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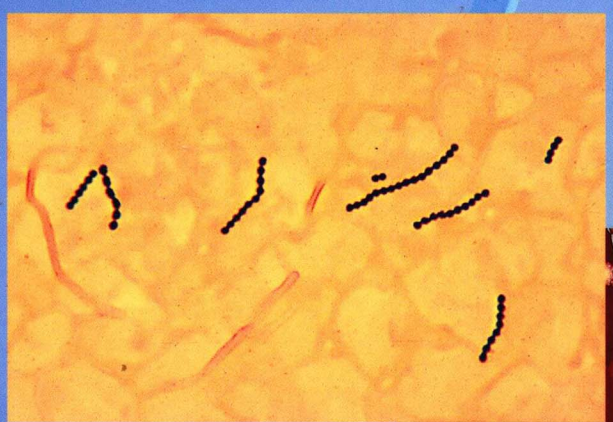
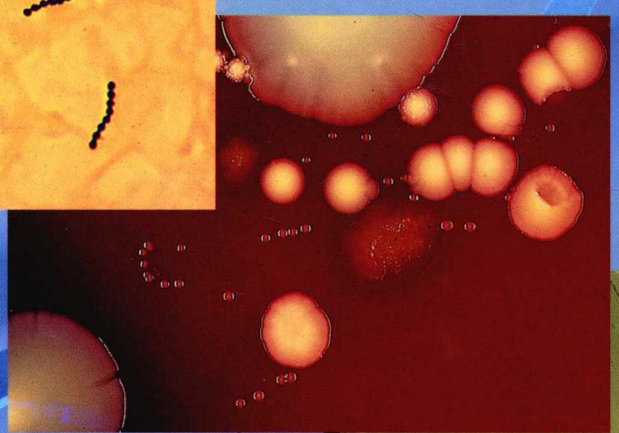
SECOND EDITION

Color Atlas of Medical Bacteriology





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Preface

At the dawn of the third millennium, when the names of Watson and Crick are more familiar to students than Koch and Pasteur, when the jargon terms NAAT, RFLP, and MALDI-TOF are part of everyday conversation, and when many hands-on identification methods are part of history, who needs an atlas of bacteriology?

Traditionally, microbiology—especially medical bacteriology—has been dependent on the subjective interpretation of a Gram stain or growth on an agar plate. While there are several excellent textbooks on the subject, they usually are composed of written descriptions of microorganisms with few images. In part, we were motivated to publish the first edition of this atlas by the challenge to find illustrations for our own lectures and laboratory presentations. But since medical bacteriology is dynamic, with changes occurring frequently, especially in classification, nomenclature, and methodology, we accepted the challenge of an update. Following uncountable hours of planning and several missed deadlines, this second edition went to press. We extend a special thanks to ASM Press for their patience and understanding.

With this second edition we have attempted to provide updated illustrations of typical Gram stains, colony morphologies, and biochemical reactions of bacteria most frequently encountered in the clinical laboratory. Furthermore, since the time of our first edition, the continuous and alarming emergence of antibiotic resistance has resulted in the need for more antimicrobial susceptibility testing. As well, the rapid development of molecular techniques has brought these principles into wide use in medical bacteriology. Accordingly, we have written two additional chapters to incorporate these concepts.

Each chapter has a brief introduction to provide some context for the illustrations; for in-depth background on individual organisms, the reader should consult one of the many excellent textbooks and manuals available. This second edition was structured with reference to a number of sources, listed below, and particularly to the *Manual of Clinical Microbiology*, 10th edition (MCM10). However, we are responsible for any errors that appear. The number of photos of a particular organism does not necessarily correlate with the frequency of its isolation or its clinical relevance. Certain bacteria have variable, distinctive, or unique pictorial characteristics, and we have tried to provide a representative sampling of these. We hope you will find this atlas a useful reference tool.

With the increasing use of nucleic acid techniques, the remarkable forms, shapes, and colors of bacteria in the laboratory are rapidly being replaced by signals only measurable by instruments. The time is very near when we will be showing our grandchildren many of the images in this atlas that have become a distant memory. In the meantime, let us enjoy the beauty of the colorful bacterial world.

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We would like to thank many individuals who have made this book possible. First and foremost, we want to thank all the staff in the Division of Medical Microbiology at the University of California, Irvine Medical Center. In the laboratory, "The Book" became an obsession that for some was almost the equivalent of finding the Holy Grail. Now that it is complete, we can all relax! We also want to thank our colleagues at the Orange County Health Department and in particular Douglas Moore, Paul Hannah, Douglas Schan, and Tamra Townsen. Over the years we have worked with them on a daily basis on public health-related issues. Their help with the first edition of this atlas covering highly pathogenic organisms was invaluable. We also would like to acknowledge the contributions of Alan G. Barbour, Philippe Brouqui, J. Stephen Dumler, Ted Hackstadt, Barbara McKee, James Miller, and Christopher D. Paddock, who provided key images and specimens and made our work much easier. Additionally, we would like to thank AdvanDX, Inc., Anaerobe Systems, BD Diagnostic Systems, bioMérieux, Inc., Dade Behring Inc., EY Laboratories, Hardy Diagnostics, Thermo Scientific Remel Products, Inc., and Roche Diagnostics Systems for contributing various organisms, media, and reagents.

