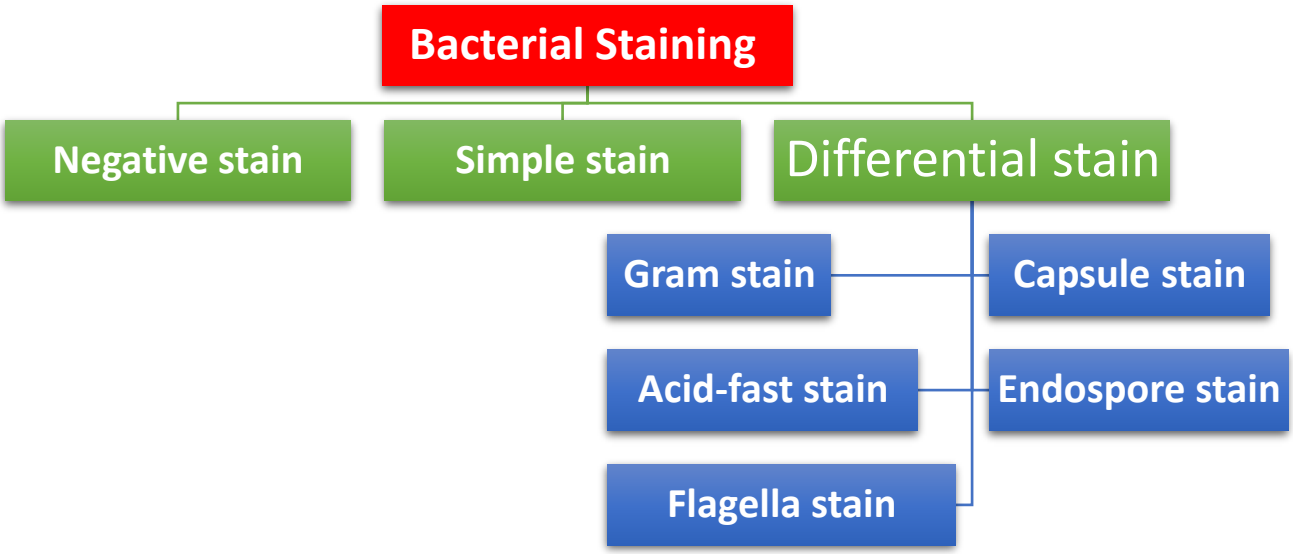


Since living bacteria are generally colorless and almost invisible because of their lack of contrast with the water in which they may reside, staining is necessary in order to make them readily visible for observation of intracellular structures as well as overall morphology.

Negative Staining

Principles : Negative, indirect, or background staining is achieved by mixing bacteria with an acidic stain such as nigrosin, India ink, or eosin, and then spreading out the mixture on a slide to form a film. The above stains will not penetrate and stain the bacterial cells due to repulsion between the negative charge of the stains and the negatively charged bacterial wall. Instead, these stains either produce a deposit around the bacteria or produce a dark background so that the bacteria appear as unstained cells with a clear area around them.

Procedure: as in the figure (1):

