Practical Microbiology

Cihan University
Medical Laboratory Analysis

Lab 6: Inspection (Microscopic examination: wet mount, smear preparation & simple staining)

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MSc: Microbiology

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Objective

➢Each student should apply direct microscopic examinations to:

✓Wet mount motility test.

✓Prepare microbial (bacterial) film or smear.

✓Simple staining (basic & acidic stains).

✓Determine shape, size and arrangement of microbial cells.

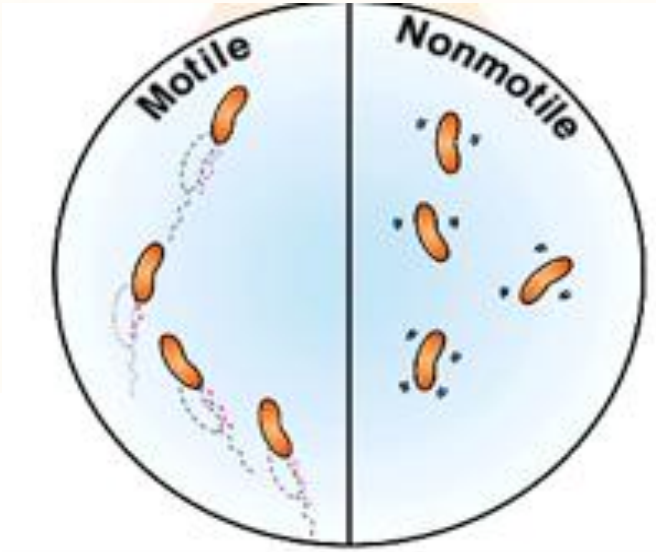
Microscopic examinations

- There are two principal ways of preparing a microbial specimen for observation with light microscope:
- ✓ **Unstained smears (wet preparation):** to examine the motility.
- ✓ **Stained smears:** to study the size, shape, arrangement and staining affinity of the microbes.

Motility (wet mount)

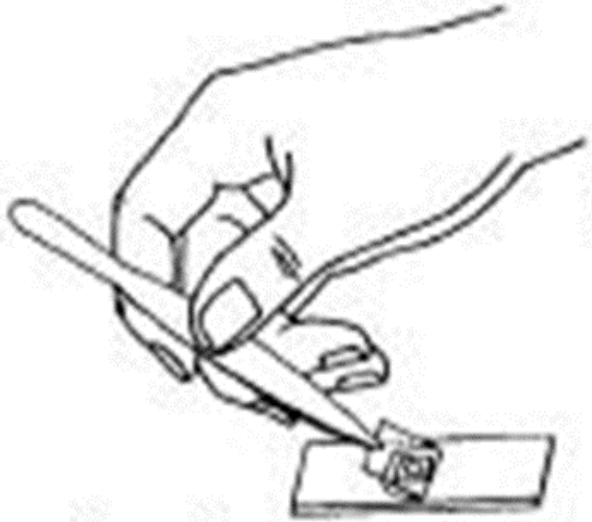
- Many microbes show no motion and are termed nonmotile.
- However, in an aqueous environment, these same microbes appear to be moving erratically (**Brownian movement).**
- Microbial true motility are regular and directional such as forward motion.
- **Procedure:**
- Place a few loopful of the microbe from a broth culture or colony (with a drop of water) from an agar plate on a clean slide.
- With 45 angle, place the edge of a cover slide on the slide so that it touches the edge of liquid.
- Slowly lower down the cover to prevent air bubbles formation.
- Examine from low to high power.
- In addition to being able to determine the presence or absence of **motility**, this method is useful in determining **cellular shape and arrangement.**

Wet mount



Add a drop of water to a slide.

Place the specimen in the water.



Place the edge of a coverslip on the slide so that it touches the edge of the water.

Slowly lower the coverslip to prevent forming and trapping air bubble

Smear & preparation procedure

➢**Smear:** is a thin microbial film over the surface of a slide.

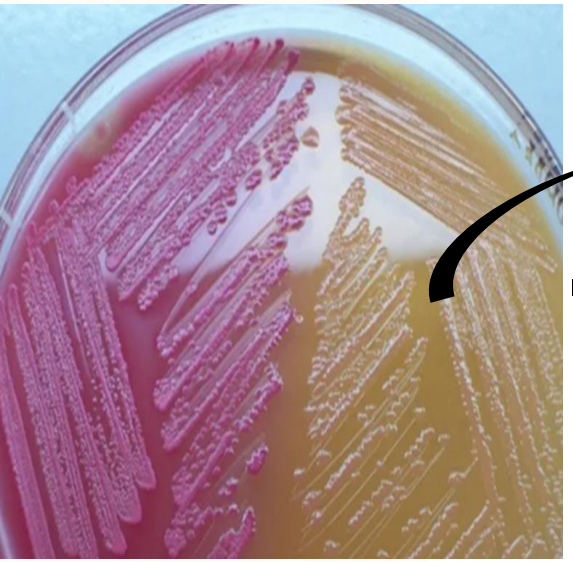
✓If the microbes (bacteria) are growing in a liquid medium (broths, milk, saliva, urine, etc.), one starts by placing one or two loopful or drops of the liquid medium directly on the slide use **inoculating loop** to disperse the organisms.

✓From solid media such as nutrient agar, blood agar, stool, etc. one starts by placing one or two drops of water on the slide and then uses a **inoculating needle** to transfer and disperse the small colony in the water.

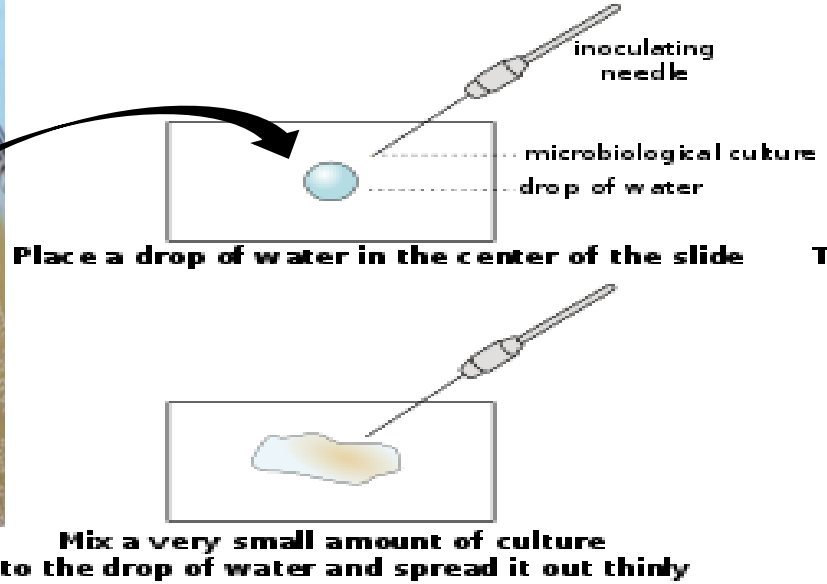
✓Spread the microbes on large area on the slide that they are adequately separated from one another (create milky suspension).

✓Cell fixed to the slide by slowly passing the slide several times over the flame (heat fixing) to prevent washing of the microbes during staining process.