Technical Note

The microscopic pictures were taken with a Zeiss Universal microscope (Carl Zeiss, Inc., West Germany) equipped with Zeiss and Olympus (Olympus Optical Corp., Ltd., Japan) lenses. The macroscopic images were captured with a Contax RTS camera with a Carl Zeiss S-Planar 60 mm f/2.8 lens and a Nikon EL camera with a Micro-Nikkor 55 mm f/3.5 lens. Provia 100F and 400F Professional Fujichrome film (Fuji Photo Film Co., Ltd., Tokyo, Japan) and Kodachrome 25 Professional film (Eastman Kodak Co., Rochester, N.Y.) were used for most of the images. The final magnification of the Gram and acid-fast stains is ×1,200.

Contents

Staphylococcus and *Micrococcus*

1

Members of the genera *Staphylococcus* and *Micrococcus* are characterized as being catalase-positive, gram-positive cocci in pairs and clusters. These organisms commonly colonize the surface of skin and mucosal membranes of mammals and birds. *Micrococcus* is generally considered to be a saprophyte, while *Staphylococcus*, particularly *Staphylococcus aureus*, is an important and frequently encountered human pathogen. The genus *Staphylococcus* includes organisms with a low G+C content. In contrast, *Micrococcus* and the genera *Alliococcus*, *Rothia*, and *Kocuria*, all members of the family *Micrococcineae*, are of less human importance and have a high G+C content.

The species of *Staphylococcus* most frequently associated with human infections are *S. aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, and *Staphylococcus schleiferi*. Of these, *S. aureus* is the most virulent, being a major cause of morbidity and mortality. It 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, endocarditis, scalded-skin syndrome, and toxic shock syndrome. The last two are caused by toxigenic strains. *S. aureus* is also capable of producing food poisoning due to the elaboration of enterotoxins in foods such as potato salad, ice cream, and custards. Intense vomiting and diarrhea usually occurs within 2 to 8 h after ingestion of food containing the toxin.

An increasing problem with *S. aureus* is its resistance to antimicrobial agents, in particular methicillin. In the majority of methicillin-resistant *S. aureus* (MRSA) strains, this is due to an alteration in penicillin binding protein PBP2a, which is encoded by the *mecA* gene. Overproduction of β-lactamase accounts for a smaller percentage of MRSA strains. In recent years, *S. aureus* strains with decreased susceptibility to vancomycin have been identified. These strains are referred to as VISA (vancomycin-intermediate *S. aureus*) or, when addressing their susceptibility to the glycopeptide class of antimicrobials as a whole, GISA (glycopeptide-intermediate *S. aureus*). 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. A molecular test to directly detect the *mecA* gene and 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, screening agar plates incorporating 6 μg of either oxacillin or vancomycin are commercially available to screen for MRSA and VISA strains, respectively. Selective chromogenic agars have facilitiated the detection of MRSA from nares specimens. Due to the importance of

rapidly identifying blood cultures positive for *S. aureus*, peptide nucleic acid fluorescent in situ hybridization (PNA FISH) and nucleic acid amplification protocols have also been developed. These tests can be performed directly from blood cultures positive for gram-positive cocci in pairs and clusters.

Coagulase-negative staphylococci (CNS), in particular *S. epidermidis*, are recognized as the leading cause of nosocomial infections, with immunocompromised hosts at increased risk. Because CNS are members of the normal skin and mucosal membrane flora, 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 CNS is their ability to form a biofilm on the surface of indwelling or implanted foreign bodies, 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 CNS human pathogens, *S. lugdunensis* and *S. schleiferi* have been implicated in serious infections including endocarditis, septicemia, arthritis, and joint infections. *S. lugdunensis*, which at times can behave more like *S. aureus* than CNS, has been associated with aggressive infections that have a high mortality rate; therefore, rapid recognition of this species is important for initiation of appropriate antimicrobial therapy. While other species of CNS have been implicated in a variety of infections, they occur with less frequency.

Micrococcus spp. are common inhabitants of the skin and 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.

