mannitol. Pictured here is a plate inoculated with a methicillin-susceptible *S. aureus* strain (left) and a MRSA strain (right). The methicillin-susceptible *S. aureus* strain failed to grow. As with most *in vitro* testing for methicillin susceptibility, oxacillin (not methicillin) is used because of its higher stability.

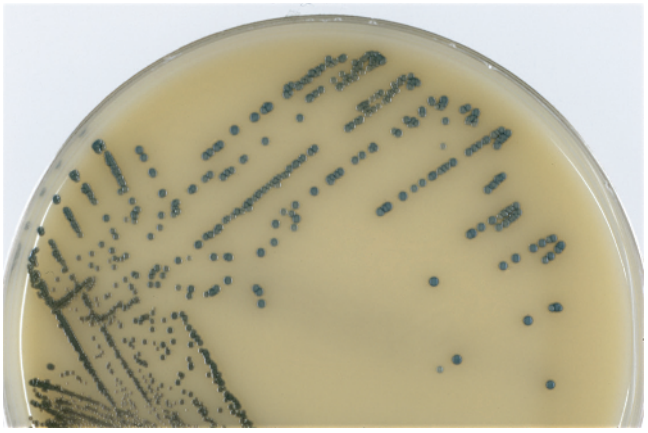
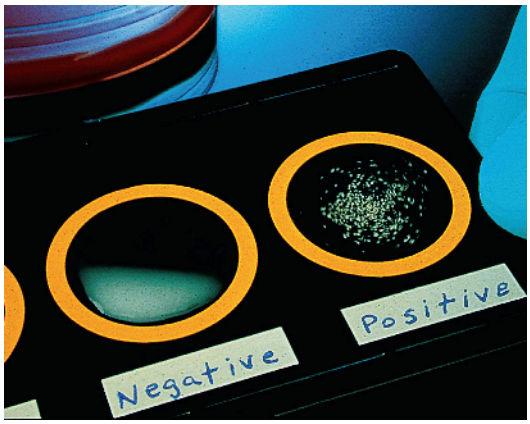


Figure 1-14 Spectra MRSA. Shown is a chromogenic medium used to detect MRSA, Spectra MRSA (Thermo Scientific, Remel Products, Lenexa, KS), which is both selective and differential. When the chromogenic substrate incorporated into the inhibitory agar is degraded by the enzymatic action of MRSA, the colony takes on a denim blue color. Shown here is an overnight nasal culture from which MRSA was isolated.



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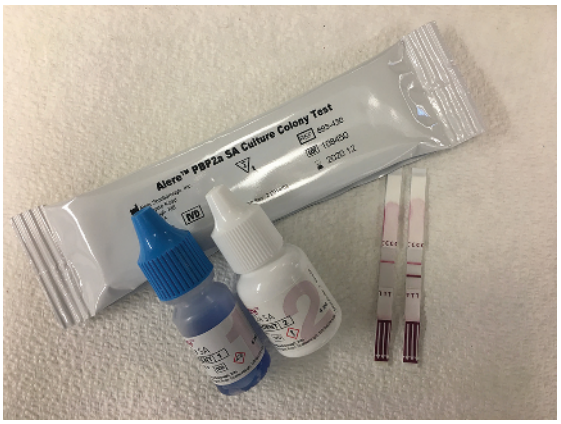


B

Figure 1-15 Assays to detect PBP2a found in MRSA. (A) The product of the *mecA* gene, which results in methicillin resistance, is an altered penicillin-binding protein, PBP2a. Monoclonal antibody to this altered protein was used to coat latex particles, which were then used in the Oxoid agglutination assay to detect PBP2a. (B) Shown is the Alere PBP2a SA Culture Colony Test (Alere Scarborough, Inc., Scarborough, ME), a lateral flow assay that utilizes monoclonal antibodies for the detection of PBP2a. Both formats are rapid tests that are used once the organism is isolated on solid medium.

