

Corso di aggiornamento

Tubercolosi: un impegno globale

Diagnosi laboratoristica di tubercolosi Parte I -

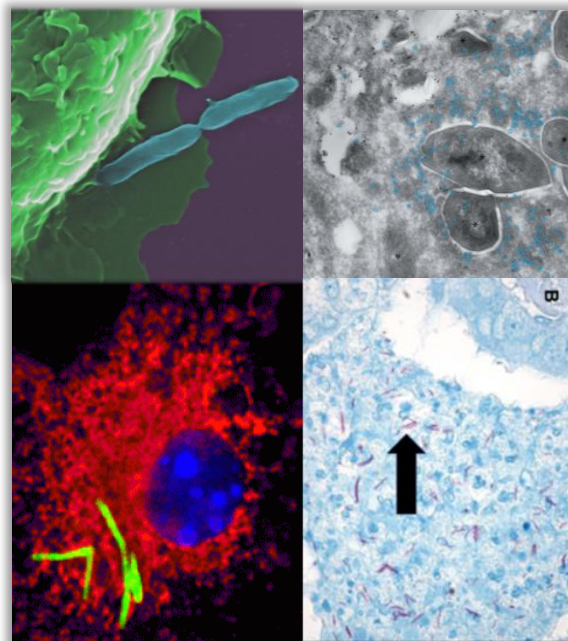
Giovanni Delogu



UNIVERSITÀ
DI PARMA



SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Parma



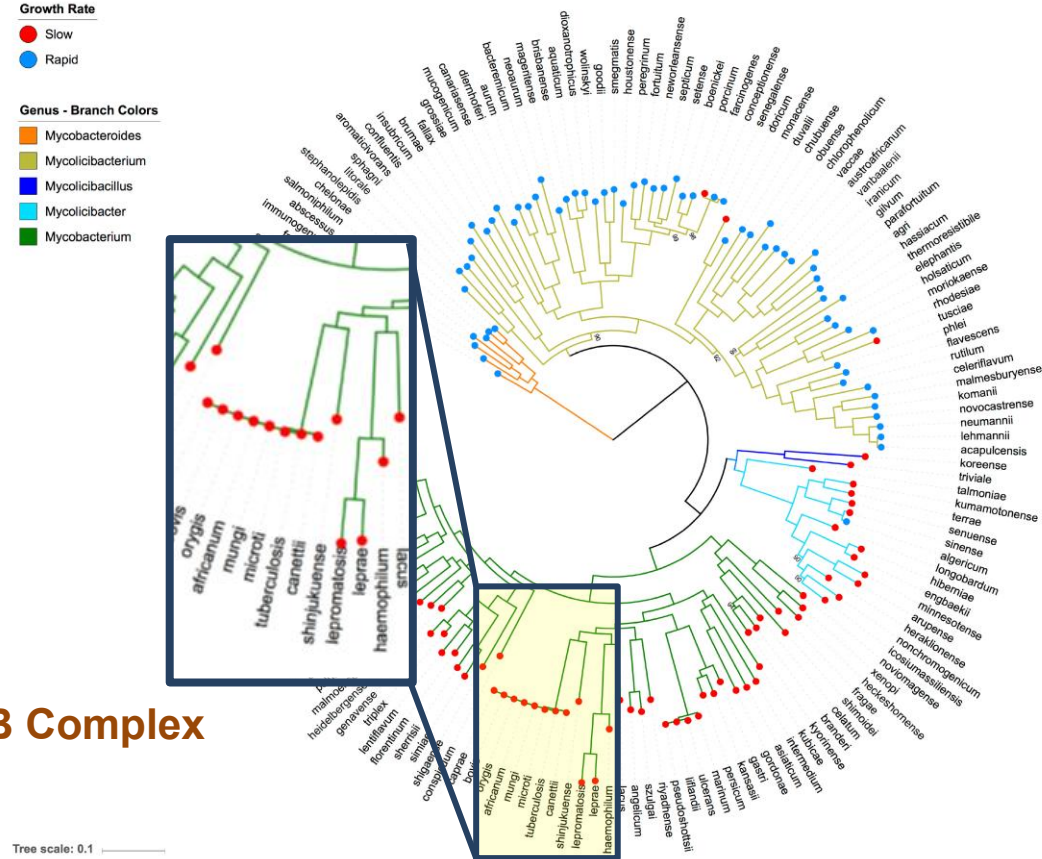
Outline

- The TB Bacillus
- Natural history of Mtb infection;
- The spectrum of TB conditions;
- Tools to detect and monitor Mtb infection and TB disease;

Mycobacteria

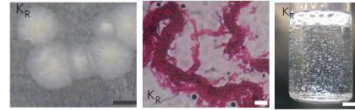
- Commonly found in the environment, often in association with protozoa;
- Some species emerged as important pathogens for mammals and other animals.
- Large genetic differences between the different species due to extensive HGT in most species.

Phylogenetic tree of all well characterized *Mycobacterium* species.



