


# Diagnostic challenges of Tuberculosis





**Tuberculosis (TB) is an ancient, infectious and communicable illness sometimes called white death.**

**TB had been the Catastrophic disease in past a today's challenge, and will be the future threat.**



# Mycibacteria:

**Mycobacteria are especial group of bacteria which possess a hug lipid rich capsule in outer membrane. Although cell wall structure mimics G(+)<sup>1</sup> but they are neither gram negative nor gram positive.**




# Microbiology ( brief ) of M.tb

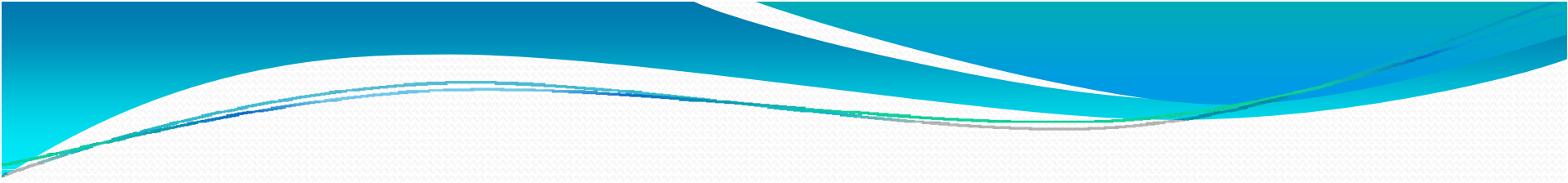
**M.tb is an-aerobic, non sporformer, non-motile, facultative intracellular bacilli.**

**The capsule offers the following charecters to it.**

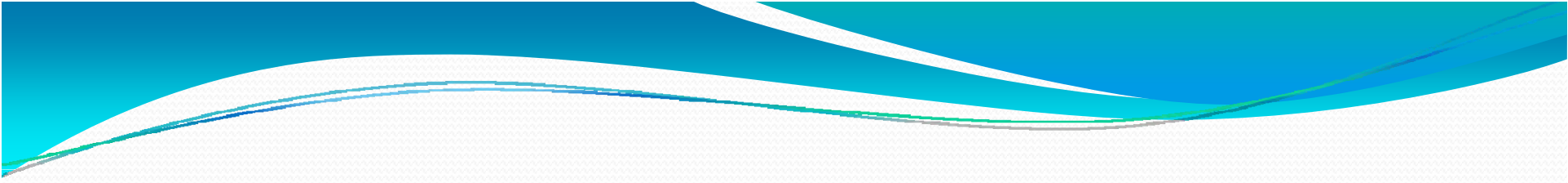
**The aniline dyes can't enter the cell wall, but when the bacterium are stained with carbol fuschin they resist decolorization and are seen as bright red rods in a blue counterstain. This character is called Acid fastness.**



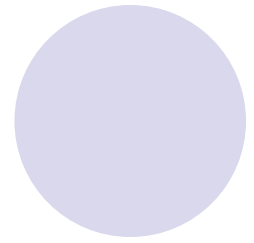
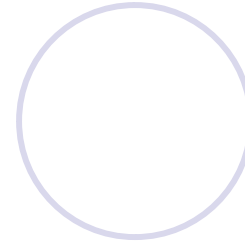
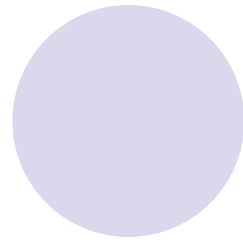
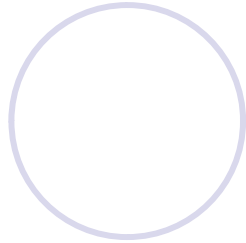
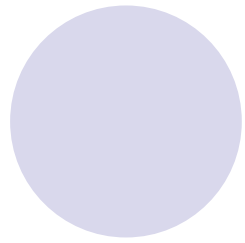
**Due to lipid capsule nutrients can't enter readily, So a slow metabolic rate (6-8 weeks) for observable colonies on egg-based medium (LJ) and long generation time (18-20 hr) or more.**



**M.tb can resist adverse environmental stress (Darkness, Humidity) and also resist against disinfectants, and chemotherapeutic agents, after prions, bacterial spore, M.tb is the most resistant agent.**

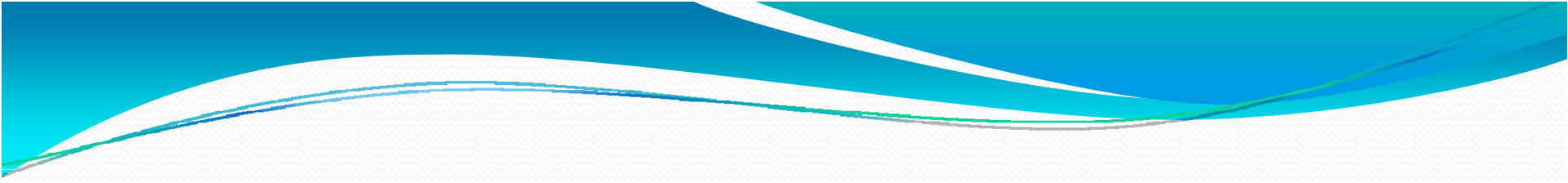


**The lipid Capsule made up of estrified fatty acids (Mycolic acid, Waxes, etc:) which are virulence determinants and other cell structure component such as arabinogalactan are antigenic determinants.**



# **Laboratory Diagnosis and challenges**





**M.tb can disperse in the Lab and environment via respiratory droplets ( aerosols 1-3  $\mu$ ) particles during Lab work up; smearing, Concentrating and culturing aerosols are very small particle which may contain 1-10 bacilli are highly infectious. So personal protective equipment (PPEs) should be used by lab workers.**



**During Lab working the workers should able to use  
Biological safety cabinets- BSC- class II and/or III)  
and Biological safety level-BSL-II.**

**So BSC and BSLs are obligations of TB lab.**



**In province and research TB lab other regulations BSC III and BSL-3 are mandatory when working with MDR, XDR and XXDR M.tb.**



# Laboratory Diagnosis:

- a. Staining: As mentioned earlier M.tb Can't be stained with alkaline aniline dyes, The most popular staining is Acid fast staining either Hot method (Ziehel–Neelson) and/or cold staining (Kynnyoun)**



**In order to see M.tb, at least  $10^4$ CFu/ml of organism should be present in the specimen.**

**Because stained smears are observed by  $\times 100$  lens, all fields can't be surveyed.**

**In Acid fast staining dead and alive bacteria are stained.**

**Thin smear result in false Negative and Thick smears may possess some alive bacilli in the depth and possibility of contamination of lens, and work place.**



**Acid fast staining is simple, not expensive and can be done in field laboratories and reliable in screening sputum smears, especially in endemic area.**



**Flouochrome staining: auramin and rhodamin.**

**M.tb when stained with flouochrome dyes floursing a bright yellow emition and can be observed by a LED lamp or flouresent microscopy.**

**The flourchrome stained smear are observed by  $\times 10$  lens. So all microscopic field can be reviewed.**

**In this staining method tissue fragments may also take the stain and false positive result is produced.**



موفق و سلامت باشید