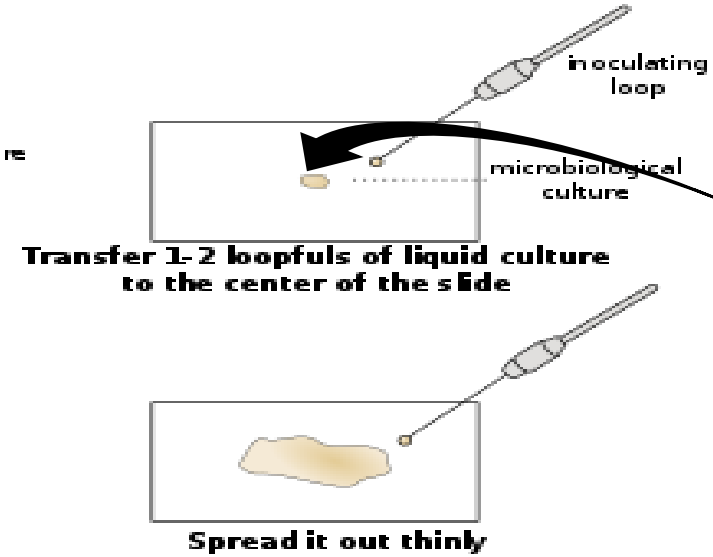
✓Or fix by chemical fixation: Cover with methanol for 1 minute.

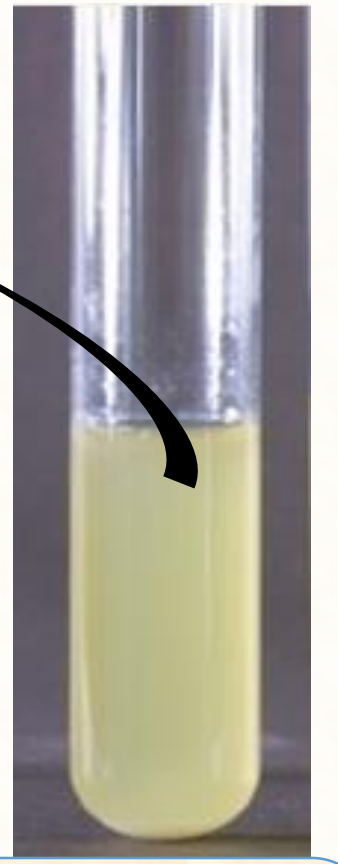


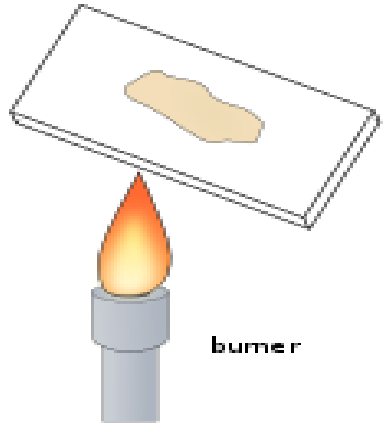
Smear from solid medium



Smear from liquid medium







From solid media, take only a very small amount of colony to make a good smear.

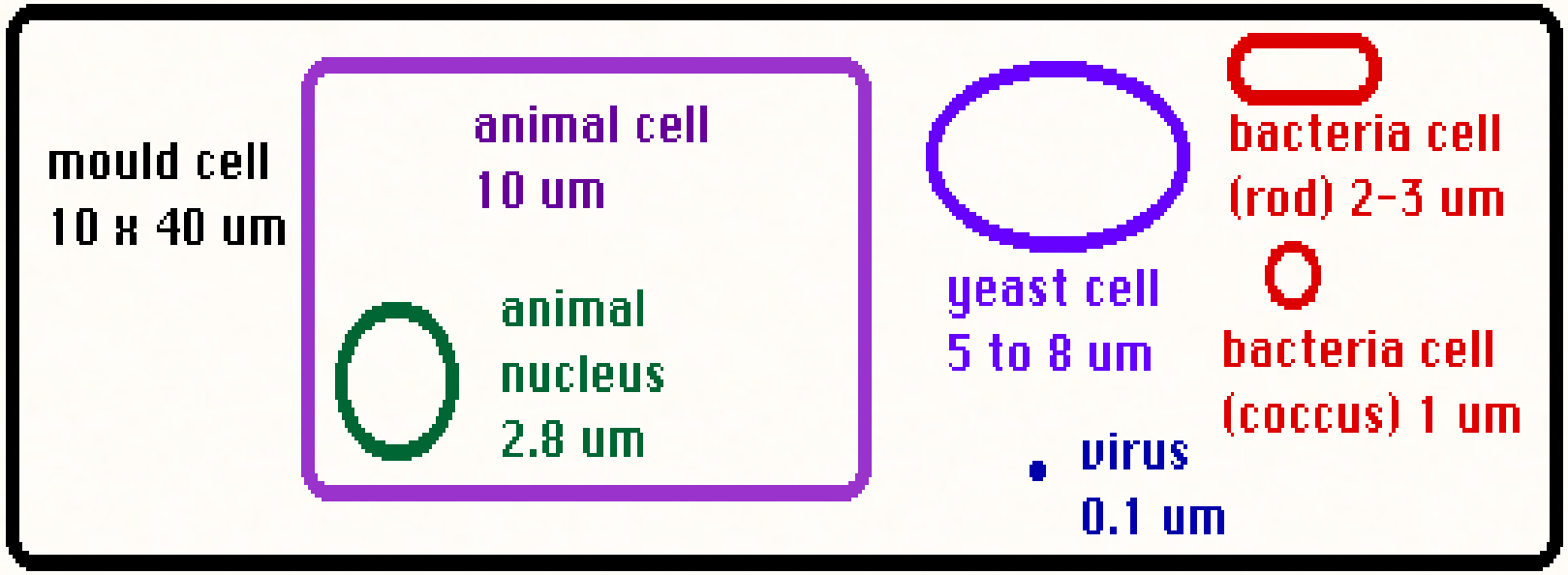
Avoid standing the slide over the flame as heating could melt the microbial cells and coagulate proteins.

Simple stain

- In his technique only one stain is used.
- Used to study **size**, **shape** and **arrangement** of microbes.

Size

- Generally microbes cell size range from 0.1-10 µm in diameter & 2-30 µm in length.



Shapes

➢ **There are different shapes could be seen under the light microscope:**

✓Cocci (coccus): spherical or round shape. *Staphylococcus, Streptococcus*

✓Bacilli (bacillus) rod shape. *Bacillus, Clostridium,* Enterobacteriaceae

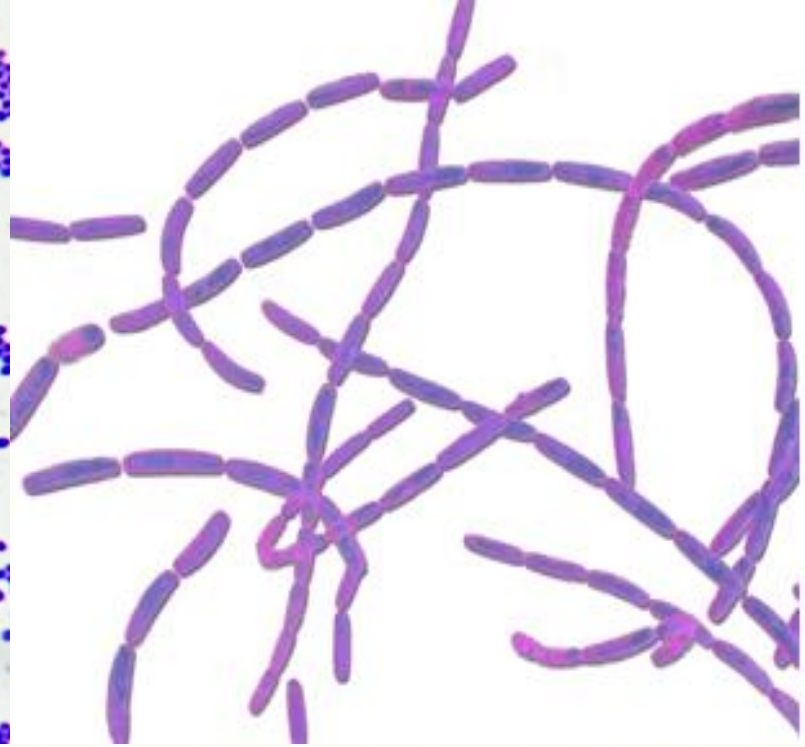
✓Coccobacilli: slightly elongated cocci or oval shape. *Yersinia*