MTB Complex

***Mtb* is a bacterium specifically adapted to humans, and then mammals, which evolved to exploits T cell host immune responses to promote transmission**

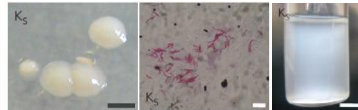
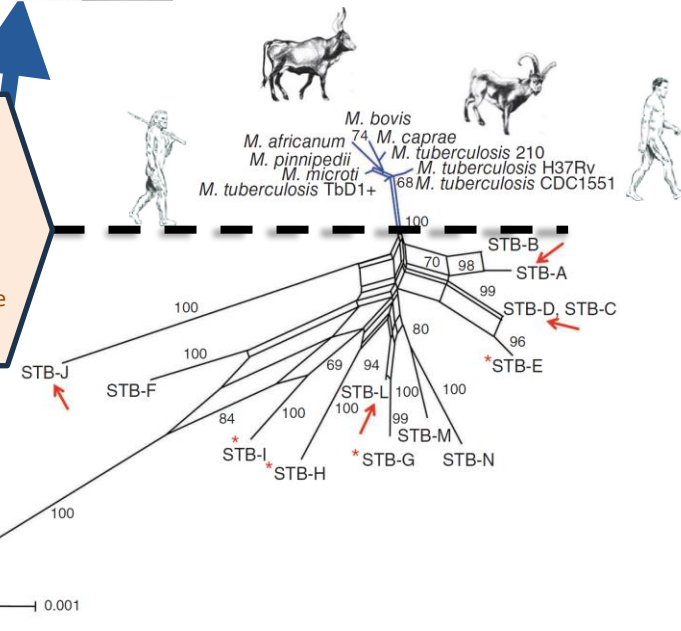


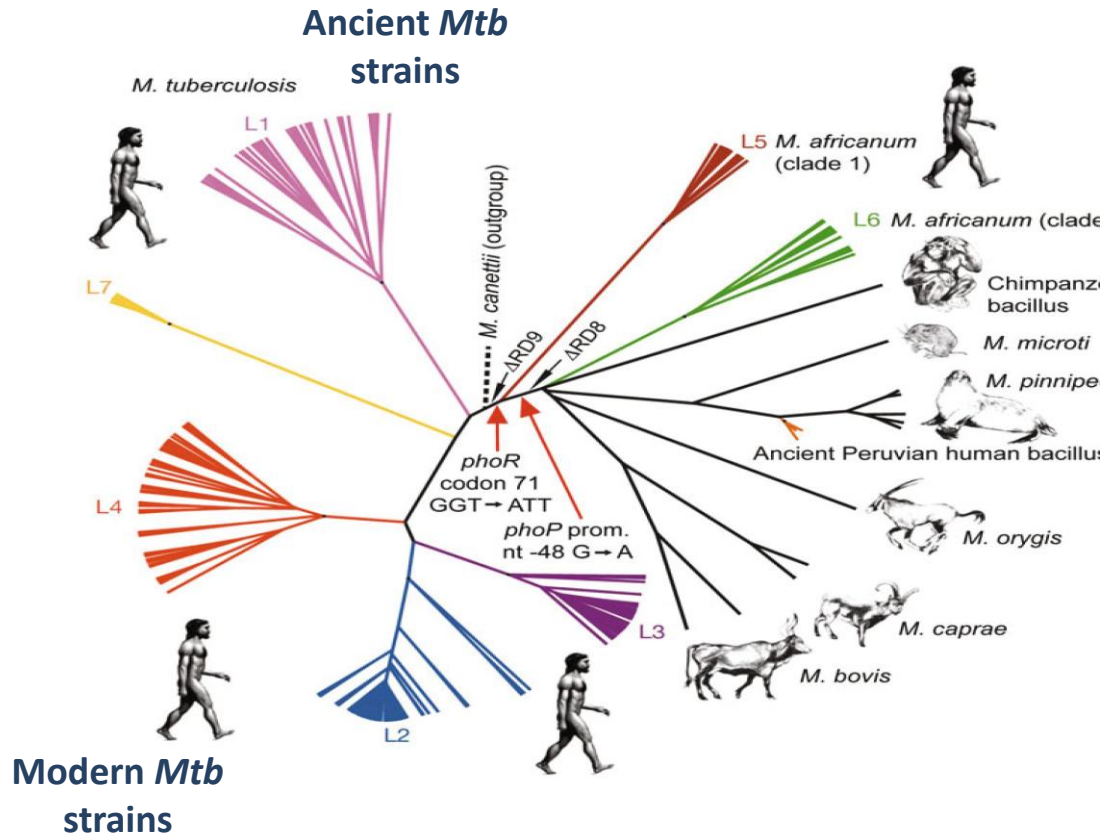
MAIN PHENOTIPIC CHANGES

- *Mtb* OM enriched in PDIM;
- Lack of the major porin MSPA;
- Loss of lipo-oligosaccharides (LOS);
- Amplification PPE and PE proteins as surface antigens and porins;
- Increase impermeability;
- Super-conserved T cell epitopes