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 MRSA strains. CNS, in particular *S. epidermidis*, produce white colonies; however, other CNS strains and species can have colonies with a slight cream pigment. In general, CNS 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, and here 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 CNS. However, since other organisms can grow on this medium and strains of CNS can also ferment mannitol, additional testing is required. Chromogenic media selective and differential for MRSA are more commonly used when 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 made in 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 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 CNS, *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 a latex particle that has been coated with plasma, immunoglobulin, and 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 CNS isolates.

Strains of *S. aureus* that produce a weak coagulase reaction can be further tested by the DNase 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. When testing for the thermostable endonuclease, a suspension of the organism is boiled before being placed on the DNA plate.

CNS can be identified to the species level on the basis of 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 CNS. While most of the CNS of clinical importance are novobiocin susceptible, *S. saprophyticus* is novobiocin resistant. Other tests that can be used to differentiate among the species are phosphatase, production of acetoin, polymyxin susceptibility, pyrrolidonyl arylamidase activity (PYR test), and acid production from carbohydrates.

Micrococcus and related species, 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 CNS, 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 *Staphylococcus* from *Micrococcus*. In general, *Staphylococcus* is resistant to bacitracin (0.04-U disk), in contrast to *Micrococcus*, which is susceptible, while the opposite is found with furazolidone (100-μg disk) and lysostaphin (200-μg/disk), where *Micrococcus* is resistant.

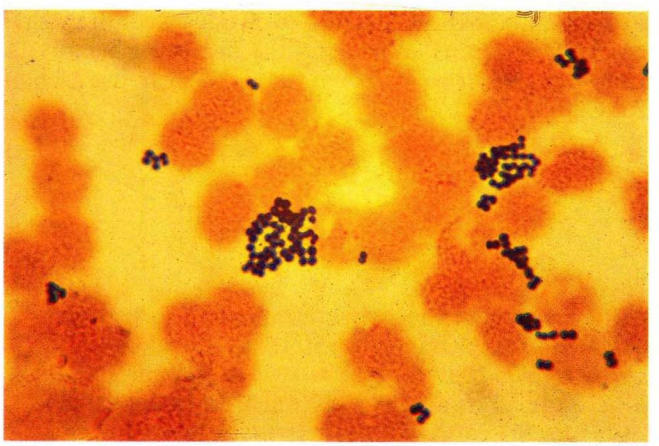


Figure 1-1 Gram stain of *Staphylococcus aureus*. A Gram stain of a positive blood culture shows gram-positive cocci in grape-like clusters. On subculture to solid media, *S. aureus* was isolated.

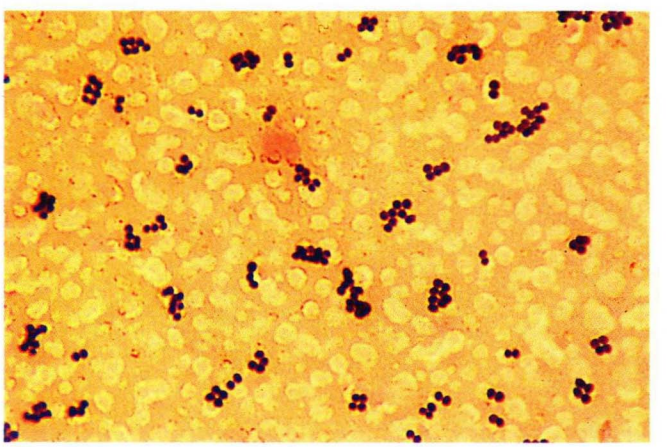


Figure 1-2 Gram stain of *Micrococcus luteus*. *M. luteus* organisms are gram-positive cocci that, like *S. aureus*, can appear in pairs and clusters. However, they also tend to form tetrads, as depicted in this Gram stain.

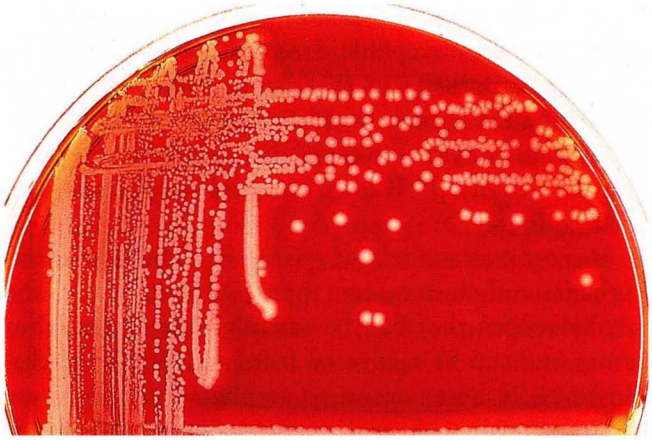


Figure 1-3 *Staphylococcus aureus* on blood agar. In the culture shown, *S. aureus* was grown overnight at 35°C on blood agar. The colonies are cream colored and opaque and have a smooth entire edge. A zone of beta-hemolysis surrounds the colony.