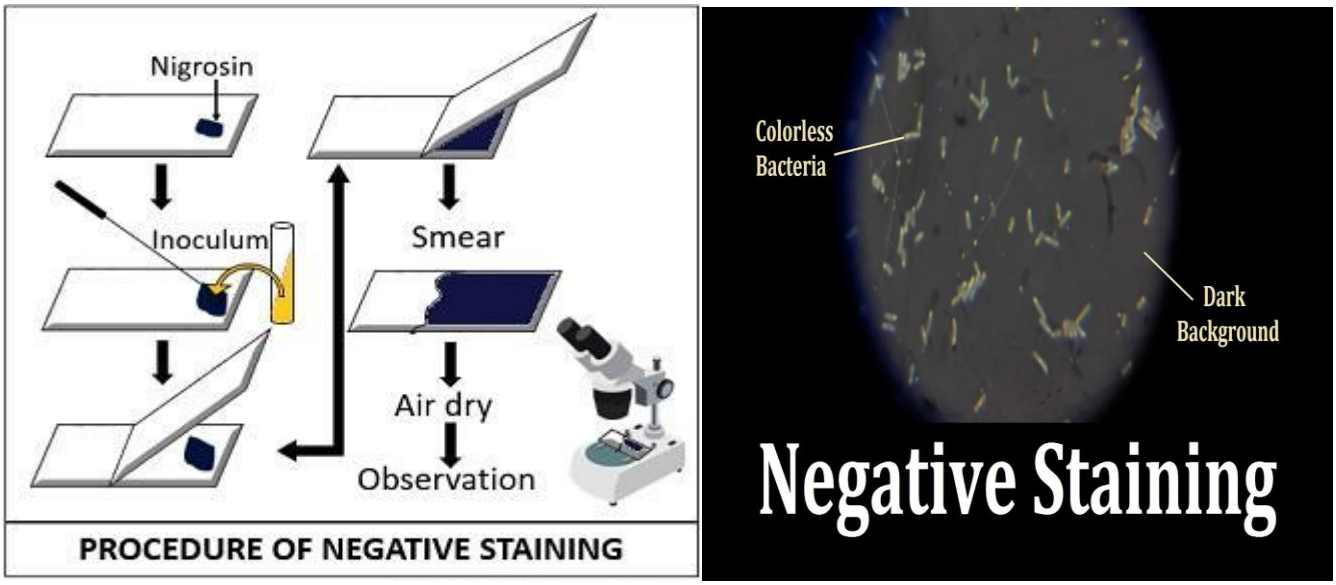


Figure (1) Negative Staining

Smear Preparation and Simple Staining

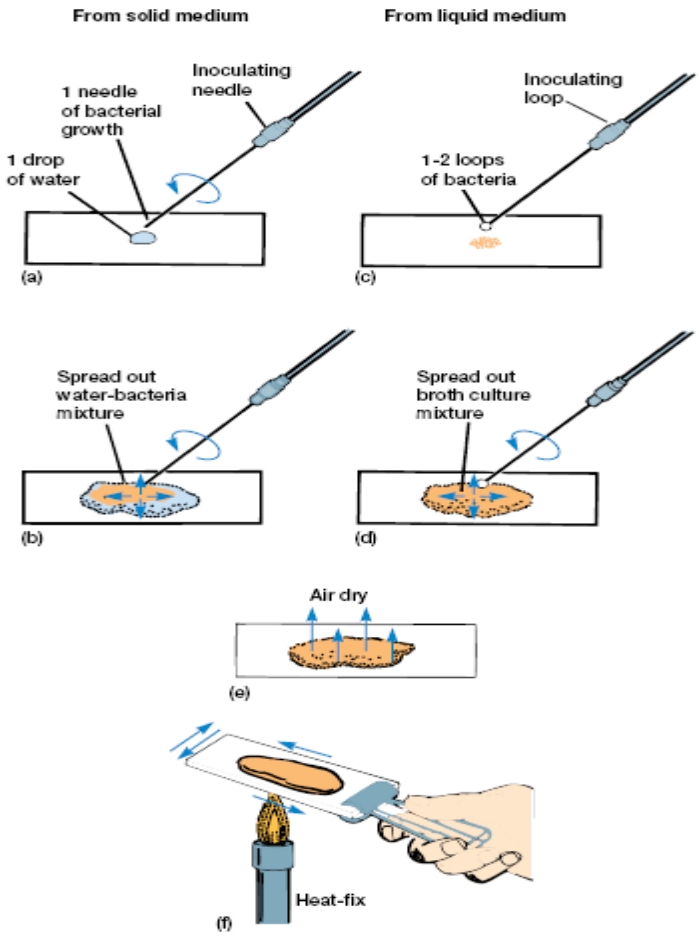


Figure (2): bacterial smear preparation

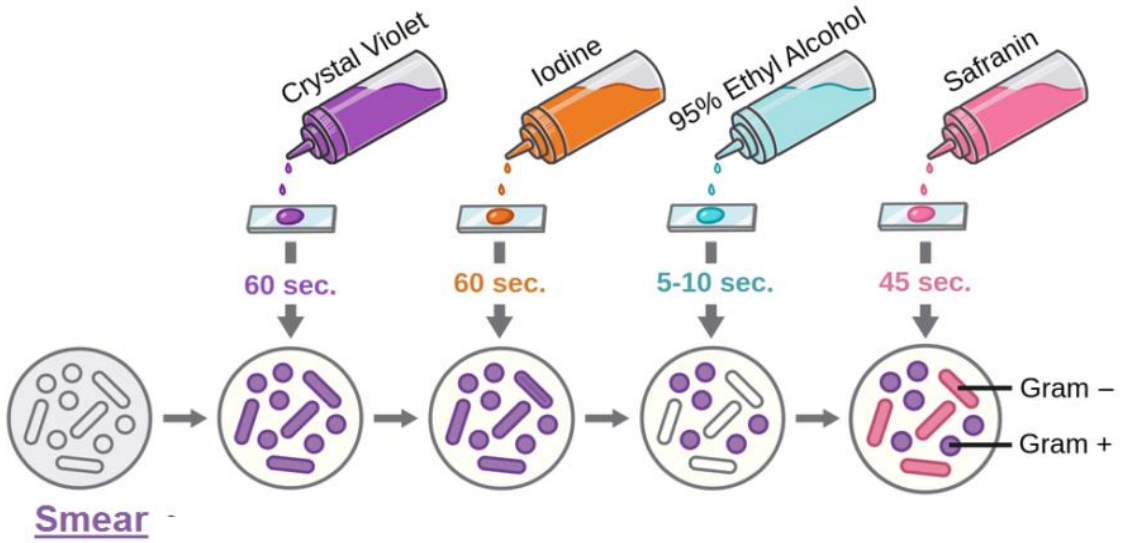
Gram Stain

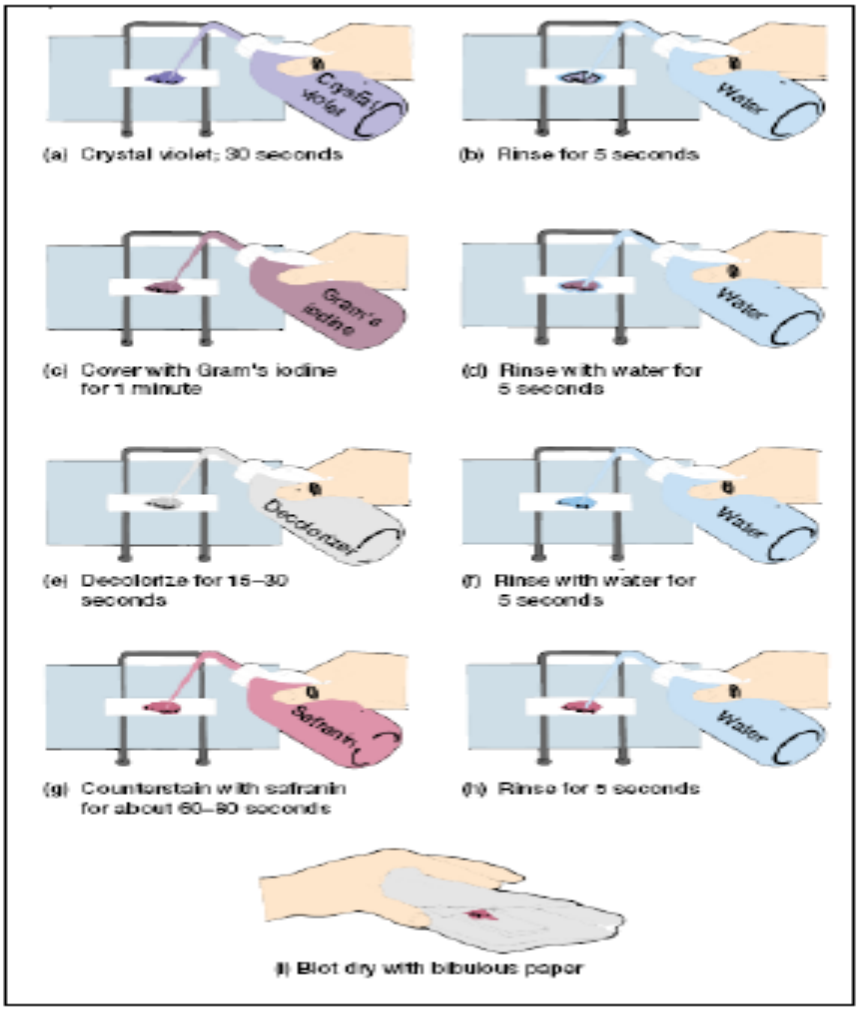
Gram staining is the single most useful test in the clinical microbiology laboratory. It is the differential staining procedure most commonly used for the direct examination of specimens and bacterial colonies because it has a broad staining spectrum. The Gram stain is the first differential test run on a bacterial specimen brought into the laboratory for specific identification. The staining spectrum includes almost all bacteria, many fungi, and parasites.