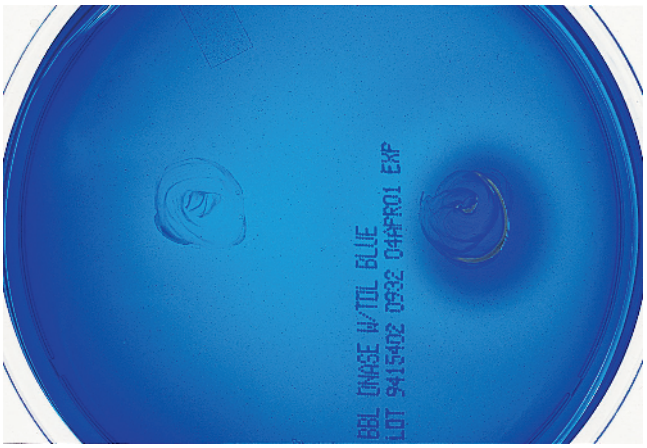


Figure 1-16 DNase plate to differentiate *Staphylococcus aureus* from CoNS. *S. aureus* produces DNase, which can degrade DNA. This property is used to aid in the differentiation of CoNS (left) from *S. aureus* (right). This is particularly useful for identification of *S. aureus* strains that produce a small amount of coagulase, thus giving an equivocal or weakly positive coagulase test. The only CoNS species that shares this property with *S. aureus* is *S. schleiferi*. In this test, a heavy inoculum of the organism is used to spot an agar plate that contains DNA and toluidine blue. If the organism produces DNase (right), the DNA is degraded, resulting in the agar turning pink in the area surrounding the inoculum due to the metachromatic qualities of toluidine blue.

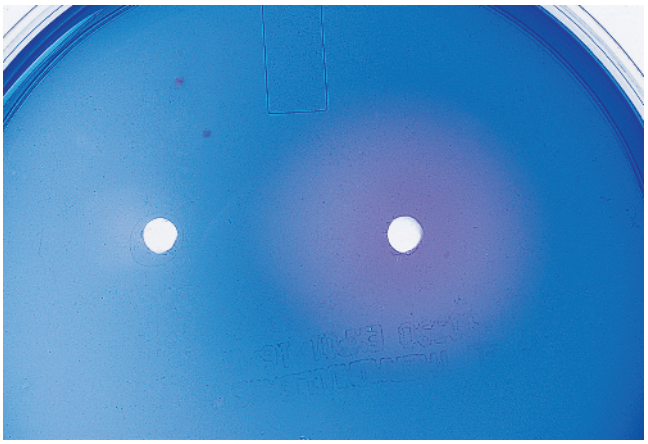


Figure 1-17 Thermostable endonuclease activity. In addition to DNase, *S. aureus* produces a thermostable endonuclease that can also cleave DNA. To test for this activity, a heavy suspension of the organism is boiled and then used to fill a well that is cut in the DNA plate containing toluidine blue. As described in the legend to Fig. 1-16, if the DNA is degraded, there is a change in the color of the agar from blue to pink. *S. epidermidis* does not produce a heat-stable endonuclease (left), whereas the *S. aureus* strain (right) does, as shown by the pink zone around the well containing *S. aureus*.