GENETIC BOTTLENECK

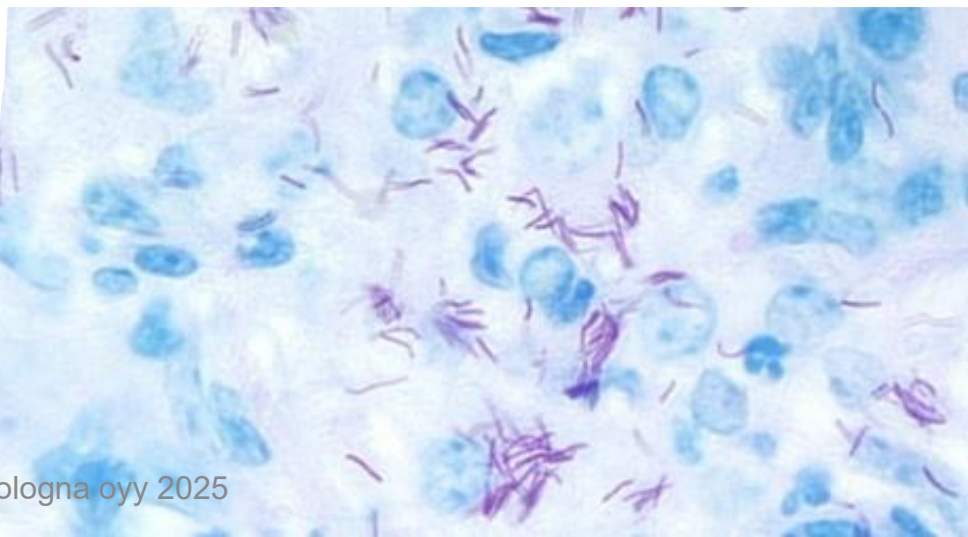
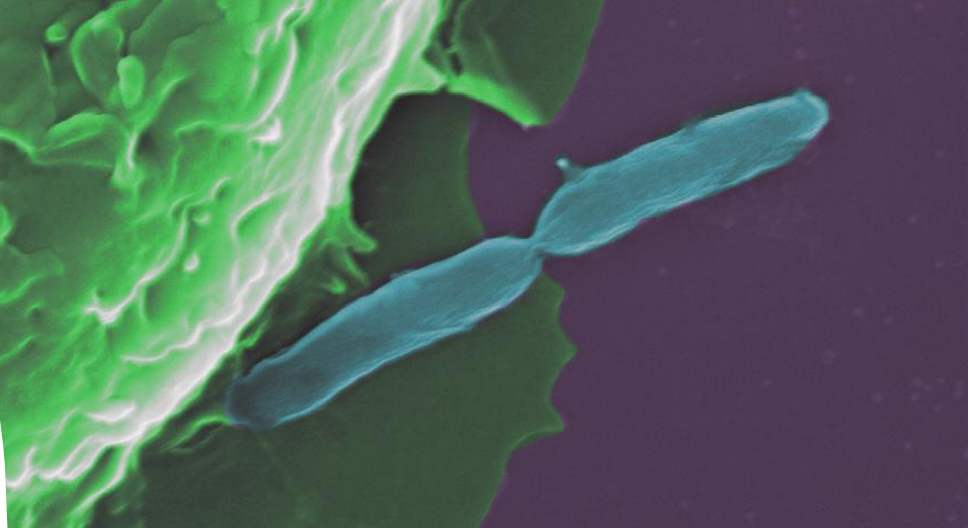
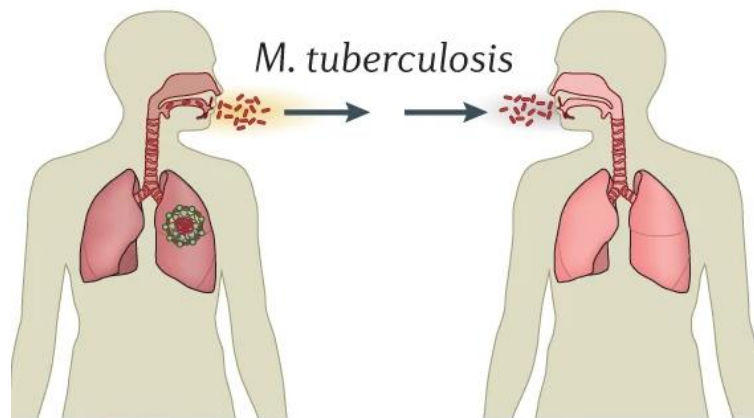
- Genome reduction;
- Clonal expansion;
- Lack of HGT;
- Amplification and diversification of *pe* and *ppe* genes;

