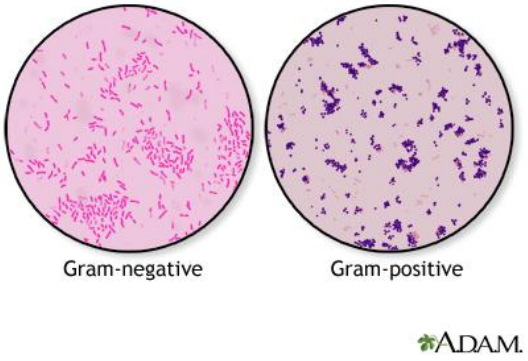
Laboratory Session 1

Laboratory Safety and Procedures

The teaching laboratory is classified as **Biosafety Level 1 (BSL-1)**. Consequently, the rules of conduct are determined by the requirements of this level. Work in this teaching lab involves pathogens that do not typically cause disease in healthy adult humans. Accordingly, standard microbiological practices are followed without the need for specific primary or secondary barriers, other than a handwashing sink.

1. Gram Stain Method

Gram staining is essential for differentiating bacteria based on the chemical and physical properties of their **cell walls**. However, this method alone is not sufficient for full bacterial identification.

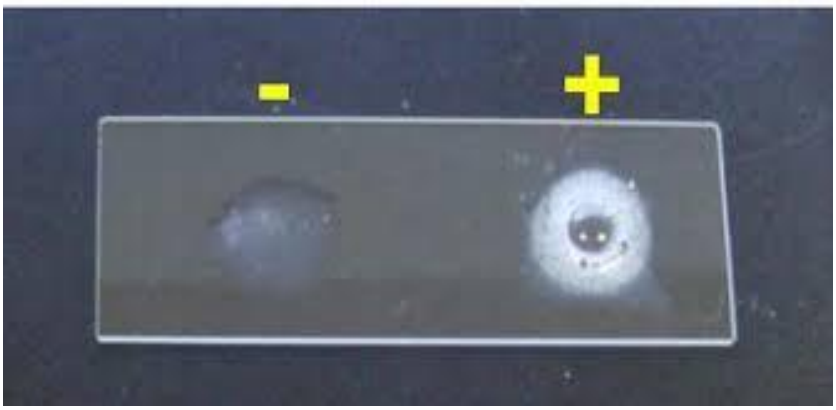


2. Catalase Activity

The catalase test is used to differentiate **Gram-positive cocci** (e.g., *S. aureus* and *E. faecalis*). All Staphylococci are catalase-positive, while Streptococci are catalase-negative. To determine if an organism is catalase-positive (*Staphylococcus spp.*), we use reference strains (ATCC) for comparison: *Staphylococcus aureus* (catalase-positive) and *Enterococcus faecalis* (catalase-negative). (See image)

Procedure: Using a loop, place a small amount of bacteria onto a dry slide and add a drop of **hydrogen peroxide** (H_2O_2).

Interpretation: The formation of **bubbles** indicates a positive reaction.



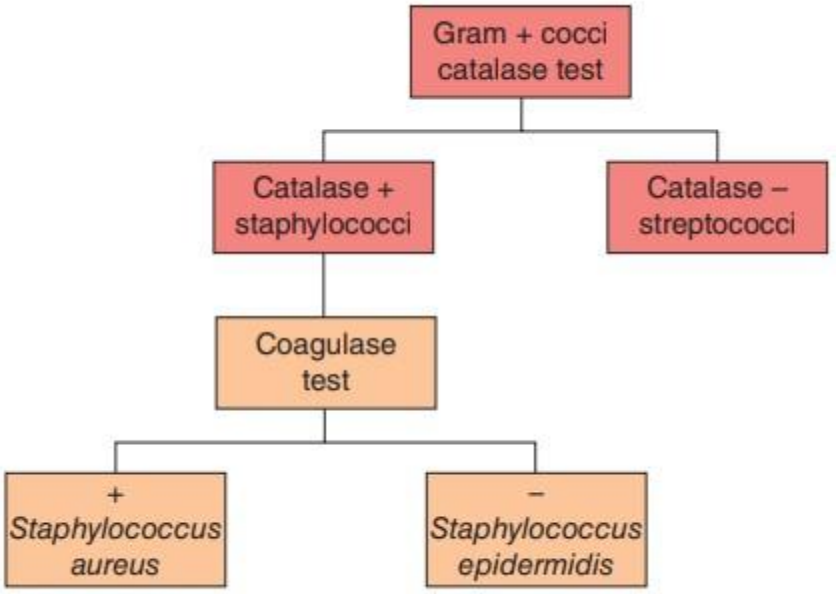


FIGURE 3-1 Algorithm for differentiating the Gram-positive coc

Laboratory3. Mannitol Salt Agar (MSA)

Mannitol Salt Agar (MSA) is a growth medium used for the identification and differentiation of *Staphylococcus* species. Therefore, it is simultaneously **selective** and **differential**.