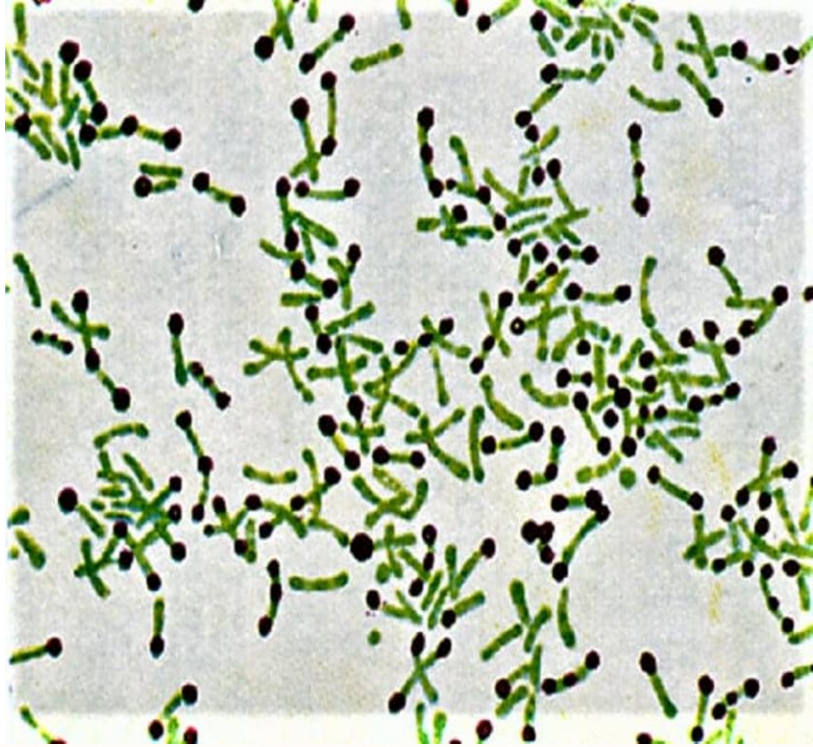
✓Curved bacilli: rod shape but curved. *Vibrio cholera*

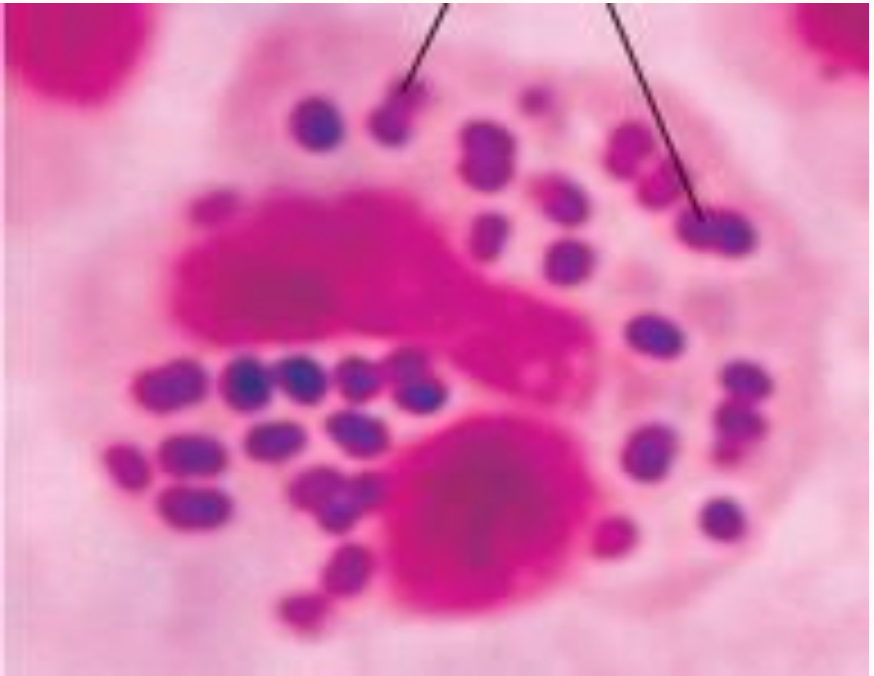
✓Spiral: helical shape. *Helicobacter pylori*

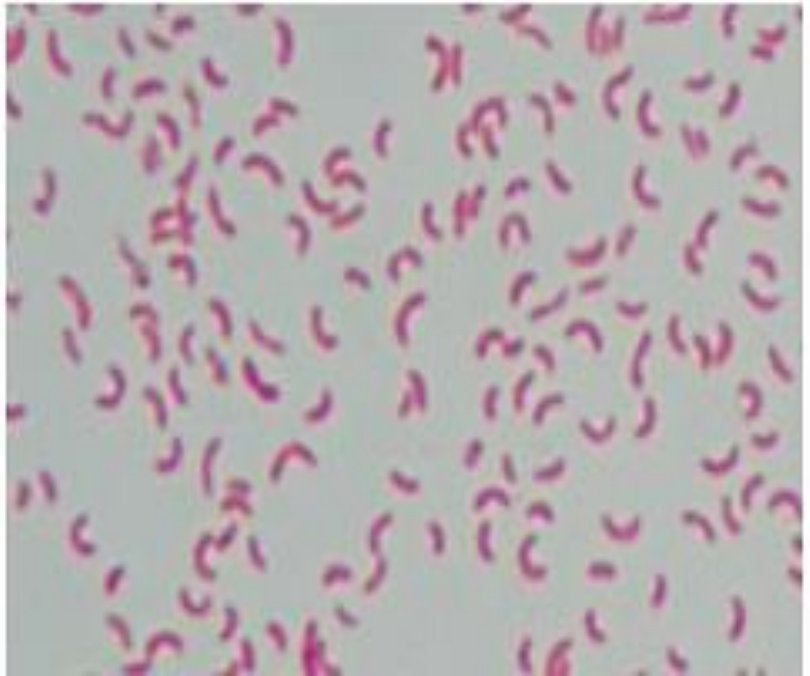
✓Club shape: coryneform. *Corynebacterium diphtheriae*

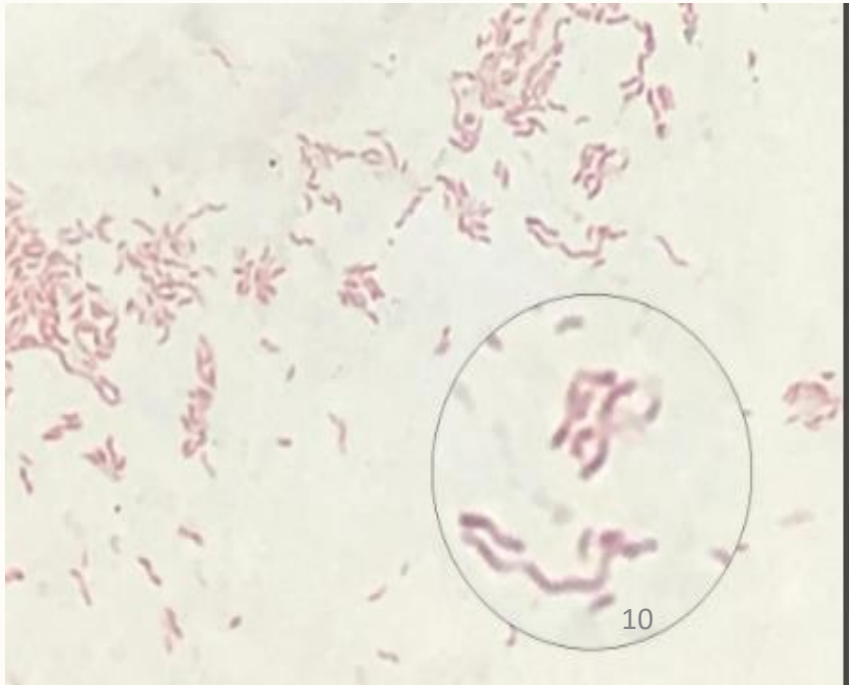
✓Branched or filamentous: branched structures like fungal hyphae. *Actinomycetes*











Arrangement

- According to their patterns of division; the cells may be stay together or separated, this produces characteristics of cell arrangement:

✓Diplococci or diplobacilli: two cells attached together.

