

Stains classified according to staining techniques :

1. Simple stains
2. Differential stains
3. Special stains

1. Simple stain:

It is the using of ONE STAIN (Basic stain) only to stain microorganisms , so M.O that present in the slide appears in ONE COLOR.

It used to get information about bacterial size, shape (cocci, bacilli, cocco-bacilli) and arrangement (single, pairs, clusters or chains).

2. Differential stain:

- This type of stain used to differentiate between two organism.
- The mainly used differential stain is GRAM STAIN.
- Gram positive(+ ve):retain to violet color of primary stain (crystal violet stain) .
- Gram negative(-ve): those that can't retain primary stain when the slide washed by alcohol but then take the red color of (safranine).

3. Special stain:

This procedure is used when a certain bacteria or some of their structures could not be stained.

Table illustrate Basic, Acidic, and Neutral stains :

Basic (+Ve Charge) Stains	Acidic (-Ve Charge) Stains	Neutral Stains
• Methylene blue • Crystal violet • Malachite green • Basic fuchsin, • Carbol-fuchsin • Safranin. • Neutral red	• Nigrosin • Acid fuchsin • India ink	• Giemsa • Leishman • Wright

Special stain include:

- A. Acid fast stain (Zeihl Neelsen stain)**
- B. Capsular stain**
- C. Spore stain**
- D. Flagellar stain**

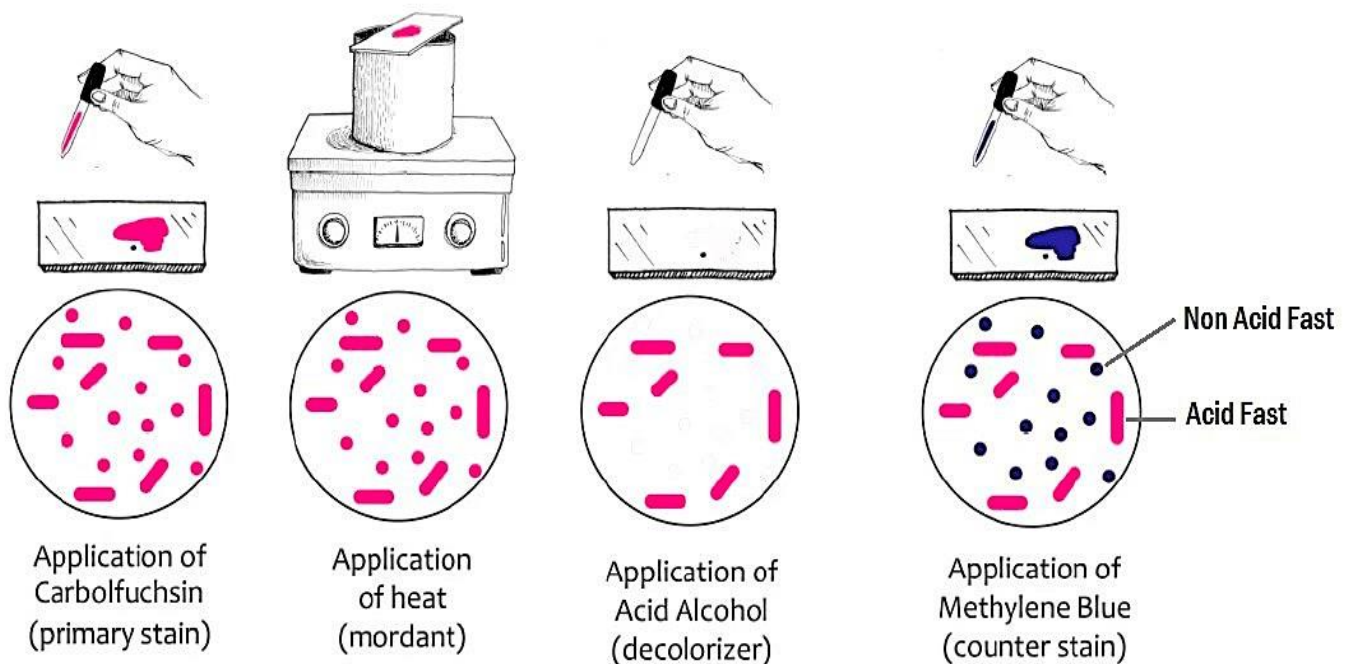
A. Acid fast stain (Zeihl Neelsen stain) :

- This technique is used for staining of *Mycobacterium tuberculosis* & *Mycobacterium leprae* (the causative agents of tuberculosis and leprosy respectively) .

