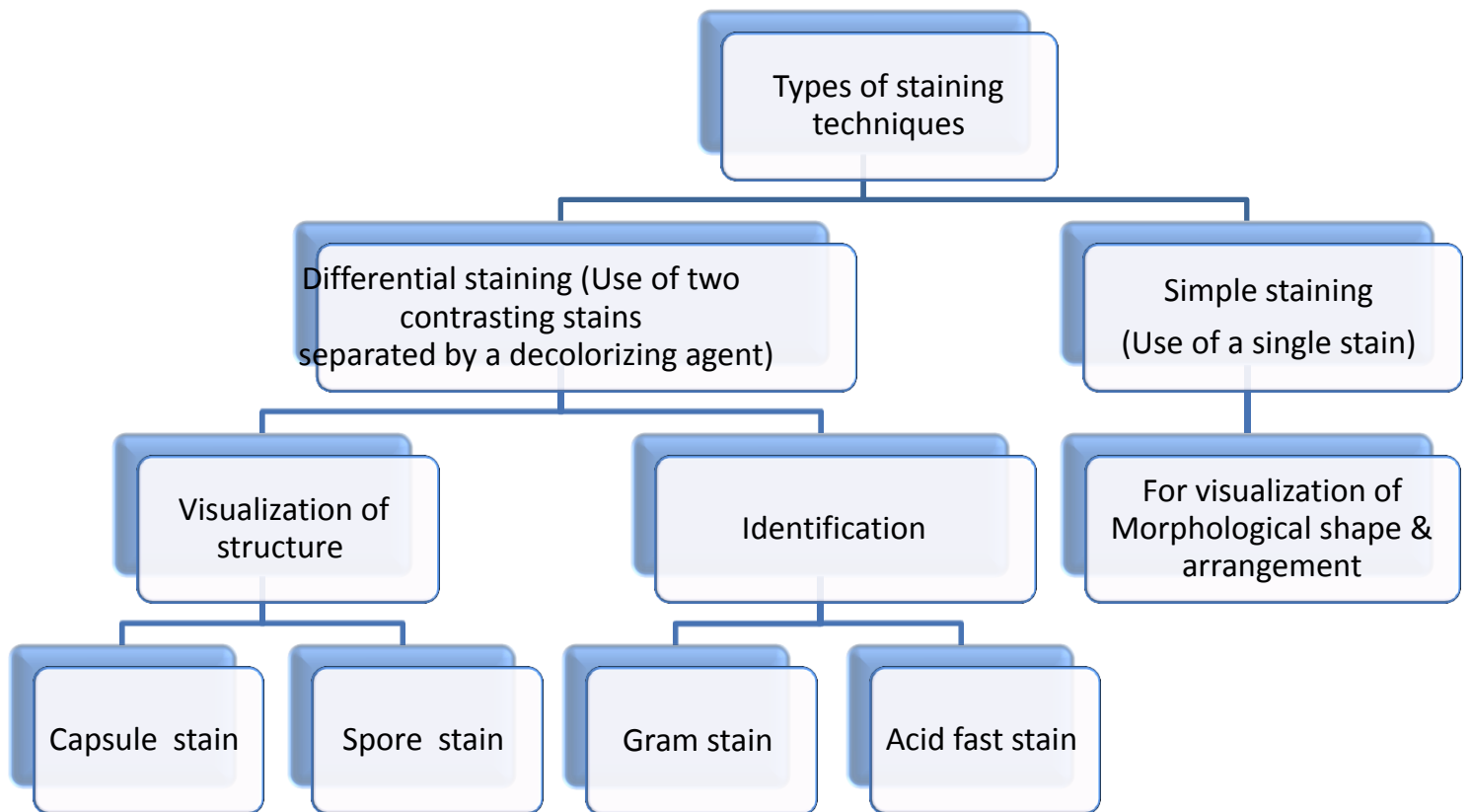


## Types of staining techniques



## Bacterial Fixed Smears and Simple Stains

Morphological stains color bacterial cells or their background to provide information about cell size, shapes, and arrangement. Unstained cells are practically transparent or colorless and lack contrast with the background, so bacteria are stained to make them more visible and to provide contrast between the bacteria and the surrounding medium.

The chemical substances commonly used to stain bacteria are called dyes. The dyes are in solutions called stains and are either acidic or basic. The acidic dyes stain the cytoplasmic components of cells that are alkaline in nature. Acidic stains carry a negative charge and because they are repelled by a like charge, they color the background surrounding negatively charged bacterial cells so the cells can be seen in outline. The presence of a negative charge on the bacterial surface acts to repel most acidic stains and thus prevent their penetration into the cell. Acidic stains are Congo red, nigrosin, and India ink. The basic dyes, such as crystal violet, safranin, and methylene blue, combine with those cellular elements that are acidic in nature. Basic stains carry a positive charge, and they are attracted to the oppositely charged, negatively charged bacterial cells.