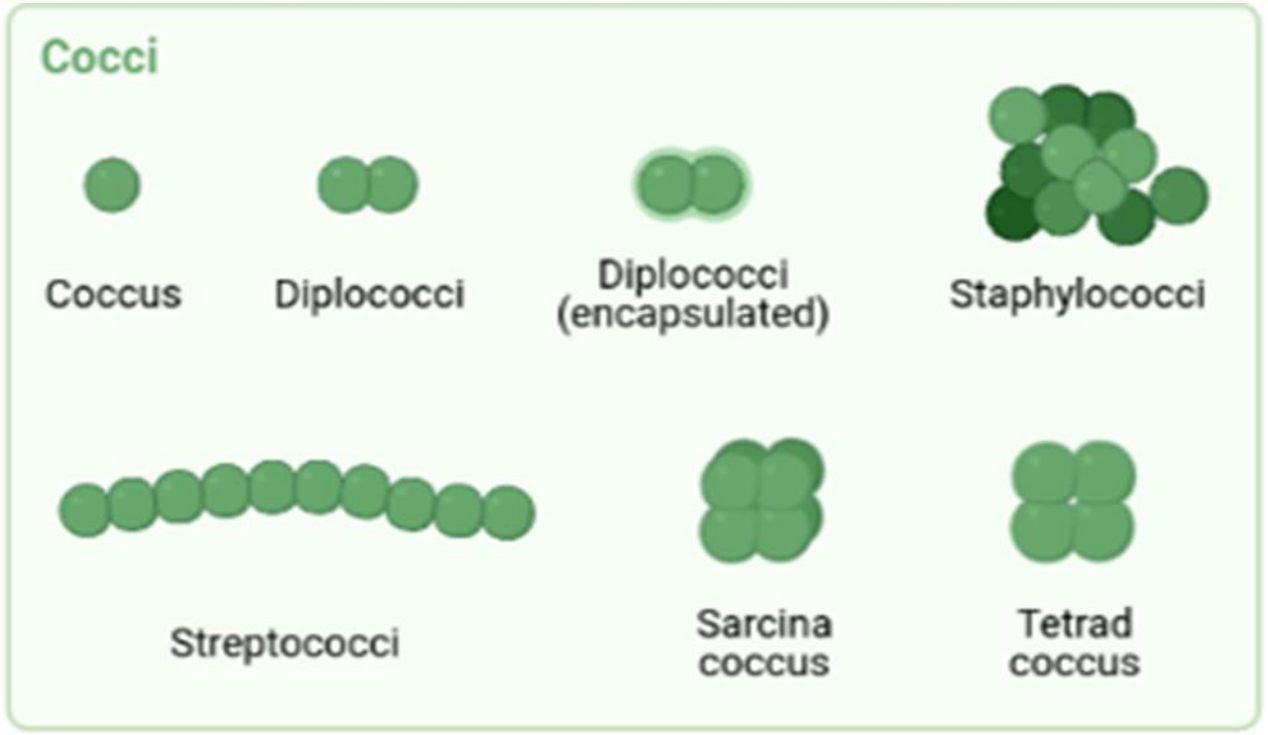
✓Tetrad cocci: four cocci cells.

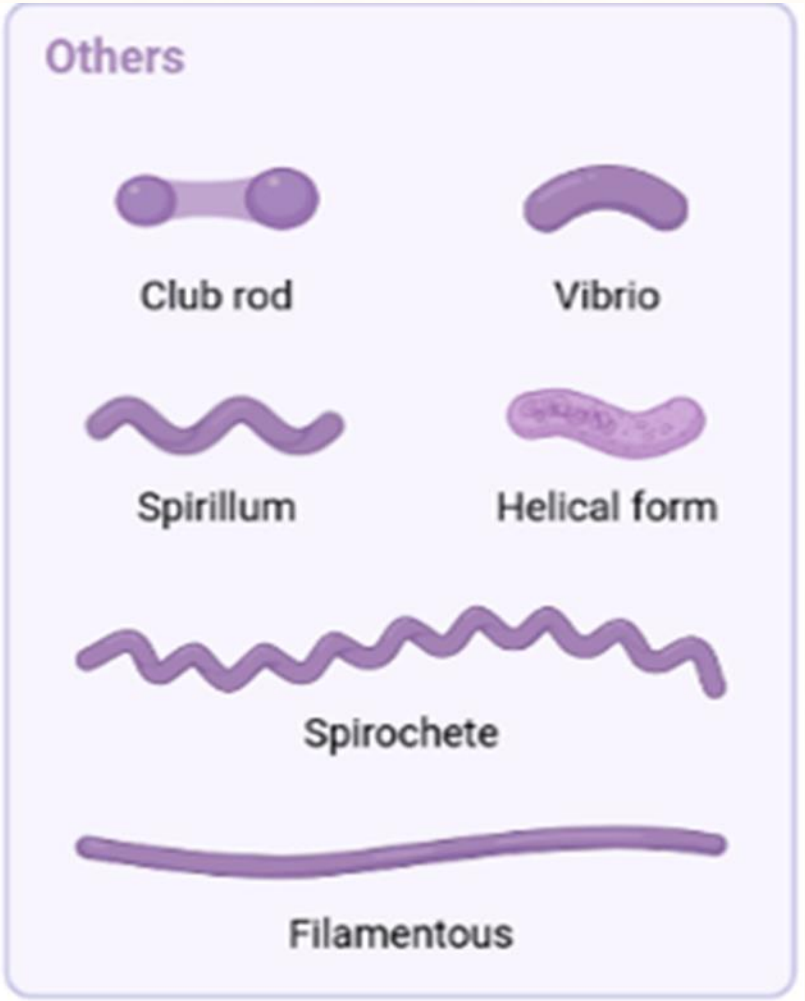
✓Streptococci: chained cocci.