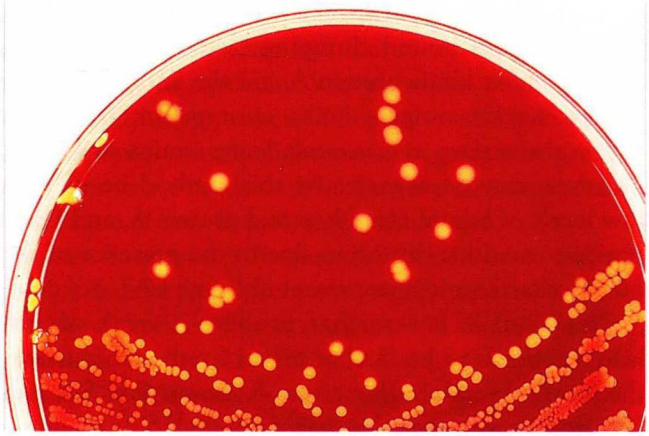


Figure 1-4 Golden pigment of *Staphylococcus aureus*. *S. aureus* is capable of producing the golden pigment that led to its species name. In practice, strains with this degree of pigment are not frequently isolated from clinical specimens. The isolate shown was incubated for 24 h on blood agar at 35°C 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*, in particular 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.

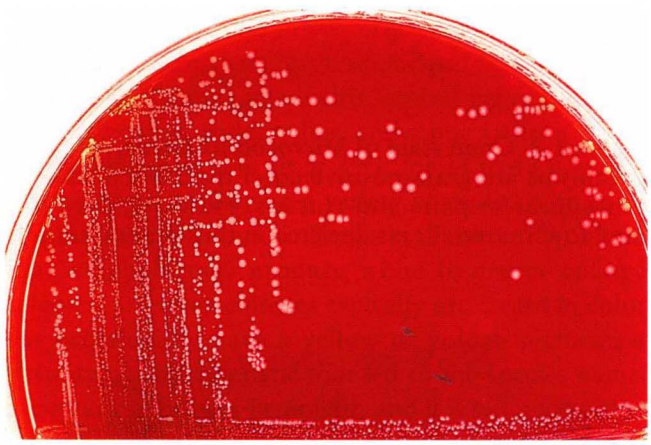


Figure 1-6 *Staphylococcus epidermidis* on blood agar. *S. epidermidis*, in contrast to both *S. aureus* and other CNS, produces a white colony with little or no pigment. The isolate shown here was grown on blood agar for 24 h at 35°C. This strain of *S. epidermidis* exhibits some variation in colony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

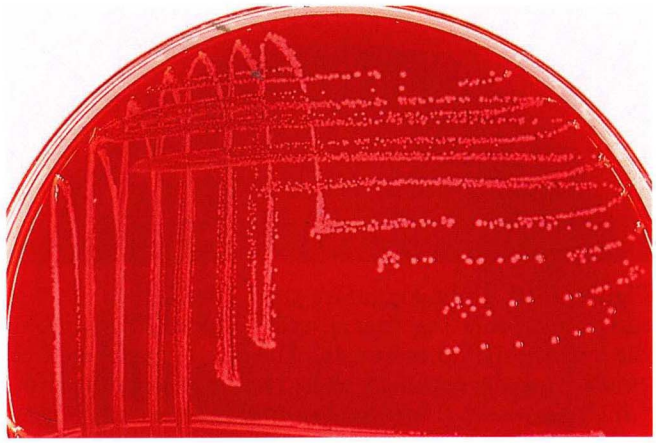


Figure 1-7 *Staphylococcus lugdunensis* on blood agar. Colonies of *S. lugdunensis* on blood agar resemble *S. epidermidis* colonies; however, they tend to be cream colored, in contrast to the typical white colonies of *S. epidermidis* (Fig. 1-6).

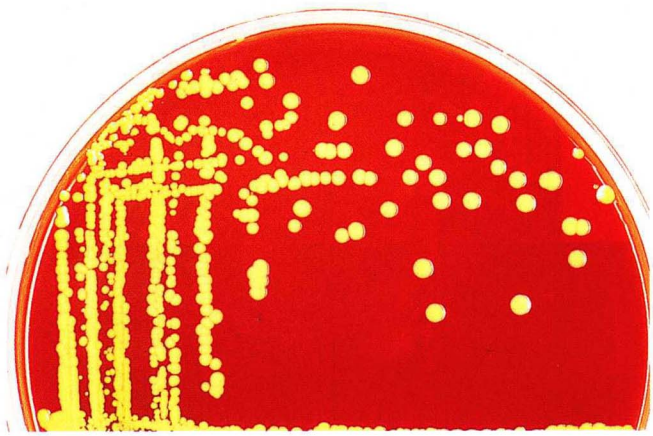


Figure 1-8 *Micrococcus luteus* on blood agar. A distinguishing feature of *M. luteus* is the vivid yellow colonies it produces. The isolate shown here was grown on blood agar for 72 h at 35°C. In general, *Micrococcus* is slower growing than *Staphylococcus*.