Principles: Simple staining depends on the fact that bacteria differ chemically from their surroundings and thus can be stained to contrast with their environment. Bacteria also differ from one another chemically and physically and may react differently to a given staining procedure. This is the principle of differential staining. Differential staining can distinguish between types of bacteria. The Gram stain is the most useful and widely employed differential stain in bacteriology.

It divides bacteria into two groups **gram negative and gram positive**.

1- basic dye crystal violet. This is the primary stain. 1min
2- treatment with an iodine solution, which functions as a mordant. 1min
3- 95% ethanol or isopropanol-acetone./ decolorizing agent . 30sec
4- counterstain is usually safranin. 1min





The end result is that gram-positive bacteria are deep purple in color and gram-negative bacteria are pinkish to red in color.

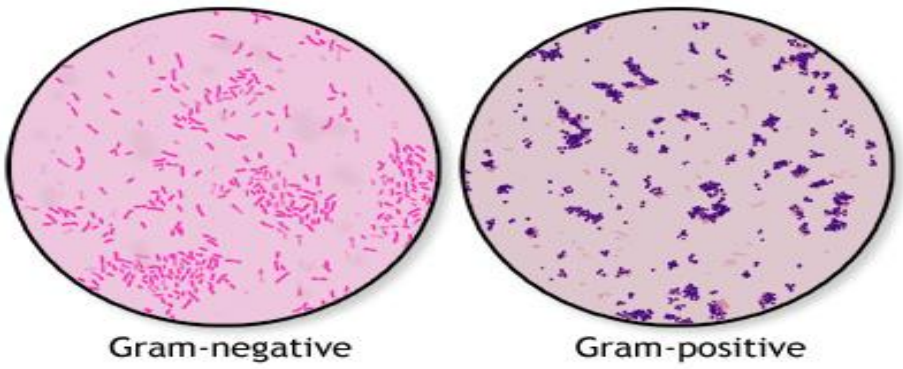


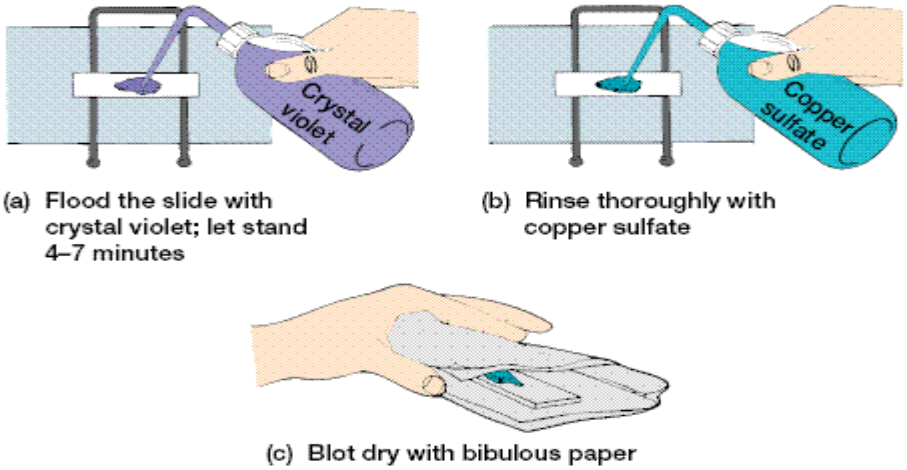
Figure (3): gram staining

Capsule Staining

Principles: Many bacteria have a slimy layer surrounding them, which is usually referred to as a capsule. The capsule's composition, as well as its thickness, varies with individual bacterial species. Polysaccharides, polypeptides, and glycoproteins have all been found in