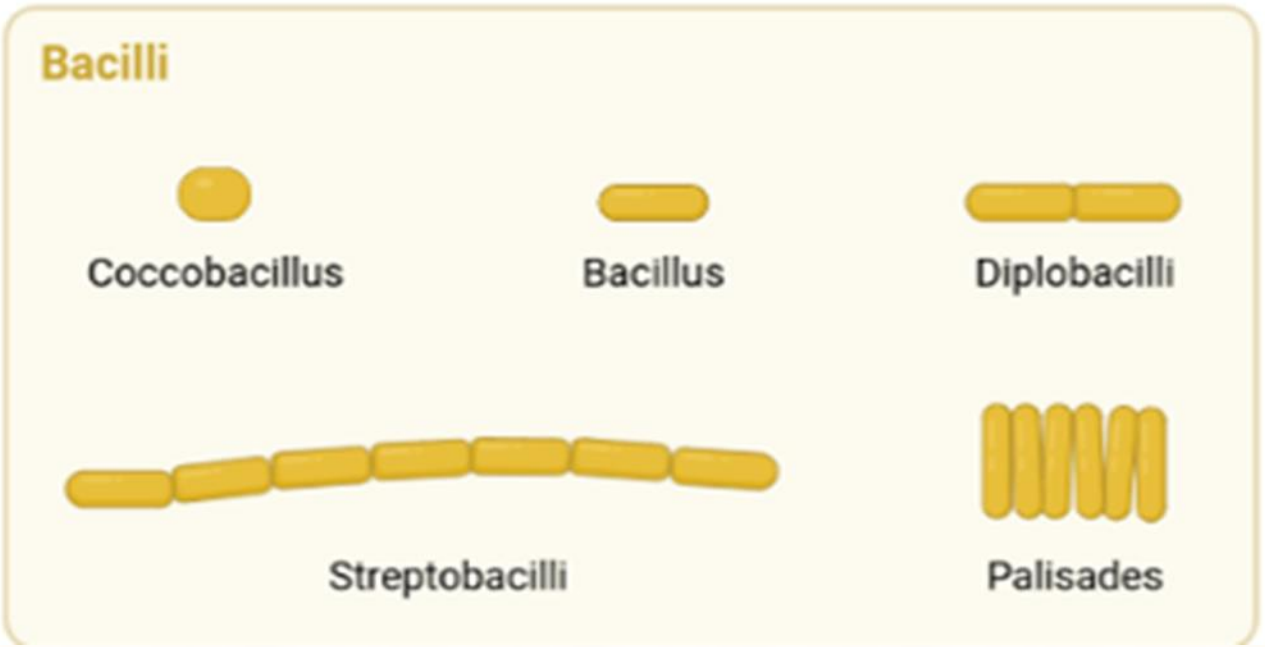
✓Staphylococci: clustered or grape of cocci.

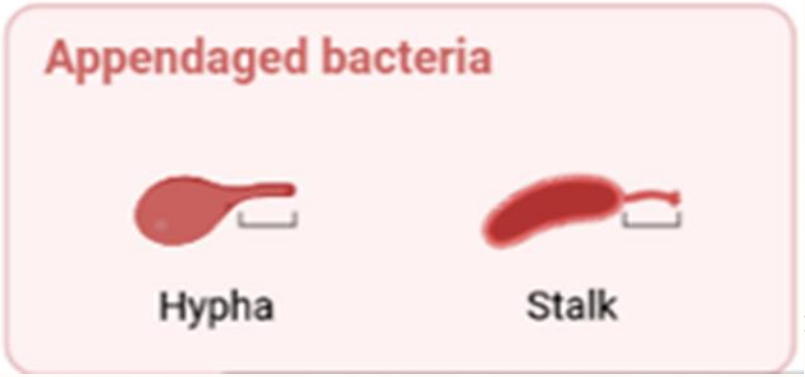
✓Streptobacilli: chained bacilli.

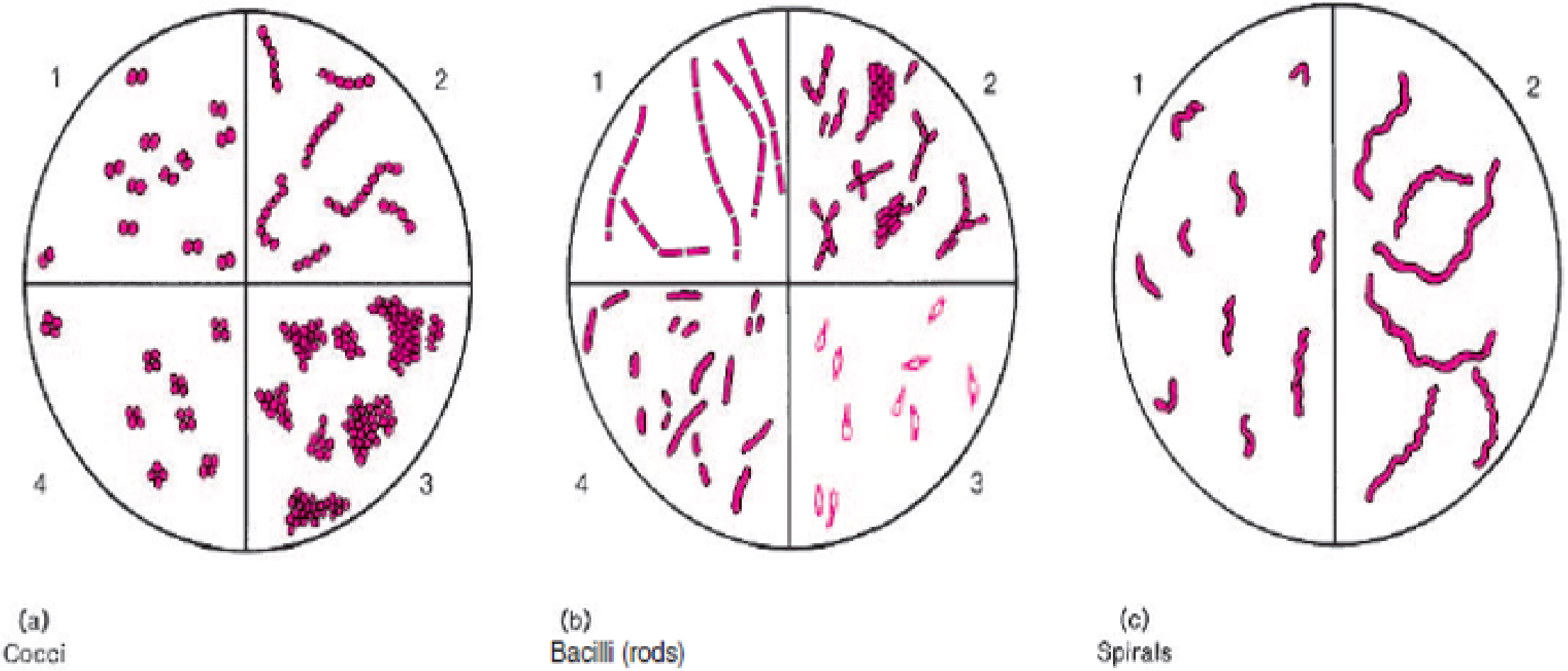
✓Palisade arrangement: cells attached together laterally.


Cocci
Coccus
Diplococci
Diplococci (encapsulated)
Staphylococci
Streptococci
Sarcina coccus
Tetrad coccus