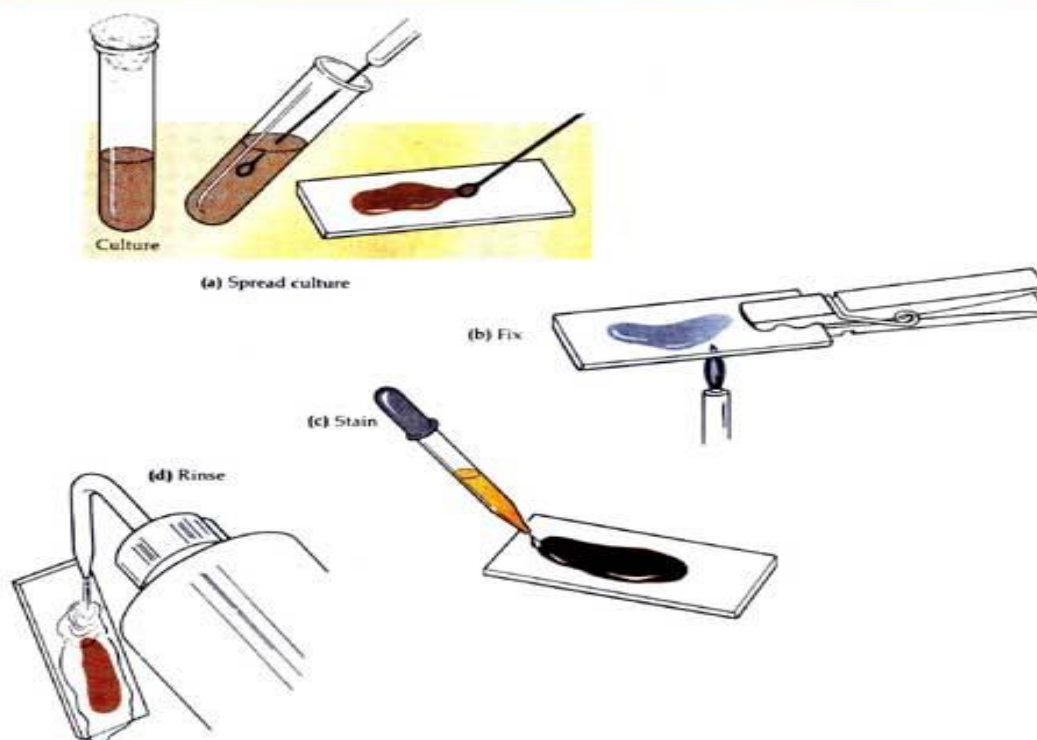
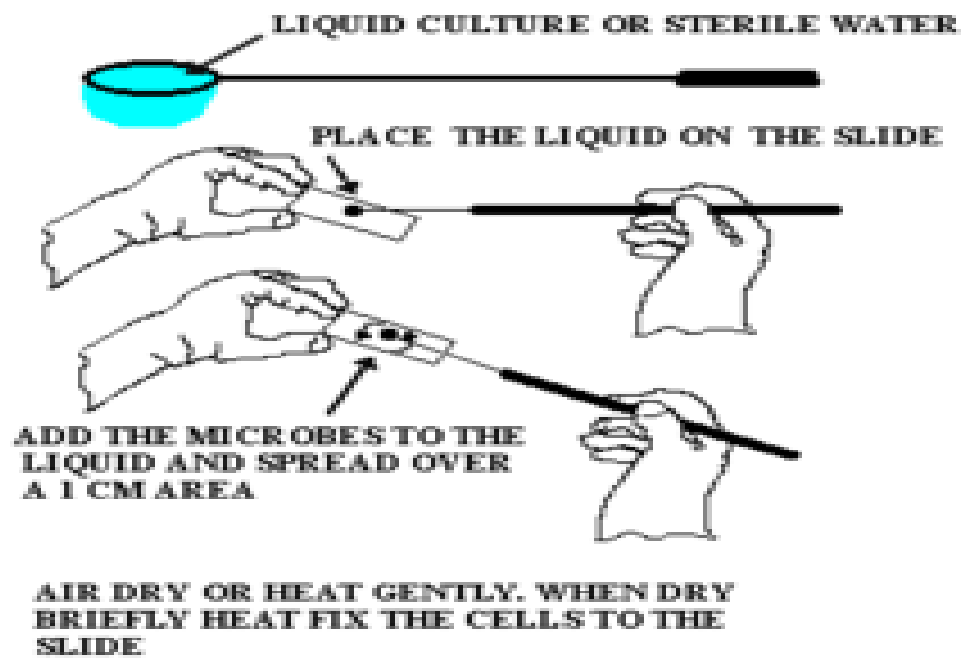
Staining procedures that use only one stain are called simple stains. Microorganisms are prepared for staining by smearing them onto a microscope slide. Bacteria are aseptically transferred from a growth on or in a culture medium to a microscope slide using an inoculating loop. Prior to staining, cells are heat-fixed to the slide. Fixation is a process of killing, immobilizing, and preserving the bacterial cells by coagulating the cytoplasmic proteins to make them more visible. In addition, the process adheres the cells to the slide so they do not wash off of the slide during staining. Basic stains are applied to bacterial smears that have been heat-fixed.