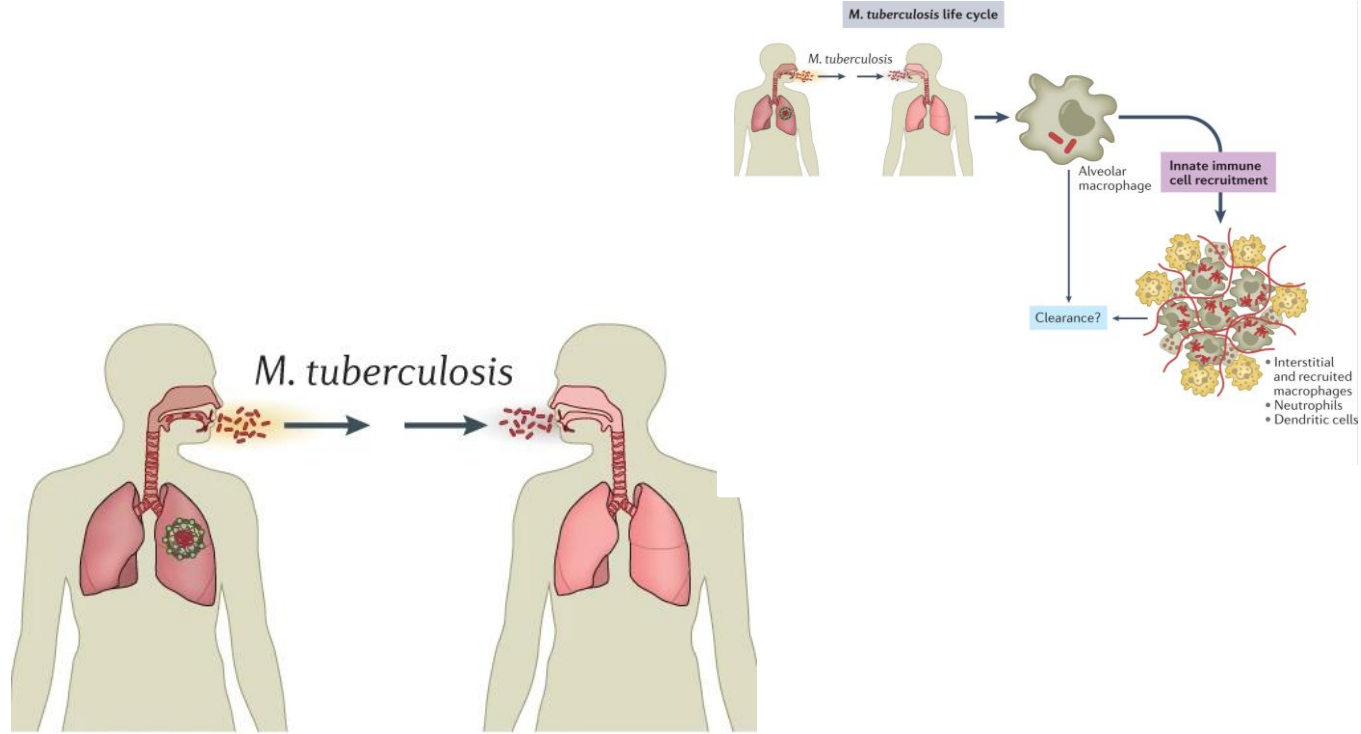


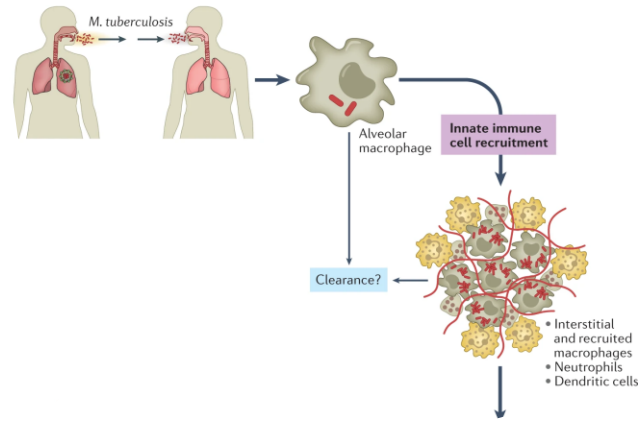


Emergence of *Mtb* as a major human pathogen occurred by clonal expansion (monomorphic slow-growing mycobacteria).

- limited genetic variability;
- “intrinsic” resistance to human (mammal) host immune responses;
- Hyperconserved T cell epitopes;

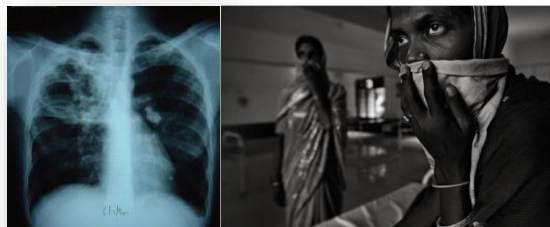
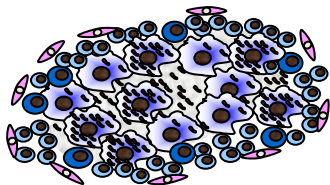




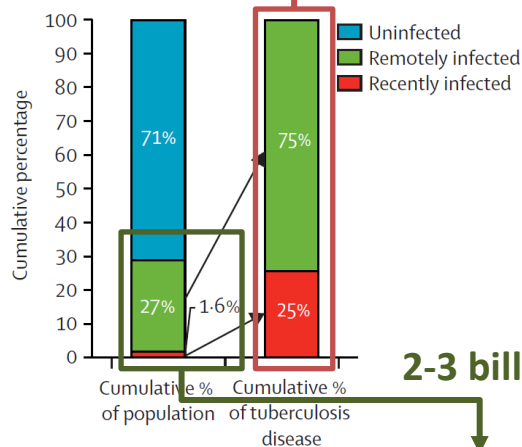


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TB DISEASE



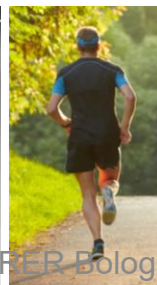
8-10 millions/year



2-3 billions

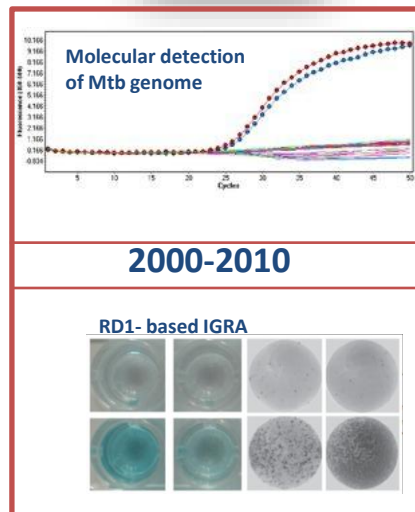
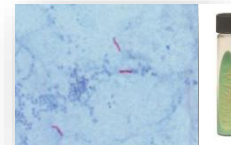


LATENT TB

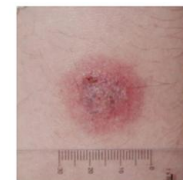


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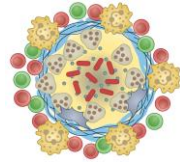
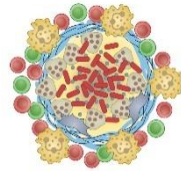
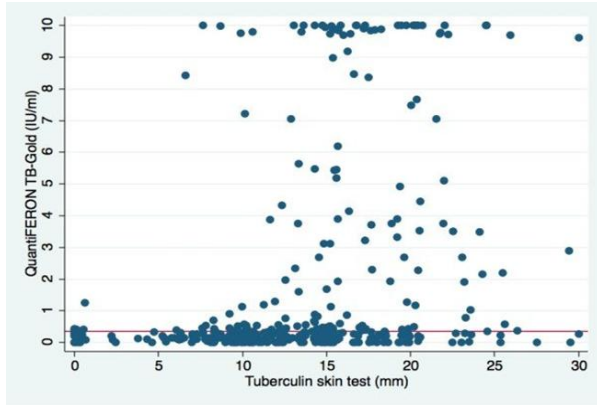
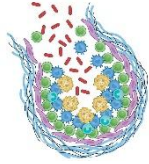
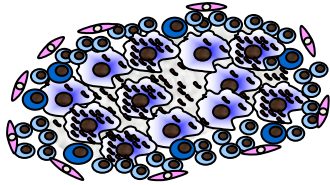
Direct
diagnosis
Microbiological