Others
Club rod
Vibrio
Spirillum
Helical form
Spirochete
Filamentous

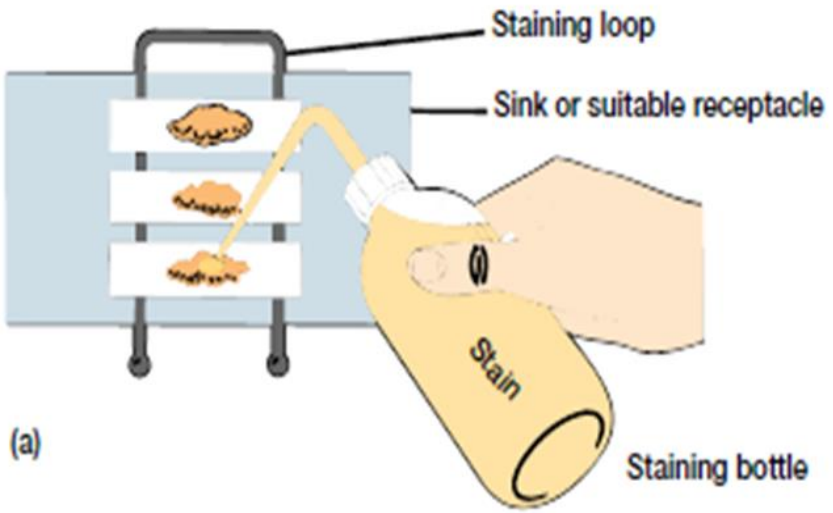

Bacilli
Coccobacillus
Bacillus
Diplobacilli
Streptobacilli
Palisades

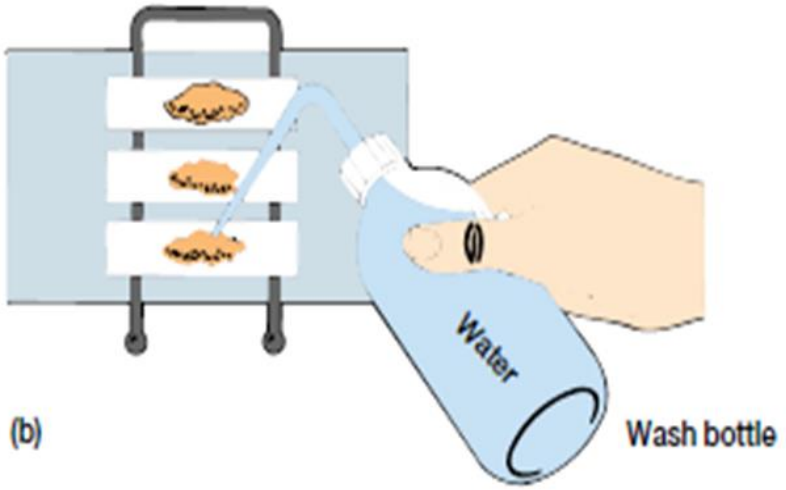

Appendaged bacteria
Hypha
Stalk

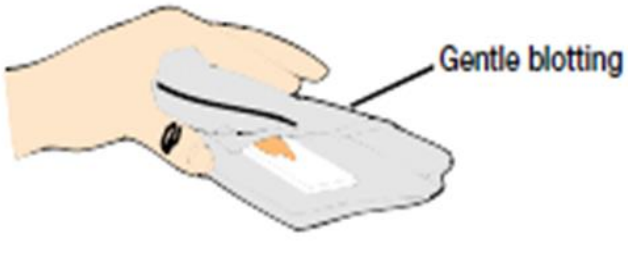


1. diplococci. 2. Streptococci (chains); 3. Staphylococci (grapelike clusters); 4. Tetrads (packets of four). (b) Bacilli (rods). 1. Streptobacilli (chains); 2. Palisades; V, X, and Y figures, clubbing; 3. Endospore-forming bacilli (note endospores as small, round, hollow, unstained areas, within or at one end of bacillary bodies); 4. A bacillus showing pleomorphism (note varying widths and lengths). (c) Spirals or Spirilla (short curved or spiraled forms with rigid bodies).

Shape		Arrangement	
Spherical	coccus (pl., cocci)	diplococcus (pairs)	
		streptococcus (chains)	
		staphylococcus (random or grapelike clusters)	
		micrococcus (square groups of four cells)	
Rod-shaped	bacillus (pl., bacilli)	streptobacillus (chains)	
Spiral	spirillum (pl., spirilla)	sarcina (cubical packets of eight cells)	
Incomplete spiral	vibrio (pl., vibrios)		
Irregular or variable shape	pleomorphic		