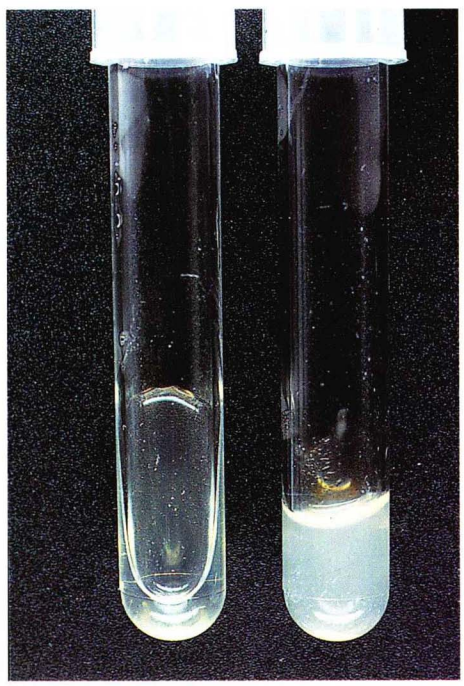


Figure 1-9 Coagulase test. A common method used to distinguish *S. aureus* from other *Staphylococcus* spp. is the tube coagulase test shown here. *S. aureus* is positive, and CNS are negative. Colonies of the isolate to be identified were emulsified in 0.5 ml of rabbit plasma. The tube was incubated 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.

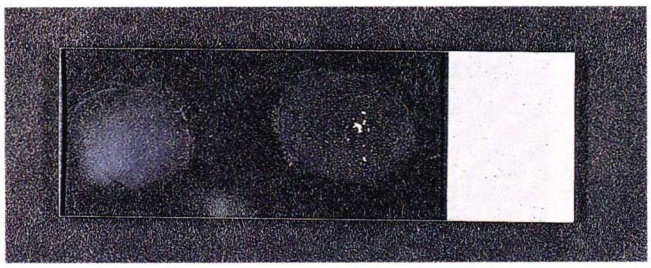


Figure 1-10 Slide coagulase test. The slide coagulase test is a rapid assay that tests for clumping factor on the surface of the organism. The test is performed by emulsifying the organism to be identified in both saline, which serves as a control for autoagglutination (left), and rabbit plasma (right).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

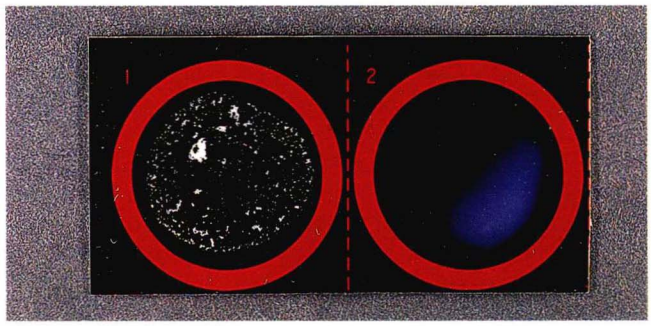


Figure 1-11 Latex test for the identification of *Staphylococcus aureus*. In the test depicted here, latex particles have been coated with antibody that can recognize bound coagulase as well as immunoglobulin that will bind to protein A present on the surface of most strains of *S. aureus*. The isolate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

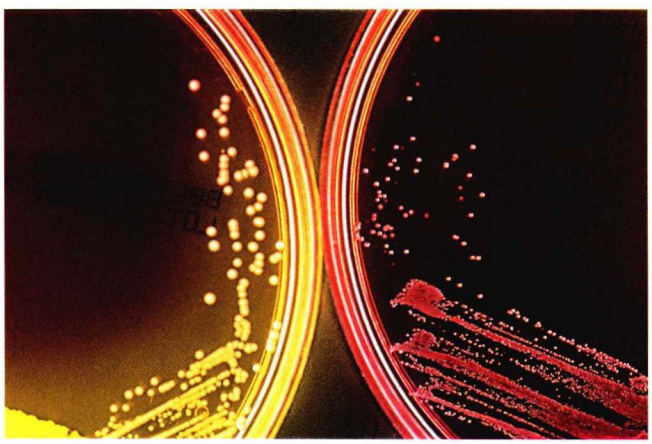


Figure 1-12 Mannitol salt agar. Mannitol salt agar is a selective and differential medium used for the isolation and presumptive identification of *S. aureus*. The high salt concentration inhibits the growth of many organisms that inhabit skin and mucosal membranes. The phenol red indicator incorporated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