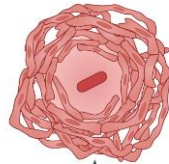
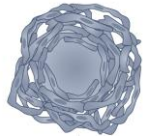
Indirect
diagnosis
Immunological



TB DISEASE

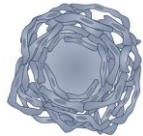
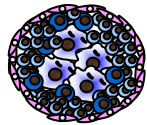
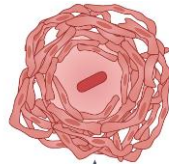
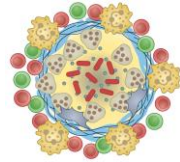
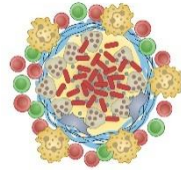
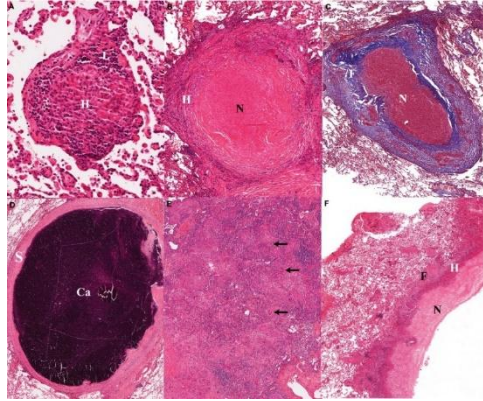
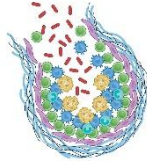
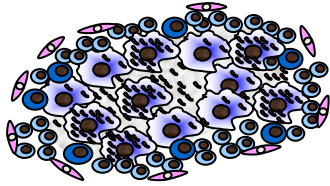