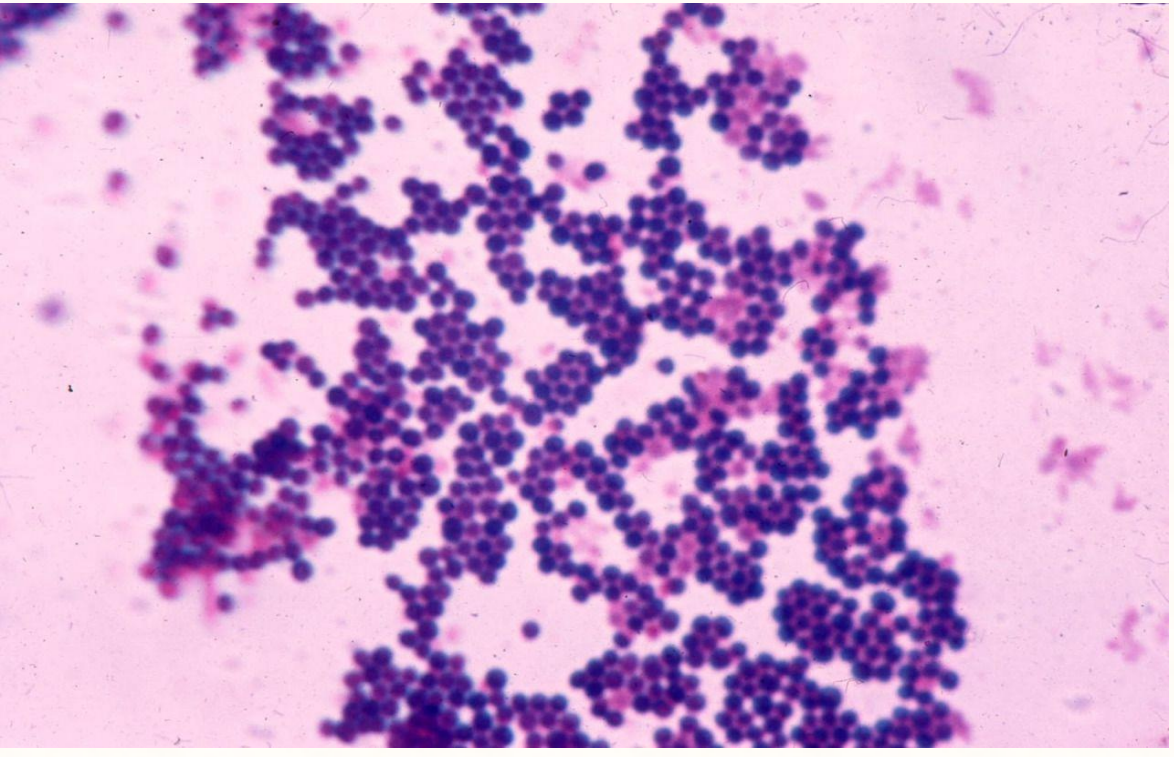


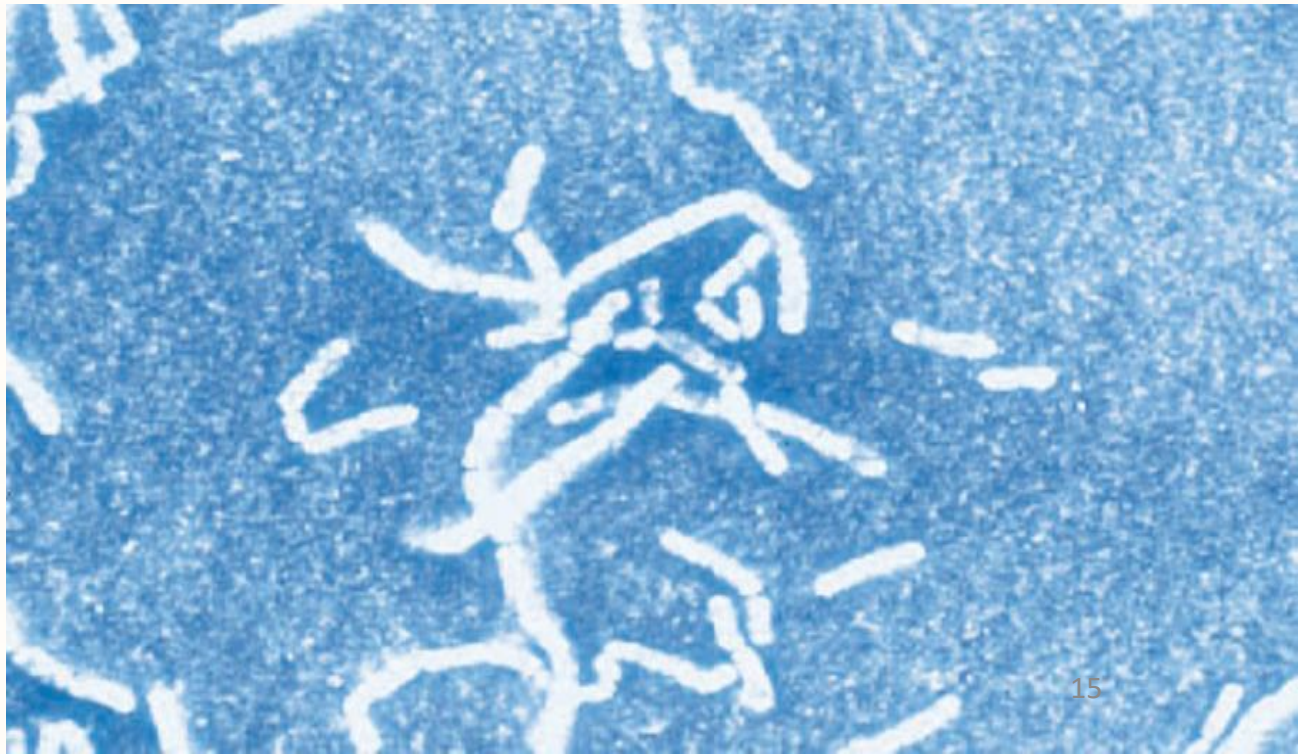
Types of stain

There are two types of stain based on their charged particles:

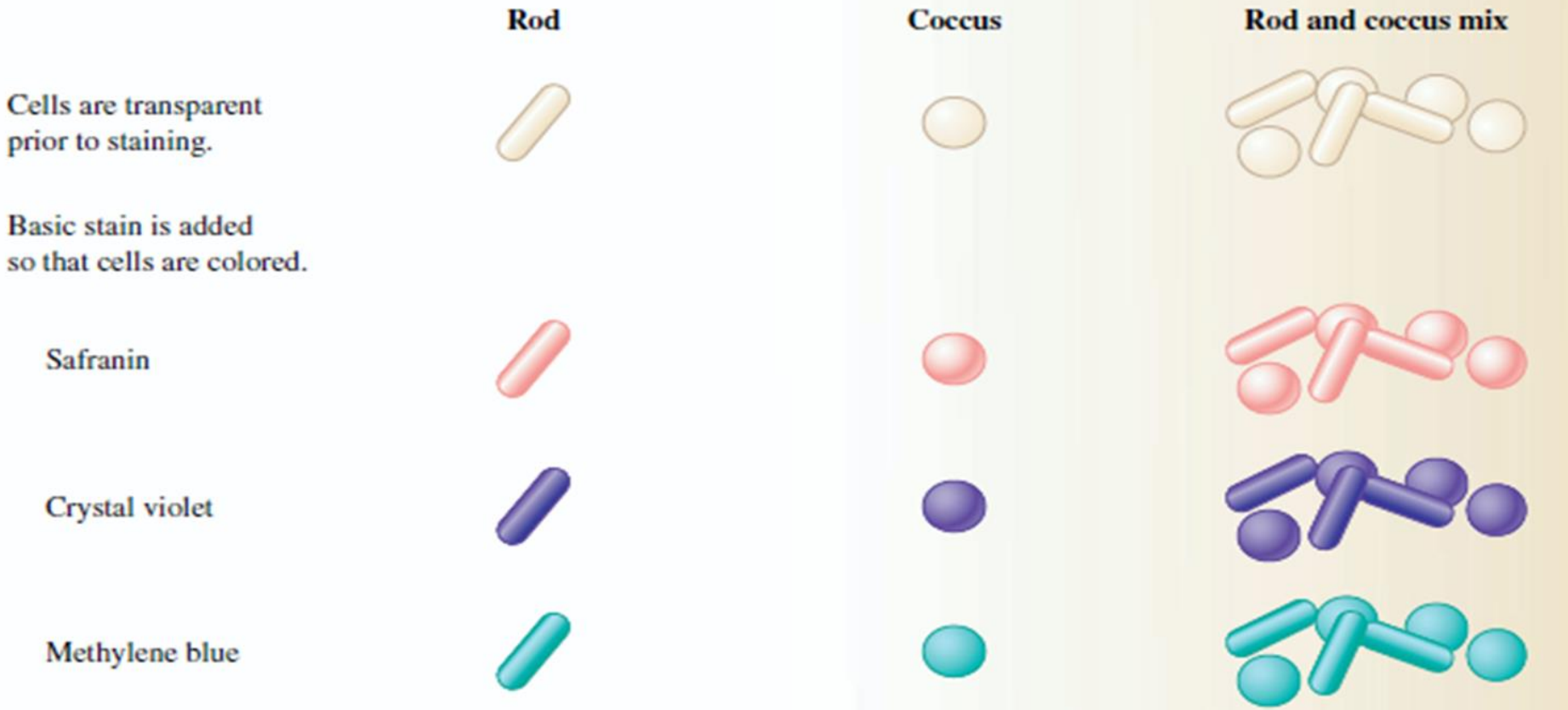
1) Basic stains: carry positive charge which color bacterial cells because they are attracted to the negatively charged cell surface (bacteria). Basic stains include crystal violet, methylene blue, and sufranin.

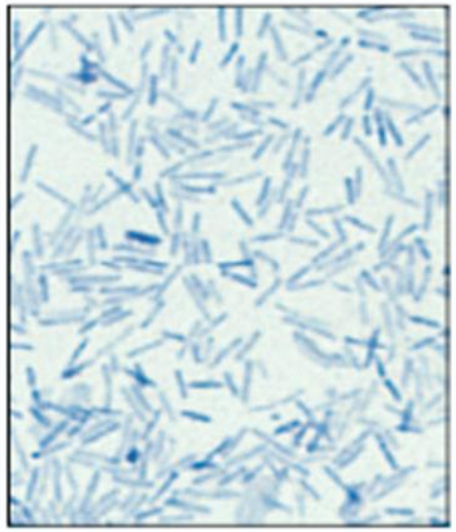
2) Acidic stain: carry negative charge that color the background surrounding bacterial cells. Acidic stains are repelled by the negatively charged bacterial surface, color only the background. Acidic stains include Congo red, nigrosin, and India ink.