Procedure: Bacteria are streaked onto MSA agar and placed in an incubator at 37°C for 24 hours.

Interpretation:

Positive Result (Yellow color): The bacteria ferment mannitol and produce acid, which changes the color of the indicator added to the medium (red turns to yellow). Example: *Staphylococcus aureus*.

Negative Result (Red/Pink color): The bacteria grow but cannot ferment mannitol; therefore, the color of the medium does not change. Example: *Staphylococcus epidermidis*.

4. Coagulase Test

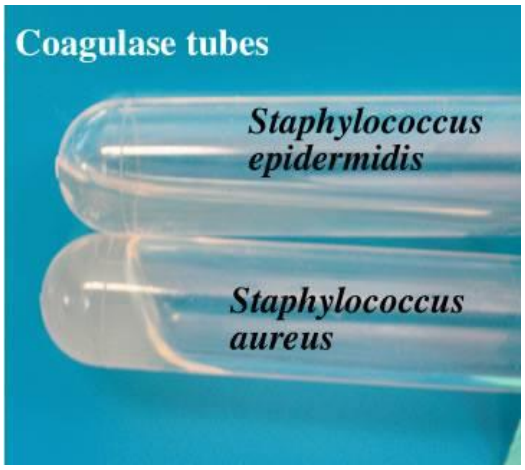
The coagulase test is used for species-level identification of Staphylococci. *Staphylococcus aureus* is coagulase-positive, while *Staphylococcus epidermidis* is coagulase-negative. Coagulase accelerates the formation of a **fibrin clot** from its precursor, fibrinogen (this clot can protect the bacteria from phagocytosis by sequestering the infected area and covering the microorganism with a fibrin layer).

Procedure: Introduce 0.5 ml of citrated rabbit plasma into a sterile test tube. Add several colonies of a pure culture of the test bacteria to the plasma. Place the tube in an incubator at 37°C. Check periodically (every 1 hour, for a maximum of 4–24 hours) for clotting.

Interpretation:

Positive (+): The plasma clots and does not flow when the tube is tilted. This identifies *Staphylococcus aureus*.

Negative (-): The plasma remains liquid and homogenous. The Staphylococcus is coagulase-negative (*S. epidermidis* or *S. saprophyticus*).