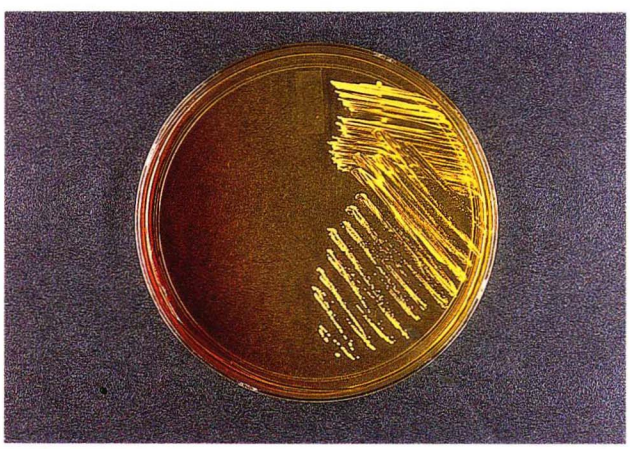


Figure 1-13 Mannitol salt agar containing oxacillin. Mannitol salt agar with oxacillin can be used to screen for the presence of MRSA in nasal specimens since the 7.5% salt and 6 μg of oxacillin in this medium inhibit most other organisms that normally colonize the nares. MRSA turns the medium yellow 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.

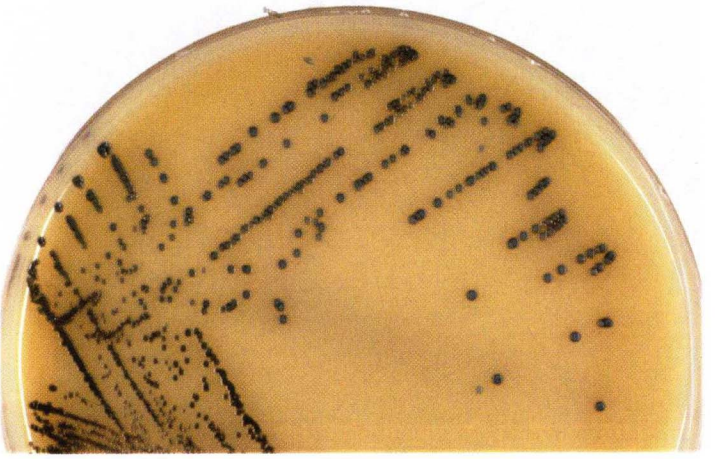
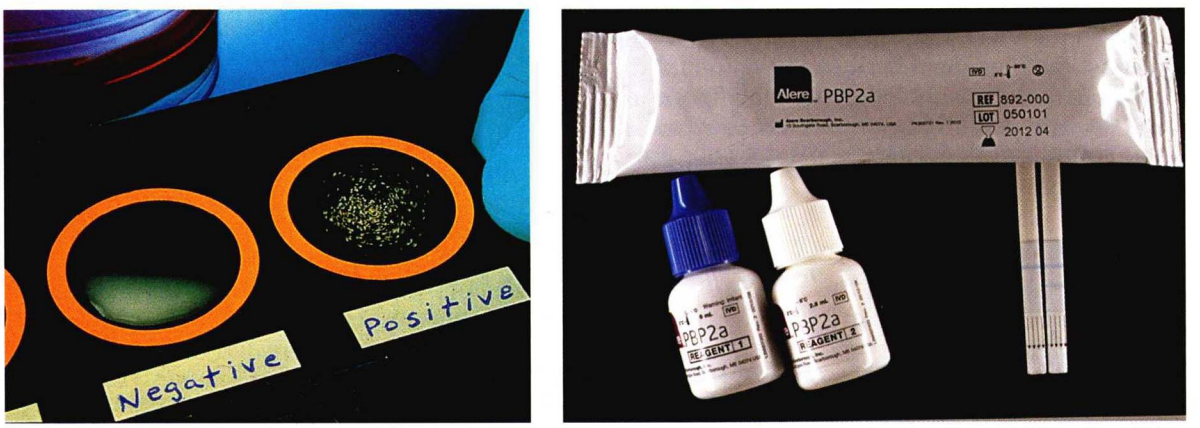