**Procedure 1:****Fixed Smear from a Solid Medium**

1. Place a drop of distilled water in the center of a clean microscope slide.
2. Using an inoculating loop, aseptically transfer a small amount of bacterial colony like *Escherichia coli* to the drop of water on the slide.
3. Using the loop, stir the bacteria into the water and spread the mixture out on the slide.
4. Allow the slide to air dry until it looks white.
5. Attach a clothespin to one end of the slide and pass it back-and-forth through the flame of the Bunsen burner until it is completely dry. Do not hold the slide in the flame, the slide will break. If the slide is not heated enough, the bacterial cells will be washed off during the staining process. If it is overheated the cells may become carbonized.






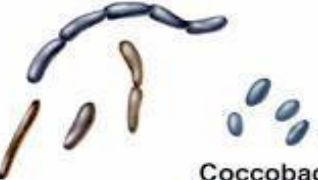











**Procedure 2:****Simple Staining**

1. Hold the side of *Escherichia coli* over a staining tray.
2. Flood the slide with either crystal violet, safranin, or methylene blue.
3. Let the stain remain on the slide 1 minute for crystal violet or safranin, 2 minutes for methylene blue.
4. Tilt the slide and let the excess stain run off into the tray.
5. Using a bottle of distilled water, gently rinse the slide until the droppings are clear.
6. Blot the slide with bibulous paper. Do not rub the slide.
7. Observe the slide under the microscope using oil immersion.
8. Dispose of the slide in a beaker of bleach.

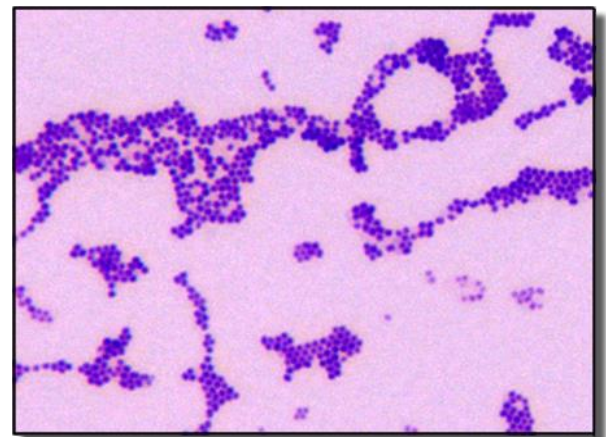
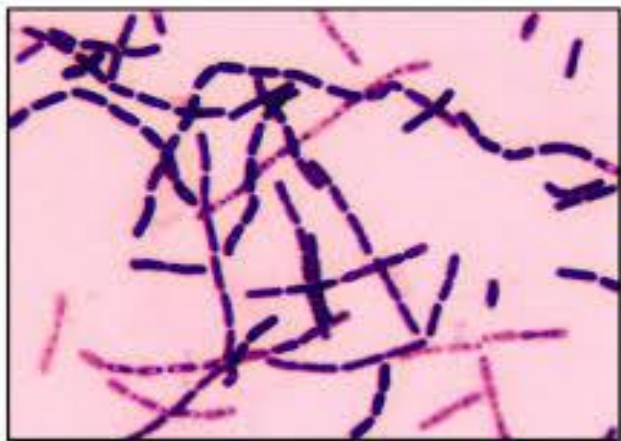


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# Bacterial shapes and arrangements

 Coccus		 Rod, or Bacillus		 Curved forms: Spirillum/Spirochete
 Diplococci (cocci in pairs)	 Neisseriae (coffee-bean shape in pairs)	 Coccobacilli		 Vibrios (curved rods)
 Tetrads (cocci in packets of 4)	 Sarcinae (cocci in packets of 8, 16, 32 cells)	 Mycobacteria	 Corynebacteria (palisades arrangement)	 Spirilla
 Streptococci (cocci in chains)	 Micrococci and staphylococci (large cocci in irregular clusters)	 Spore-forming rods	 Streptomycetes (moldlike, filamentous bacteria)	 Cocci (Spherical bacteria)

**Bacillus**  
(Rod-shaped bacteria)



Gram Positive Cocci