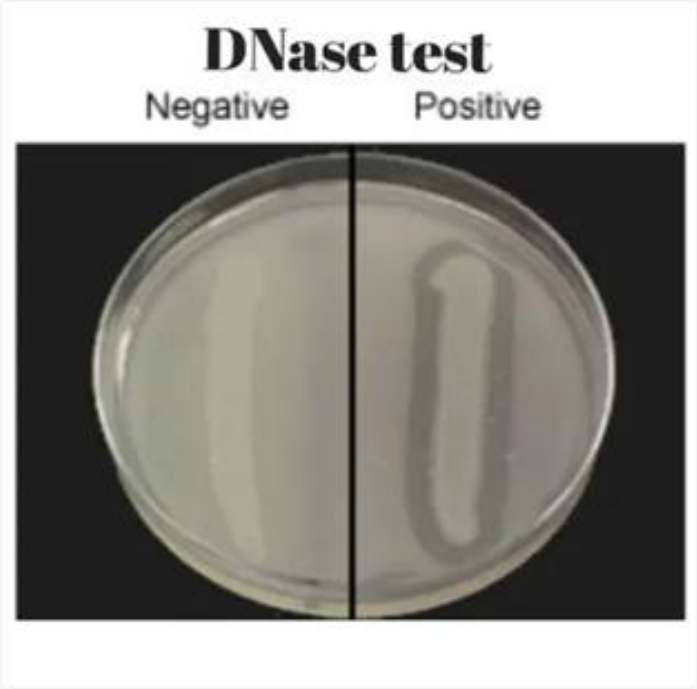


5. DNase Test

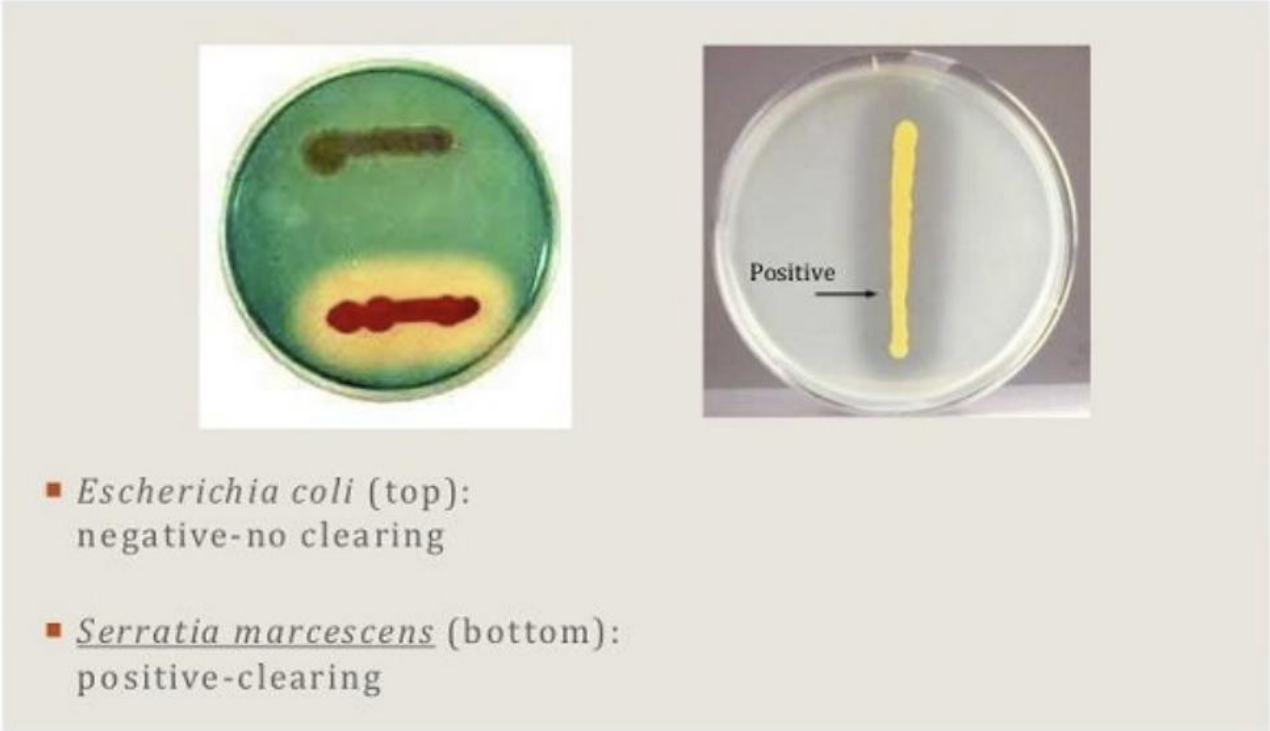
The DNase test is used to identify and differentiate microorganisms that produce **deoxyribonuclease (DNase)**, an enzyme that hydrolyzes deoxyribonucleic acid (DNA) into small fragments such as nucleotides. DNase breaks down extracellular DNA, which may help bacteria evade the host's immune response. DNase activity can be a crucial diagnostic marker in clinical microbiology, particularly in distinguishing Staphylococcus aureus (DNase-positive, coagulase-positive) from other Staphylococcal species, such as *Staphylococcus lugdunensis* (DNase-negative, coagulase-negative).

Procedure: Using a sterile inoculation loop, take a small amount of the test microorganism and transfer it to the surface of the DNase agar. Incubate the plate at 35–37°C for 24 hours, depending on the organism's growth rate. After incubation, carefully pour 1N HCl over the surface of the agar. Read the result after 5–10 minutes.

Interpretation: A **clear zone** around the bacterial growth indicates a positive DNase result, showing that the DNA has been hydrolyzed. This occurs because the hydrochloric acid causes the remaining DNA in the agar to precipitate. The result is negative if no clear zone is observed.



DNase test negative and positive after adding HCL



6. Hemolytic Activity

The hemolytic activity test (streaking on blood agar) is one of the most important diagnostic tests for bacterial identification. This test detects the bacteria's ability to break down erythrocytes through **hemolysins** they synthesize. There are three main types of hemolysis:

α-hemolysis: Incomplete. The bacteria partially destroy erythrocytes. Hemoglobin is oxidized to methemoglobin. Visually, a **green or greenish-brown zone** appears around the colony. The agar does not become transparent. (e.g., *Streptococcus pneumoniae*, Viridans group Streptococci).

β-**hemolysis:** Complete. The bacteria fully rupture (lysis) erythrocytes and the hemoglobin within them. Visually, a **clear, transparent (colorless) zone** forms around the colony. When held up to the light, the agar is transparent in this area. (e.g., *Streptococcus pyogenes* (Group A), *Streptococcus agalactiae* (Group B), *Staphylococcus aureus*).

γ-**hemolysis:** Absence of hemolysis. The bacteria possess no hemolytic activity and do not damage erythrocytes. Visually, the color of the agar around the colony remains unchanged (**remains red**). (e.g., *Enterococcus faecalis*, most Staphylococci except *S. aureus*).