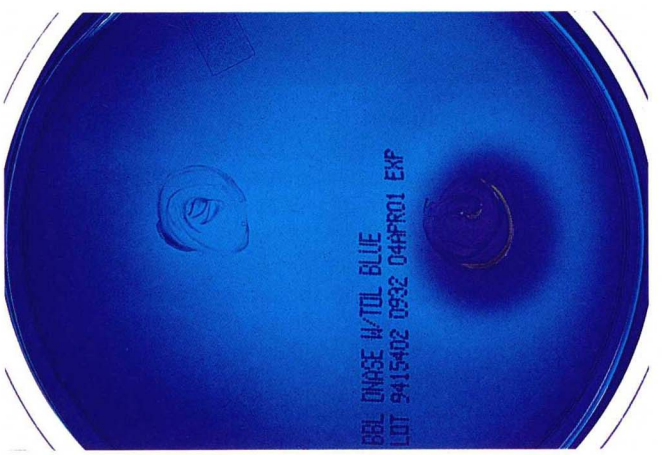
Figure 1-14 Spectra MRSA. Shown is a chromogenic medium, Spectra MRSA (Thermo Scientific Remel Products, Lenexa, KS), that is both selective and differential that can be used to detect MRSA. When the chromogenic substrate incorporated into the inhibitory agar is degraded by the enzymatic action of MRSA,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



A B

Figure 1-15 Assays to detect PBP2a protein 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 has been used to coat latex particles, which are then used in the Oxoid agglutination assay to detect PBP2a. (B) Shown is the Alere PBP2a lateral flow assay (Alere Inc., Waltham, MA) also utilizing monoclonal antibodies for the detection of PBP2a protein. Both formats are rapid tests that are used once the organism is isolated on solid media.

Figure 1-16 DNase plate to differentiate *Staphylococcus aureus* from CNS. *S. aureus* produces DNase, which can degrade DNA. This property is used to aid in the differentiation of *S. aureus* (right) from CNS (left). 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 CNS 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, the DNA is degraded, resulting in the agar turning pink in the area surrounding the inoculum due to the metachromatic qualities of toluidine blue.



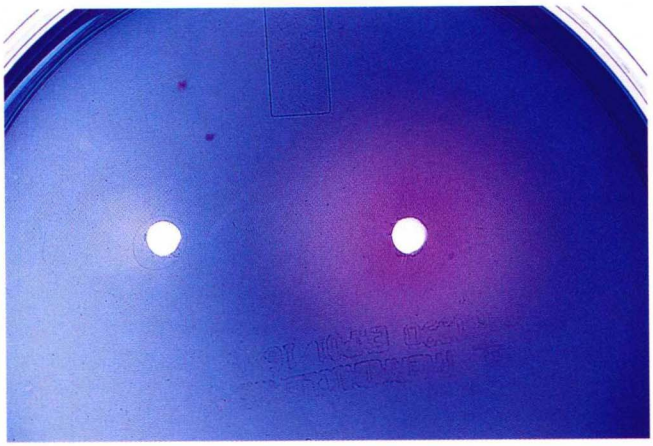


Figure 1-17 Thermostable endonuclease activity. In addition to DNase, *S. aureus* produces a thermostable endonuclease that can also cleave DNA. To test for this activity, a heavy suspension of the organism is boiled and then used to fill a well that is cut in the DNA plate containing toluidine blue. 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, as shown for the *S. aureus* strain (right). *S. epidermidis* does not produce a heat-stable endonuclease (left).

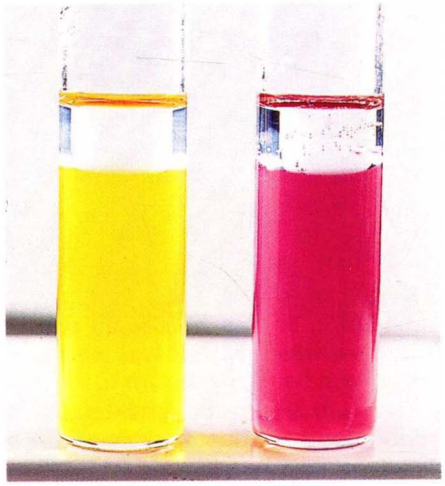


Figure 1-18 Ornithine decarboxylase test for the identification of *Staphylococcus lugdunensis*. Unlike most other CNS species, *S. lugdunensis* is ornithine decarboxylase positive. The decarboxylase medium containing 1% ornithine is inoculated and incubated overnight. Since some strains of *S. epidermidis* 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* (shown on the right) is positive due to the violet color resulting from the alkalinization of the medium.