Dyrhol-Riise AM et al (2010) BMC Infectious Diseases

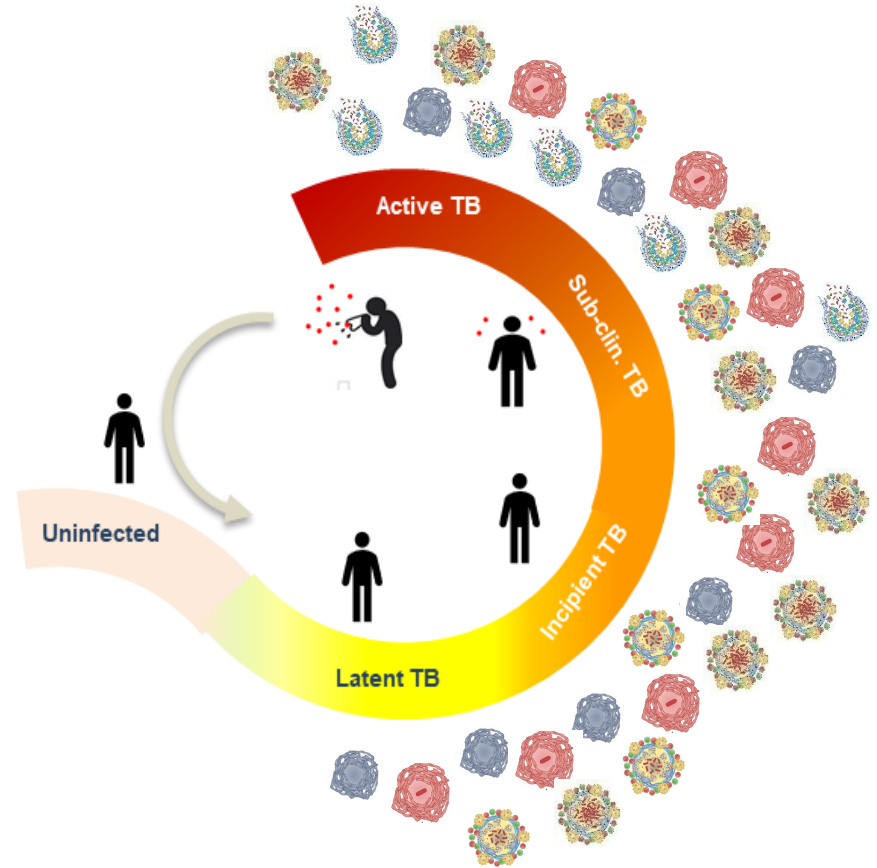


LATENT TB

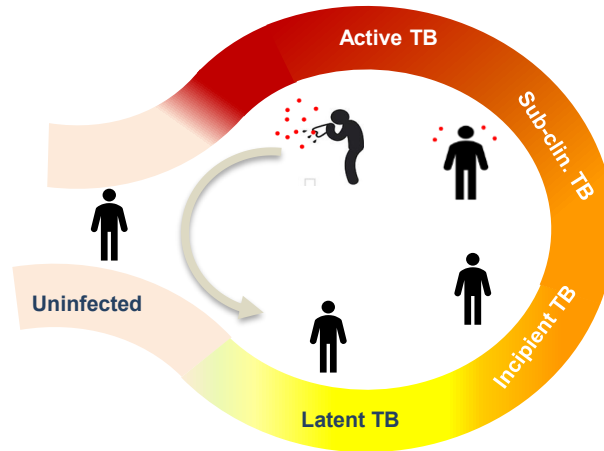
TB DISEASE



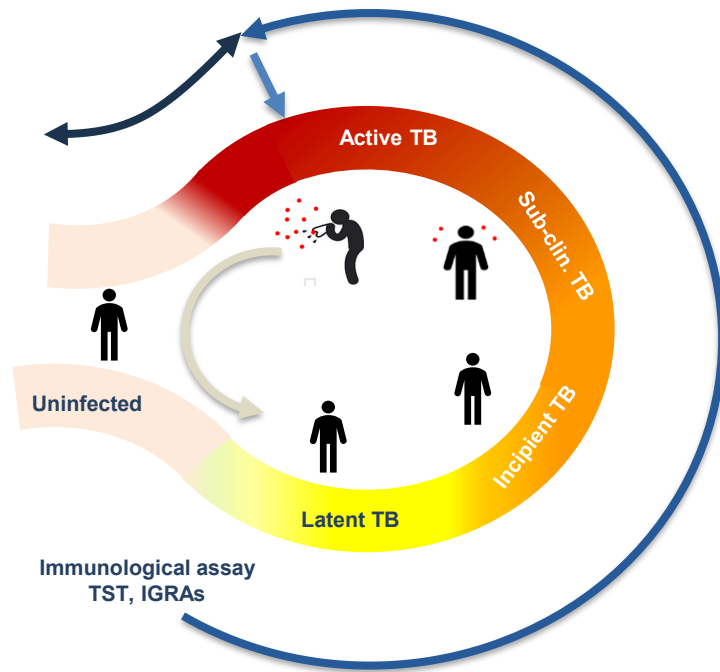
TB HETEROGENEITY

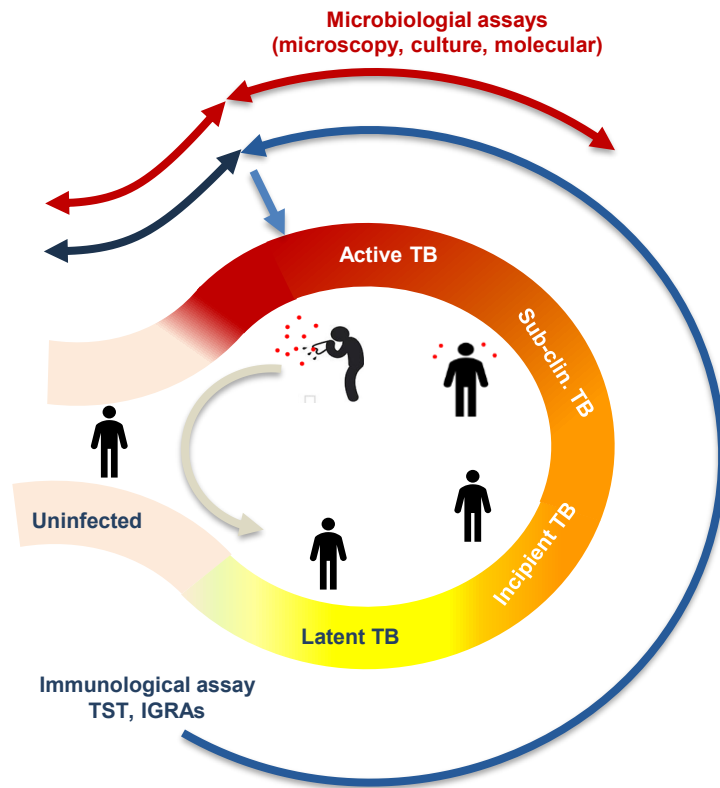


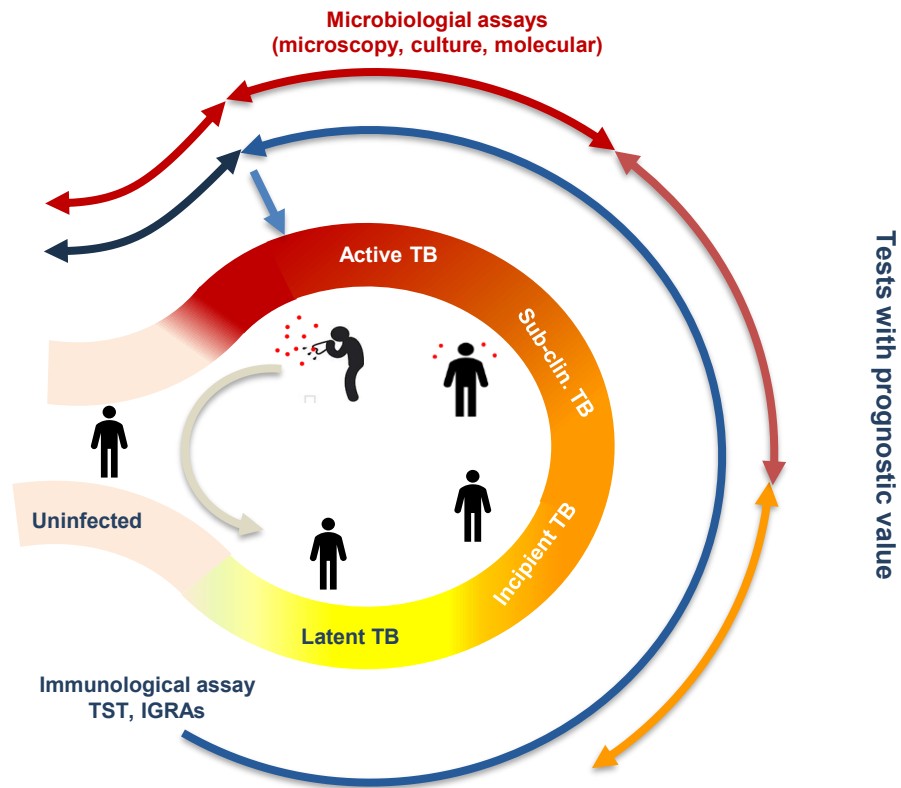
LATENT TB



Category	Definition
Uninfected	A subject with no immunological sign of TB infection. Some highly exposed subjects remain TST or IGRA negative despite the presence of an adaptive immune response against TB and are defined as “resisters”.
Resisters	Subject highly exposed to Mtb that remain IGRA ⁻ TST ⁻ . Presence of Mtb specific antibodies, IFN γ CD4 ⁺ T cells (TH17)