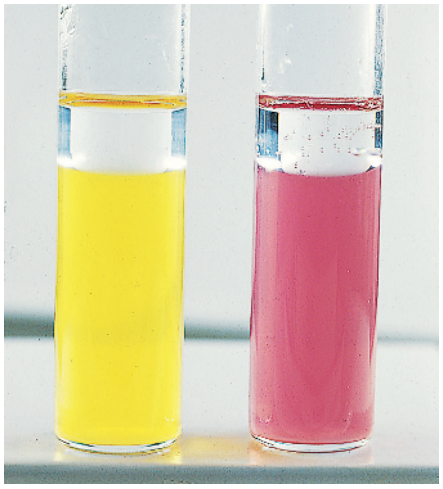


Figure 1-18 Ornithine decarboxylase test for the identification of *Staphylococcus lugdunensis*. Unlike most other CoNS species, *S. lugdunensis* is ornithine decarboxylase positive. Decarboxylase medium containing 1% ornithine is inoculated and incubated overnight. Since some strains of *S. epidermidis* can also be positive at 24 h, the specimen should be examined at 8 h, a time at which *S. lugdunensis* is positive but *S. epidermidis* is still negative. The isolate on the left, *S. saprophyticus*, is negative since it is yellow, indicating only fermentation of glucose; however, *S. lugdunensis* (right) is positive, as shown by the rose color resulting from the alkalinization of the medium.

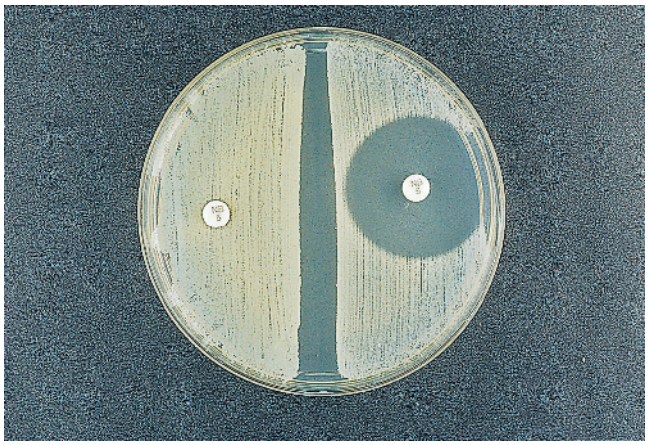


Figure 1-19 Novobiocin susceptibility. *S. saprophyticus* can be differentiated from other clinically significant CoNS isolates by its resistance to the antibiotic novobiocin. As pictured, Mueller-Hinton agar was inoculated with suspensions equivalent to a 0.5 McFarland standard of *S. saprophyticus* (left) and *S. epidermidis* (right). Novobiocin disks (5 μg) were placed on the agar surface, which was incubated for 24 h at 35°C. Zones of inhibition measuring ≤16 mm indicate novobiocin resistance, as seen with this isolate of *S. saprophyticus*, which has no zone of inhibition. In contrast, the susceptible *S. epidermidis* isolate has a large zone of inhibition around the novobiocin disk.

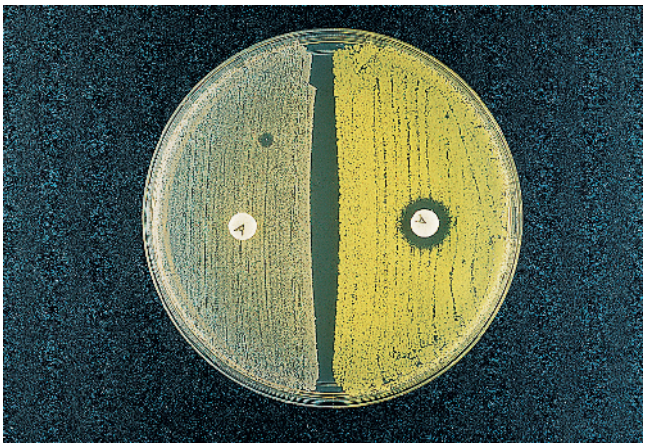
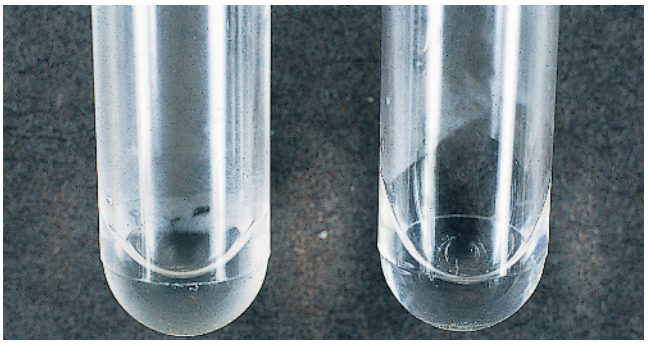