capsules. Often, a pathogenic bacterium with a thick capsule will be more virulent than a strain with little or no capsule since the **capsule protects the bacterium against the phagocytic activity** of the host's phagocytic cells.

Two convenient procedures for determining the presence of a capsule are **Anthony's** capsule staining method (figure 4) and the **Graham and Evans** procedure. Anthony's procedure employs two reagents. The primary stain is crystal violet, which gives the bacterial cell and its capsular material a dark **purple** color. Unlike the cell, the capsule is nonionic and the primary stain cannot adhere. **Copper sulfate** is the decolorizing agent. It removes excess primary stain as well as color from the capsule. At the same time, the copper sulphate acts as a counterstain by being absorbed into the capsule and turning it a light blue or pink. In this procedure, smears should not be heat-fixed since shrinkage is likely to occur, because the capsule is a highly hydrated polymer, it will shrink dramatically when heat is applied or during other procedures in the staining process, and create a clear zone around the bacterium, which can be mistaken for a capsule. **Procedure Capsule Staining (Anthony's) (Figure 4)**

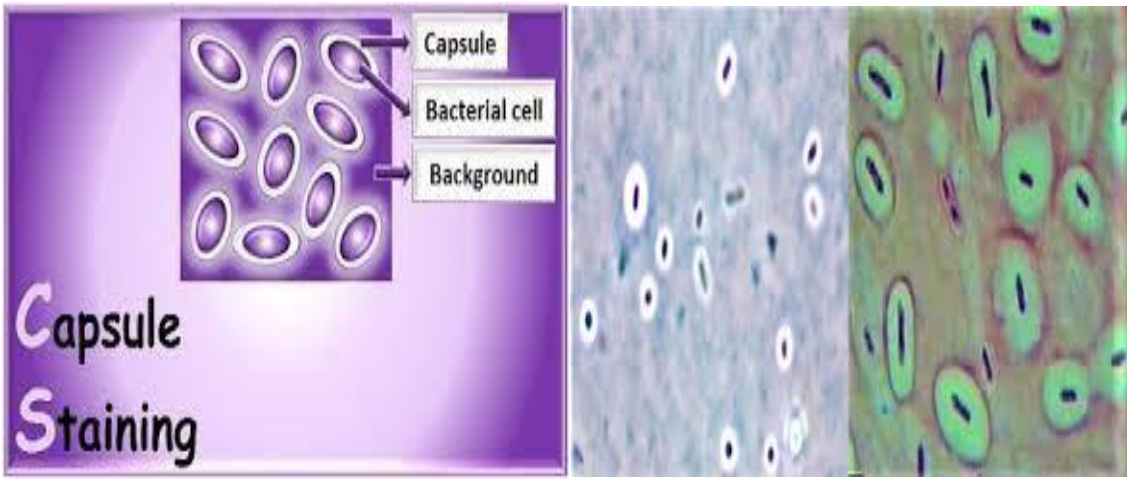
Figure 11.2 Capsule Staining Procedure.