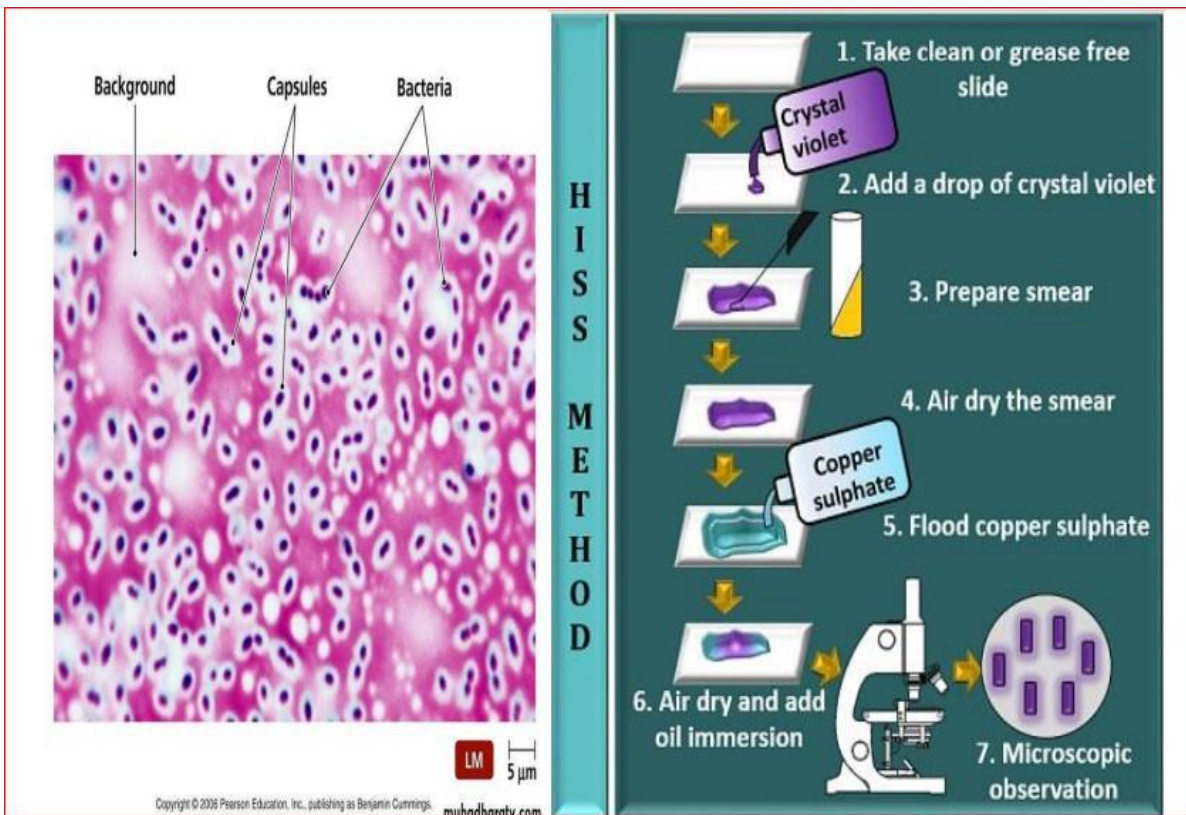
- The above bacteria possess a complex lipid (wax) in their cell wall which is called mycolic acid.
- Wax or mycolic acid which mainly gives the bacteria resistance to be stained by ordinary stains.

Ziehl-Neelsen Stain Principle, Procedure, Reporting and Mod

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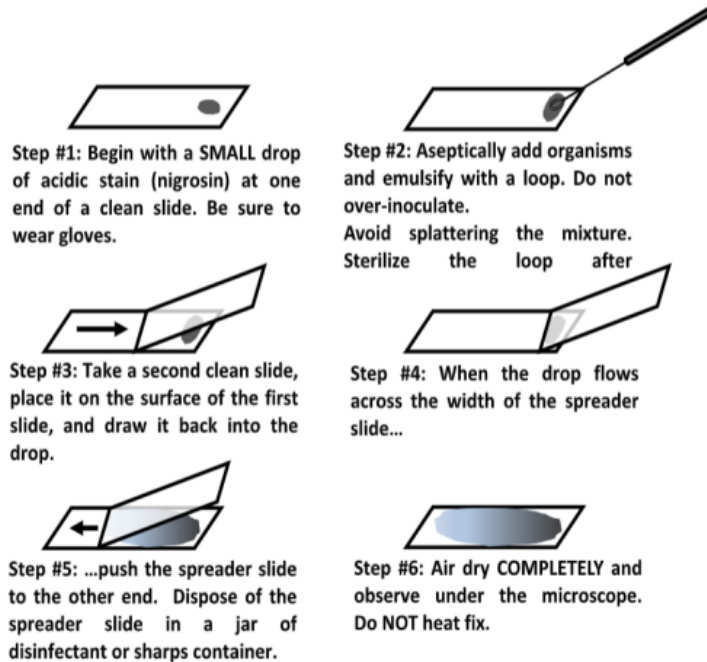
B. Capsular stain : Capsule is a gelatinous ,non ionic structure, spherical or oval shape surrounding certain of bacteria as protective extracellular structure, protect the cell from phagocytosis by phagocytic.



Negative stain India ink, Nigrosin stain

- Used to visualize MO that not easily stained e.g. cryptococcus neoformans or visualize capsule of encapsulated bacteria (eg **streptococcal pneumonia**).
- Negative stain repelled by negatively charged bacteria cell wall so only background will be stained

Negative stain procedure



Step #1: Begin with a SMALL drop of acidic stain (nigrosin) at one end of a clean slide. Be sure to wear gloves.

Step #2: Aseptically add organisms and emulsify with a loop. Do not over-inoculate. Avoid splattering the mixture. Sterilize the loop after.

Step #3: Take a second clean slide, place it on the surface of the first slide, and draw it back into the drop.

Step #4: When the drop flows across the width of the spreader slide...

Step #5: ...push the spreader slide to the other end. Dispose of the spreader slide in a jar of disinfectant or sharps container.

Step #6: Air dry COMPLETELY and observe under the microscope. Do NOT heat fix.

C- Spore staining :

Endospores position within the cell is characteristic. It can be either :

1. Central. 2. Subterminal. 3. Terminal

Genera ***Bacillus & Clostridia*** consist of keratinized protein.

The above layers give the spore protection against several hard environmental conditions, at the same time, become difficult to stain the spore with the ordinary staining techniques; therefore its staining requires a special procedure such as steaming in order to allow the stain to penetrate into the spore.