Figure 1-20 Bacitracin susceptibility. The same procedure used to test for the bacitracin susceptibility of *Streptococcus pyogenes* can be used to differentiate staphylococci, which are bacitracin resistant, from micrococci, which are susceptible. Here, *S. epidermidis* (left) is not inhibited, as shown by growth up to the disk containing 0.04 U of bacitracin, whereas *M. luteus* (right) exhibits a zone of inhibition around the bacitracin disk.

Figure 1-21 Lysostaphin susceptibility. Several species of *Staphylococcus* are susceptible to the endopeptidase lysostaphin, which cleaves the glycine-rich pentapeptide that is essential for cross bridging the cell wall. Cleavage of these basic units weakens the cell wall, making it susceptible to lysis. Depending on the makeup of this pentapeptide, specifically the glycine content, susceptibility to lysostaphin can vary. For example, while *S. aureus* is very susceptible, *S. saprophyticus* is less susceptible due to the serine content of its pentapeptide bridge. Micrococci are not susceptible to lysostaphin. As shown here, the test is performed by making a heavy suspension of the unknown organism in saline and then adding an equal volume of lysostaphin reagent. Clearing of the suspension after 2 h at 35°C indicates lysis of the organisms. In the example shown here, the lysostaphin test medium inoculated with *M. luteus* (left) remained turbid and thus was negative, in contrast to the tube on the right, which was inoculated with *S. aureus* and is positive, as shown by clearing or lysis of the bacterial suspension. This assay can also be performed as a disk diffusion test.



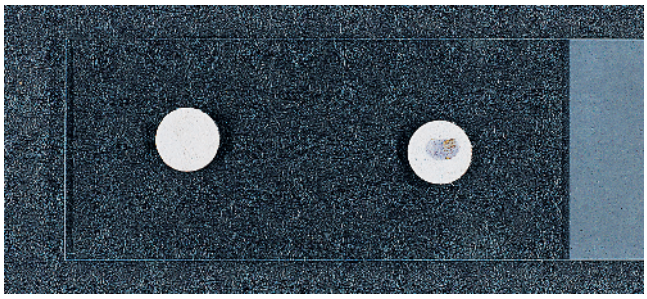


Figure 1-22 Modified oxidase test. A modified oxidase test, the Microdase test (Thermo Scientific, Remel Products), is available for differentiating micrococci from staphylococci. Micrococci possess cytochrome *c*, which is essential for producing a positive oxidase reaction, whereas clinically relevant staphylococci are oxidase negative, since they lack cytochrome *c*. In the example shown, a colony of *S. epidermidis* (left) and a colony of *M. luteus* (right) were rubbed onto a disk impregnated with tetramethyl-*p*-phenylenediamine (TMPD) dissolved in dimethyl sulfoxide. Development of a purple-blue color within 2 min indicates a positive test due to the reaction of the enzyme oxidase with cytochrome *c* and TMPD.