Latent infection	LTBI “as having evidence of TB infection and no clinical, radiological or microbiological evidence of active TB disease”
Incipient TB	Incipient TB infection is an infection that is likely to progress to active disease in the absence of further intervention but has not yet induced clinical symptoms or radiographic abnormalities.
Sub-clinical TB	Subclinical TB disease is disease that does not cause clinical TB-related symptoms but causes other abnormalities that can be detected using existing radiologic or microbiologic assays
Active TB	Active TB as “symptomatic patients with radiological or microbiological evidence of Mtb”

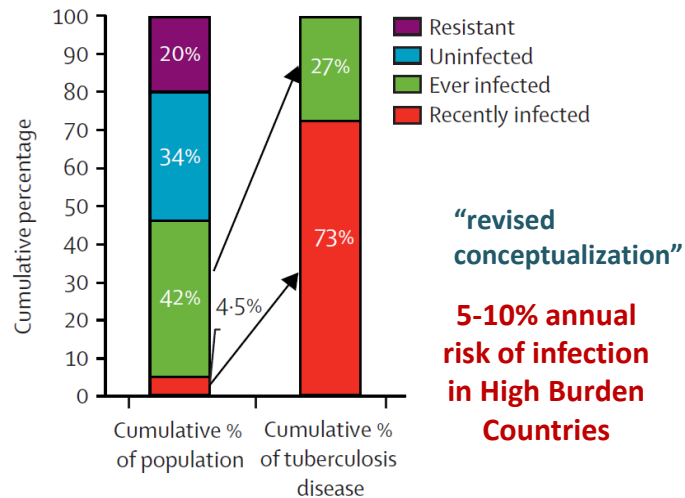
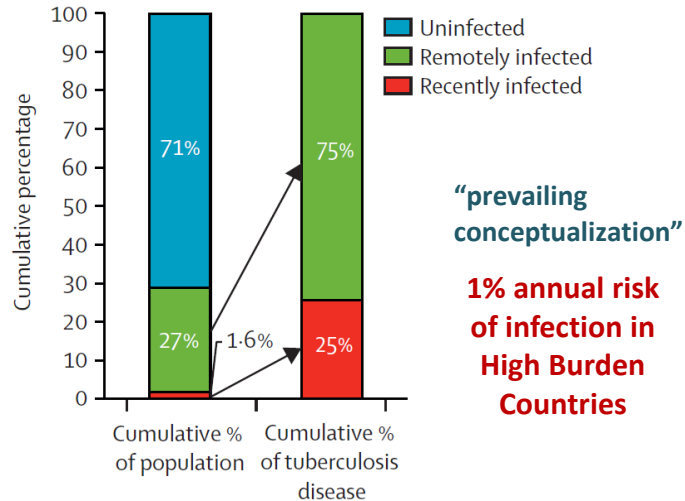
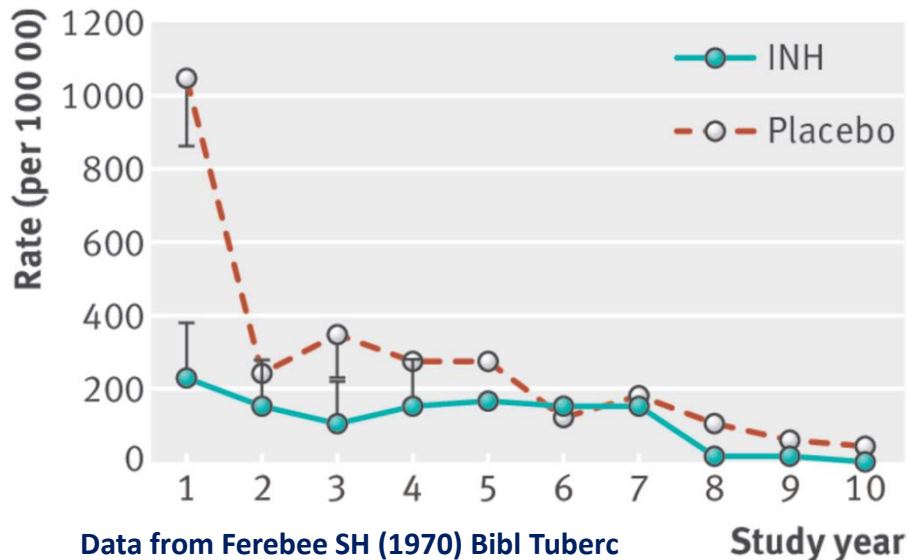