(a) Flood the slide with crystal violet; let stand 4–7 minutes

(b) Rinse thoroughly with copper sulfate

(c) Blot dry with bibulous paper



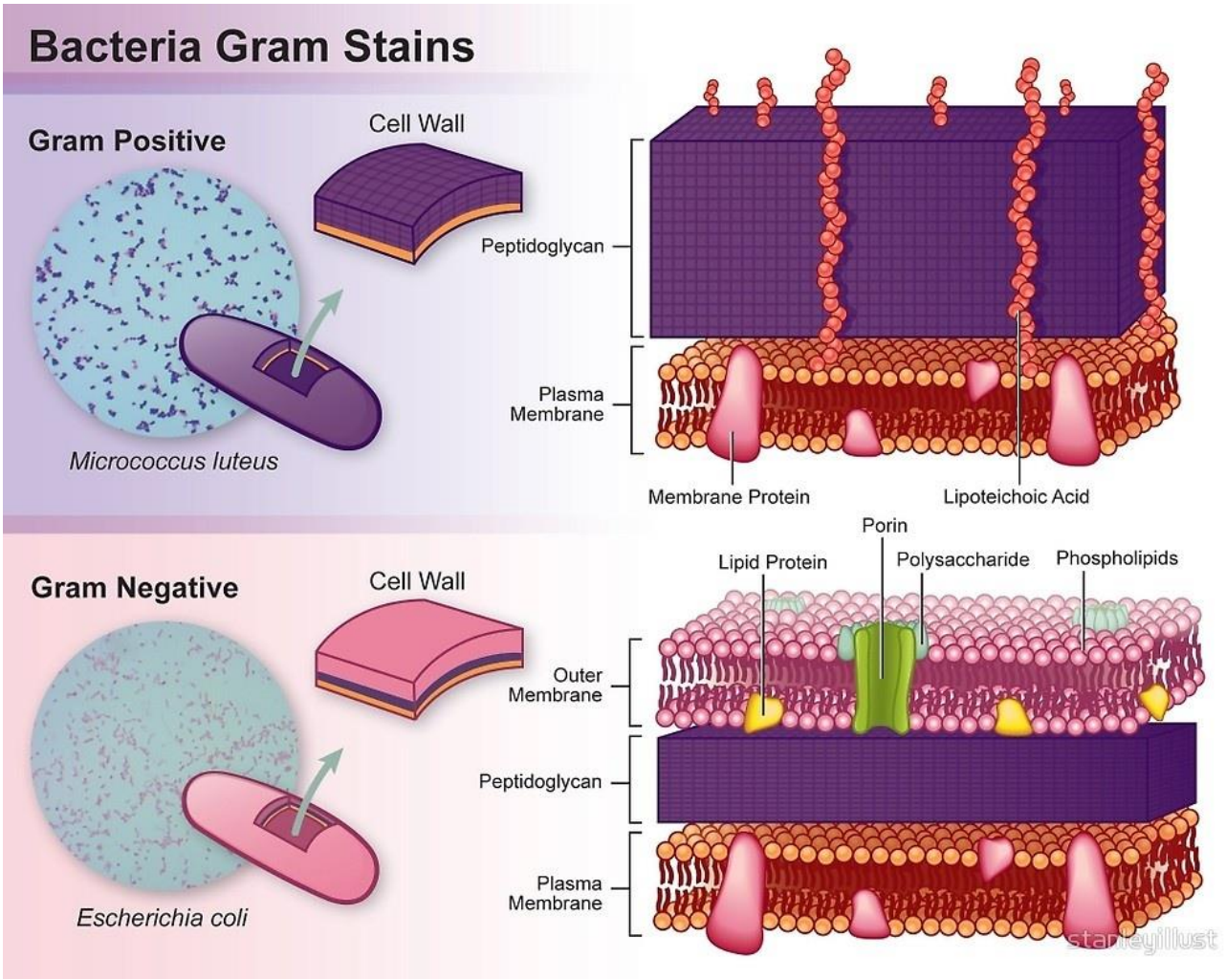


Figure (5):Comparison of the structures of gram-positive and gram-negative cell envelopes. The region between the cytoplasmic membrane and the outer membrane of the gram-negative envelope is called the periplasmic space