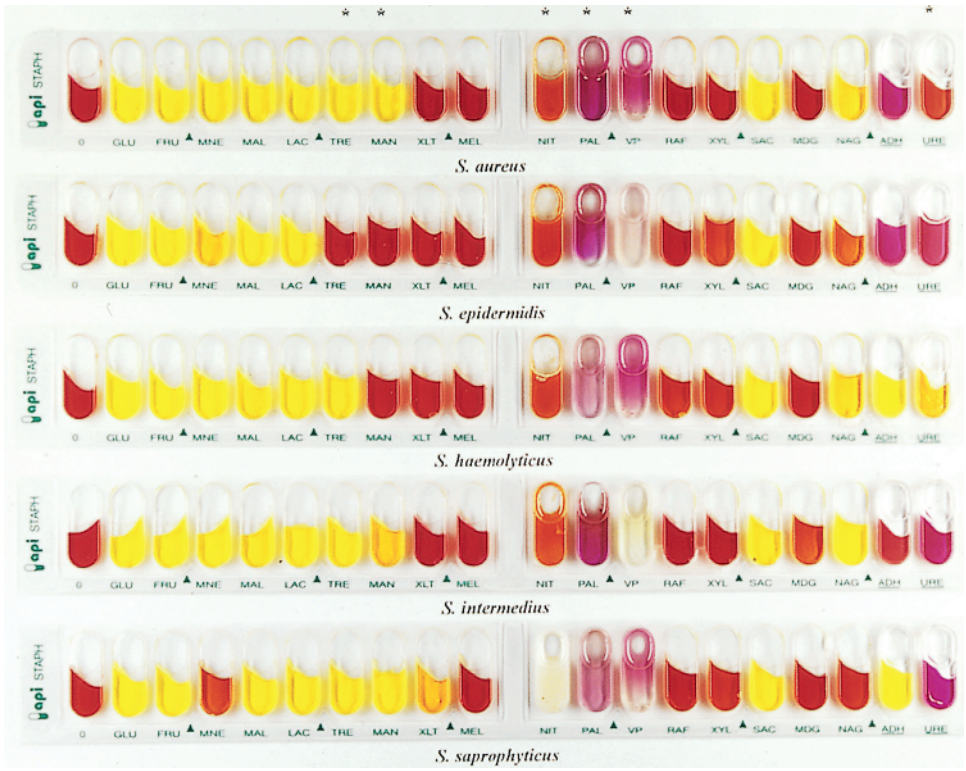


Figure 1-23 API Staph identification system. API Staph (bioMérieux, Inc., Durham, NC) is a commercial system that can differentiate among several *Staphylococcus* species. Each test strip consists of 20 microtubes, including the negative control well. Key reactions that aid in the differentiation and identification of the five *Staphylococcus* species shown are indicated by asterisks at the top.

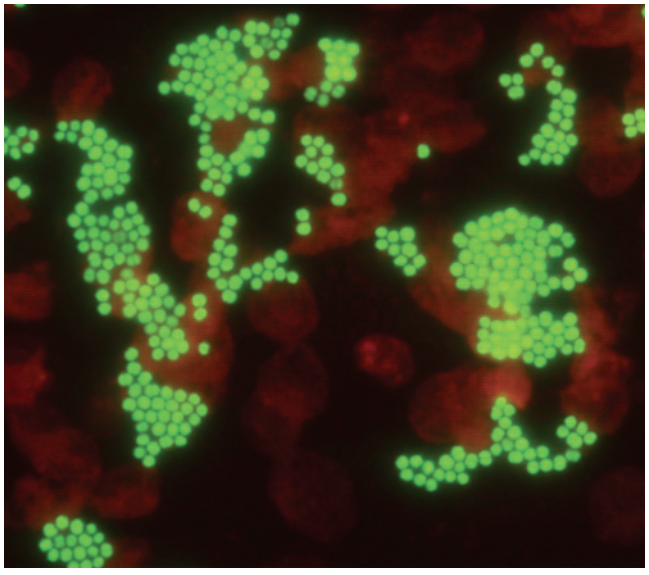


Figure 1-24 PNA FISH for the differentiation of *Staphylococcus aureus* from CoNS in blood cultures. PNA FISH (AdvanDx, Woburn, MA) is a 90-min fluorescent *in situ* hybridization (FISH) assay utilizing fluorescence-labeled peptide nucleic acid (PNA). It is performed directly from blood culture bottles that are positive for Gram-positive cocci in clusters. In the example shown, the Gram-positive cocci are *S. aureus*, which hybridized with a green fluorescent probe. (Courtesy of AdvanDx.)