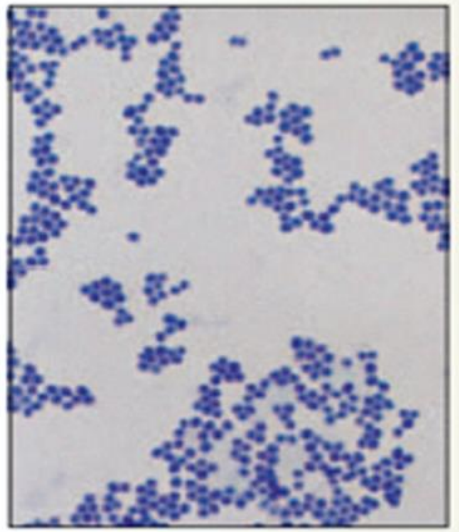


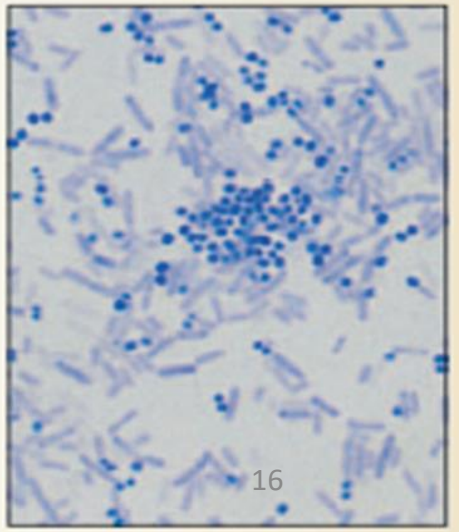


Basic stain

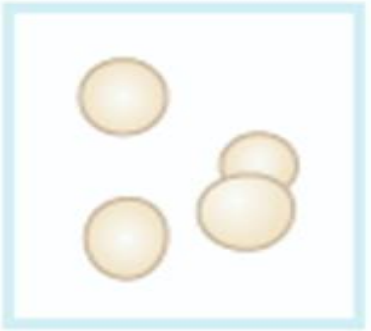
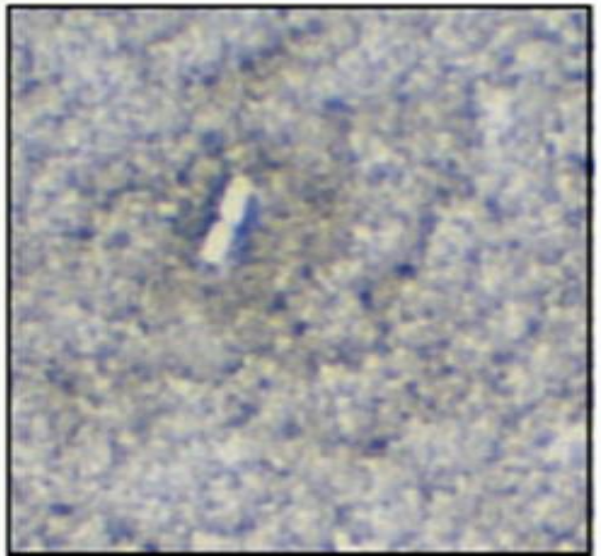
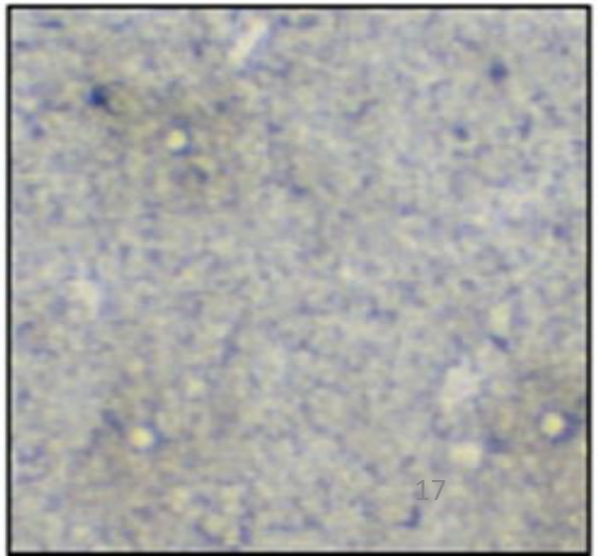

Rod
Coccus
Rod and coccus mix
Cells are transparent prior to staining.
Basic stain is added so that cells are colored.
Safranin
Crystal violet
Methylene blue







Acidic stain

	Rod	Coccus
Cells and background are colorless.		
Acidic stain colors the background; cells remain colorless.		
		

Staining of fixed smear

➢ **Procedure:**

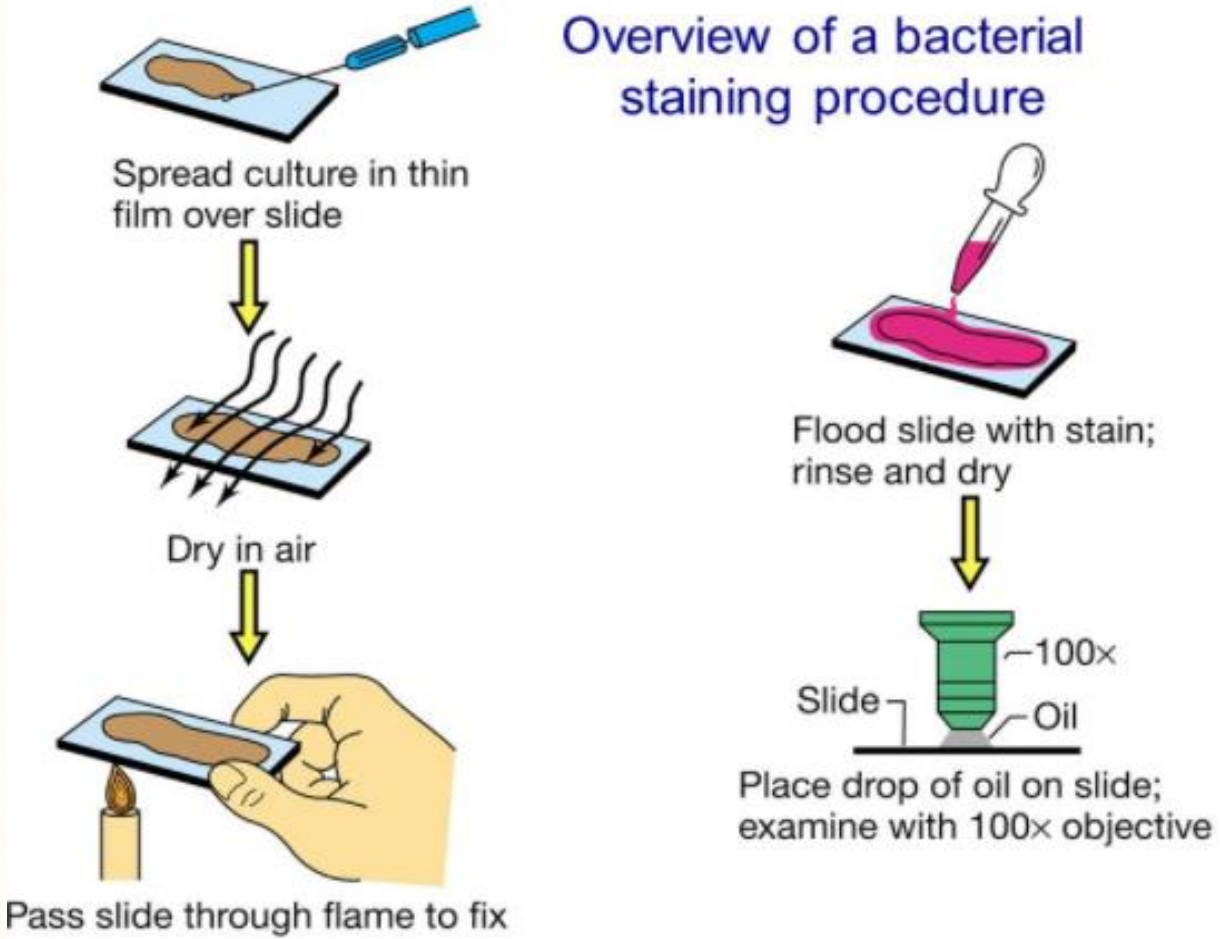
✓First, microbial smear is prepared and fixed.

✓Next, a few drops of stain applied directly to the fixed smear for 1-2 min.

✓Then, the slide rinsed off with slow running water to remove extra stain.

✓After that, let the smear to air dry.

✓Finally, examined under the microscope from 4X to 100X power (oil immersion).



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