

Acid-fast staining technique



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Acid-fast staining



➤ Is a differential staining technique .

➤ **Acid fast bacteria:**

- ✓ Such as : ***Mycobacteria*** & ***Nocardia*** species.
- ✓ Those bacteria (ex: ***Mycobacterium tuberculosis***) in C.W contain high amount of *lipid* called ***mycolic acid***.
- ✓ High level of lipid (mycolic acid) make C.W of them impermeable to most dyes>>>> so *can't be stained by simple & gram staining technique*.
- ✓ But , require high concentration of dyes & (or) heating period.



Acid-fast staining



➤ **The cause of acid-fast expression is:**

even after addition of hydrochloric acid to alc.

(a strong decolorizing agent) they don't lose the primary stain (retain the primary stain).

➤ **Common acid fast staining methods:**

- ✓ Ziehl-Neelsen technique
- ✓ Kinyoun technique.

➤ **Ziehl-Neelsen method requires :**

- ✓ Carbol fuchsin (primary stain).
- ✓ Acid alc. mix (decolorizer).
- ✓ Methylene blue (secondary stain).



Acid-fast staining



Acid fast technique theory:

- Carbol fuchsin stain has higher affinity for lipid than in acid alc. Mix. (carbolic acid solubilizes more easily in lipids than in acid alc.)
- So, after adding acid alc. (decolorizer) then methylene blue (2ry stain):
 - ✓ Some bacterial cells retain the 1ry stain and appear in **red color** >>>> those called (**Acid-fast bacteria ex: Mycobacterium tuberculosis**).
 - ✓ Others decolorized & stained with 2ry stain so appear in **blue color** >>>> those called (**Non acid- fast bacteria**).

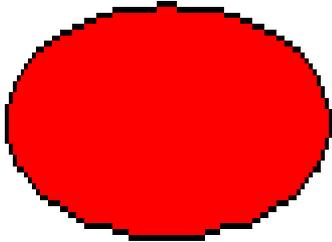


Acid-fast staining

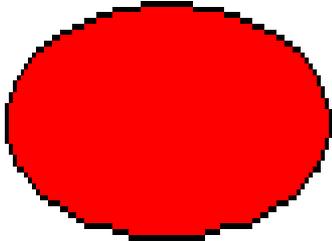
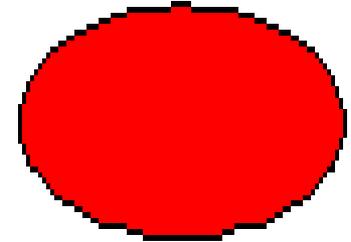


Acid Fast

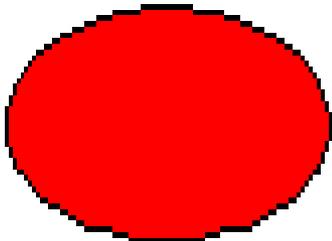
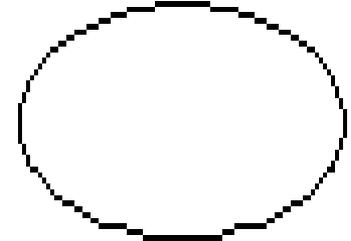
Nonacid Fast



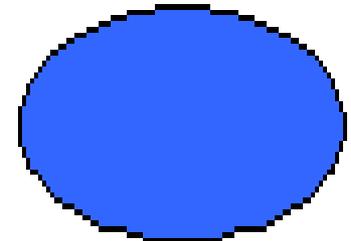
Primary Stain, Carbolfuchsin



Decolorizer, Acid Alcohol



Counter Stain, Methylene blue





Acid-fast Staining



**ACID-FAST
STAINING**



Acid-fast staining



Ziehl-Neelsen technique procedure:

- Prepare heat fixed smear.
- Flood the smear with carbol fuchsin (1ry stain) & heat for 5 min (not allow it to boil nor dry).
- Wash the slide off with water.
- Add acid-alc. Mix (decolorizer) for 1 min then wash off with water.
- Add methylene blue (2ry stain) for 1 min then wash off with water.
- Dry & add oil dps then examine under oil immersion lens.



Acid-fast Staining



Results



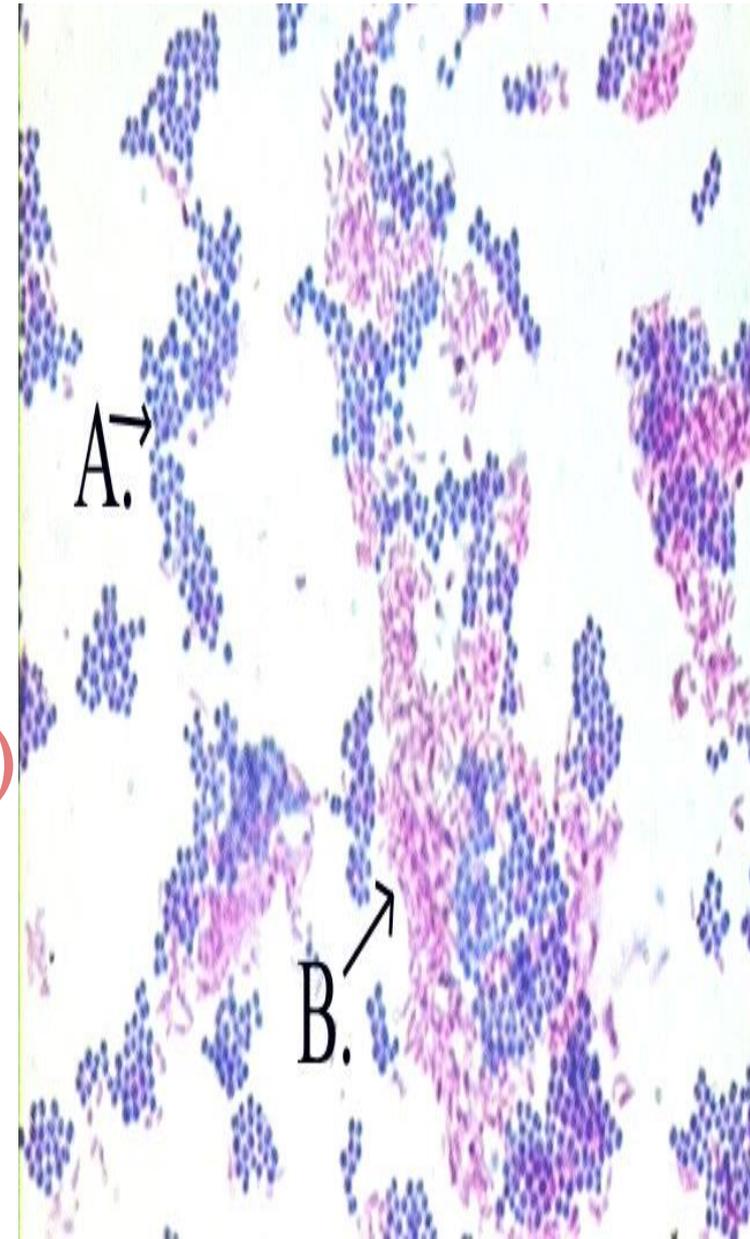
Acid-fast staining



A: **Non acid fast bacteria**

B: **Acid fast bacteria**

(ex: Mycobacterium tuberculosis)





**GOOD LUCK
&
SEE YOU NEXT LAB**