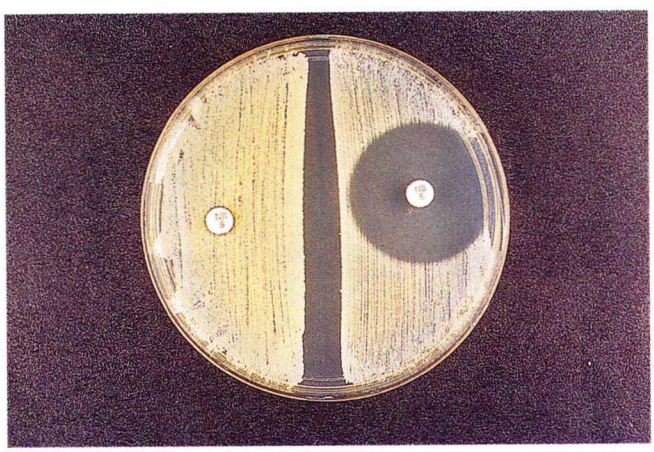


Figure 1-19 Novobiocin susceptibility. *S. saprophyticus* can be differentiated from other clinically significant CNS isolates by its resistance to the antibiotic novobiocin. As pictured, Mueller-Hinton agar was inoculated with a suspension equivalent to a 0.5 McFarland standard of *S. epidermidis* (right) and *S. saprophyticus* (left). 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.

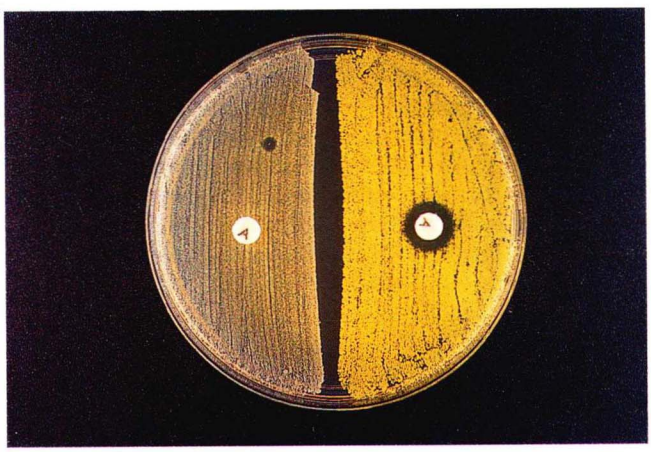


Figure 1-20 Bacitracin susceptibility. The same procedure used to test for the bacitracin susceptibility of *Streptococcus pyogenes* can be used to differentiate *Staphylococcus* spp., which are bacitracin resistant, from *Micrococcus* spp., which are susceptible. Here *M. luteus* (right) exhibits a zone of inhibition around a 0.04-U bacitracin disk, in contrast to *S. epidermidis* (left), which is not inhibited.

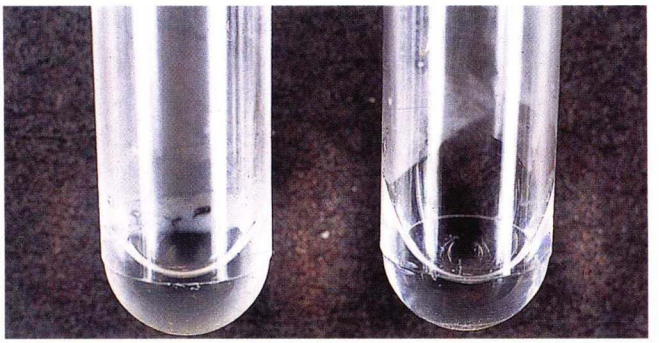


Figure 1-21 Lysostaphin susceptibility. Several species of *Staphylococcus* are susceptible to the endopeptidase lysostaphin, which cleaves the glycine-rich pentapeptide that is essential for cross bridging the cell wall. Cleavage of these basic units weakens the cell wall, making it susceptible to lysis. 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. *Micrococcus* and related species 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 *S. aureus* (right) was positive and the one inoculated with *M. luteus* (left) remained turbid and thus was negative. This assay can also be performed as a disk diffusion test.

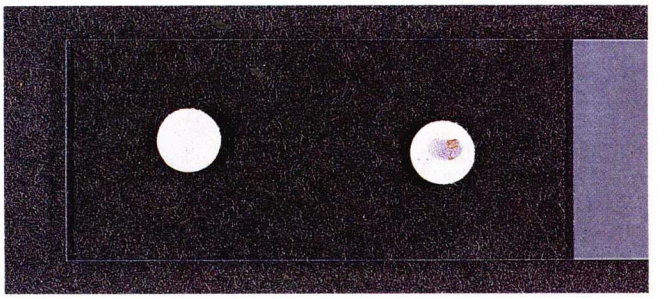


Figure 1-22 Modified oxidase test. A modified oxidase test, the Microdase test (Thermo Scientific Remel Products), is available for differentiating *Micrococcus* from *Staphylococcus*. *Micrococcus* spp. possess cytochrome *c*, which is essential for producing a positive oxidase reaction, whereas clinically relevant *Staphylococcus* spp. are microdase 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 an asterisk at the top.