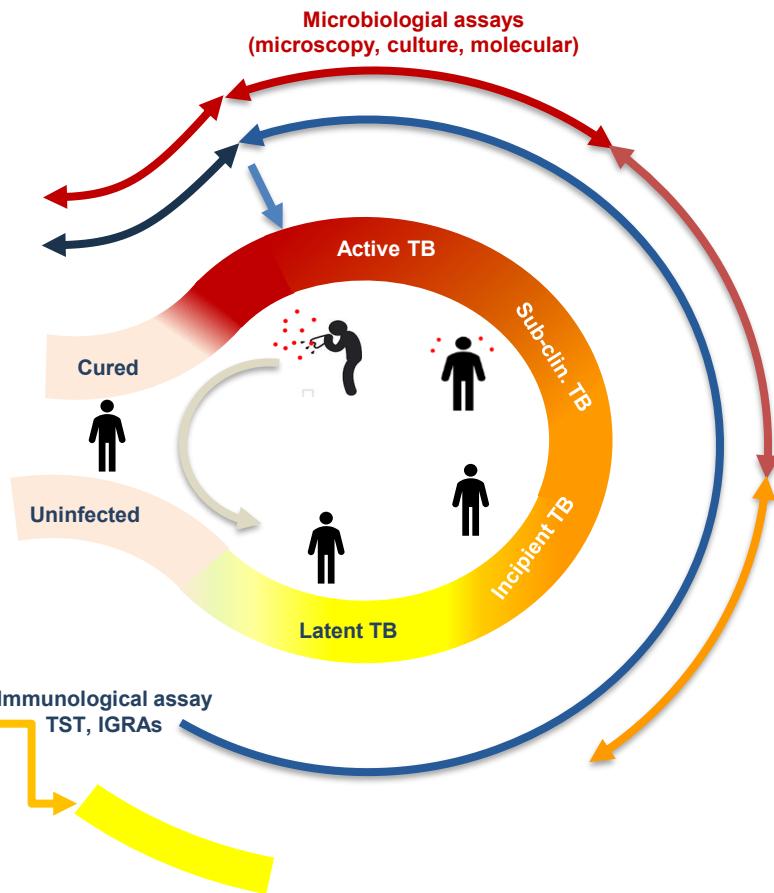




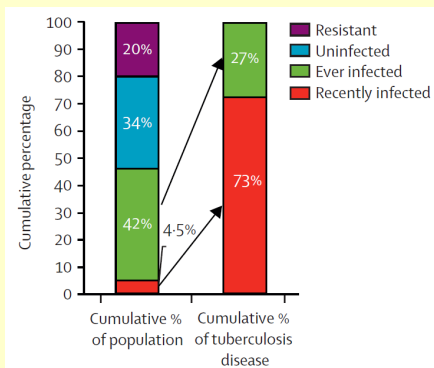
The impact of detecting INCIPIENT TB

Risk of developing TB disease is highest < 2 years after infection





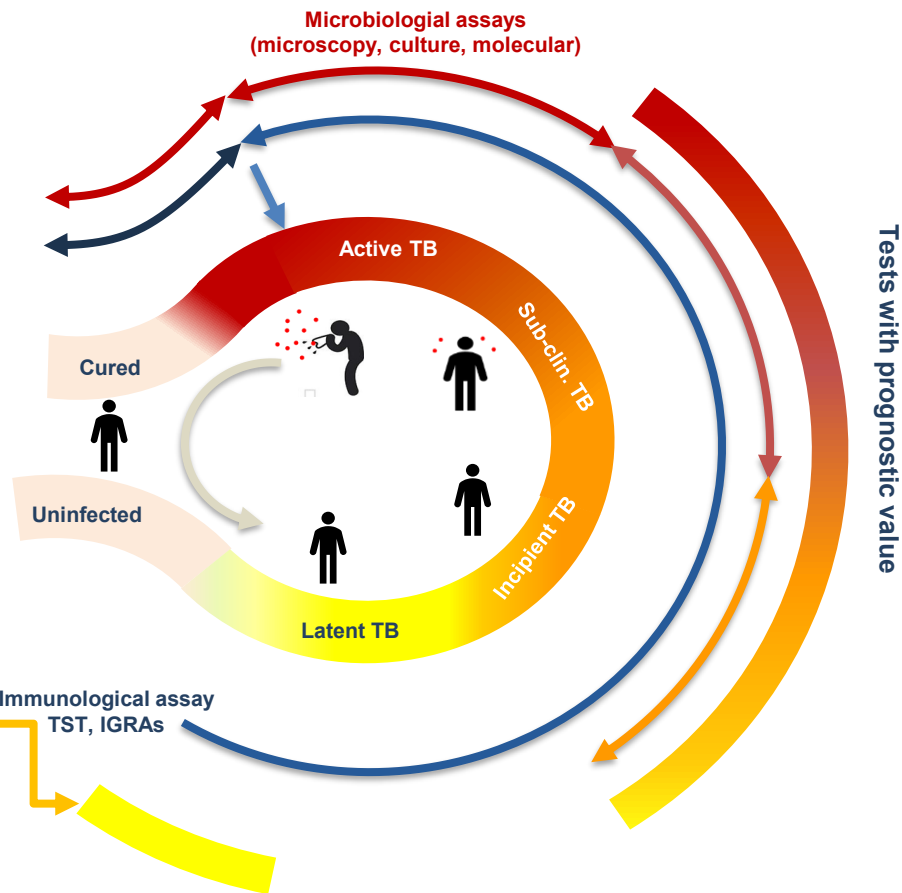
1) Detect recent *Mtb* infections



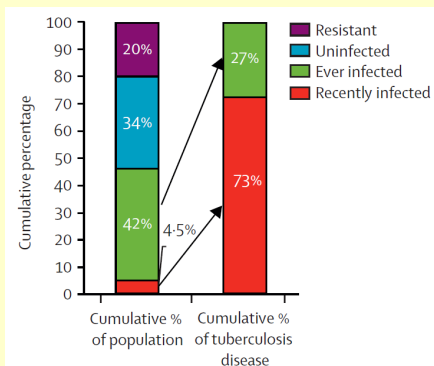
“revised conceptualization”

Dowdy DW, Behr M (2022) Lancet Inf Dis

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1) Detect recent *Mtb* infections



“revised conceptualization”

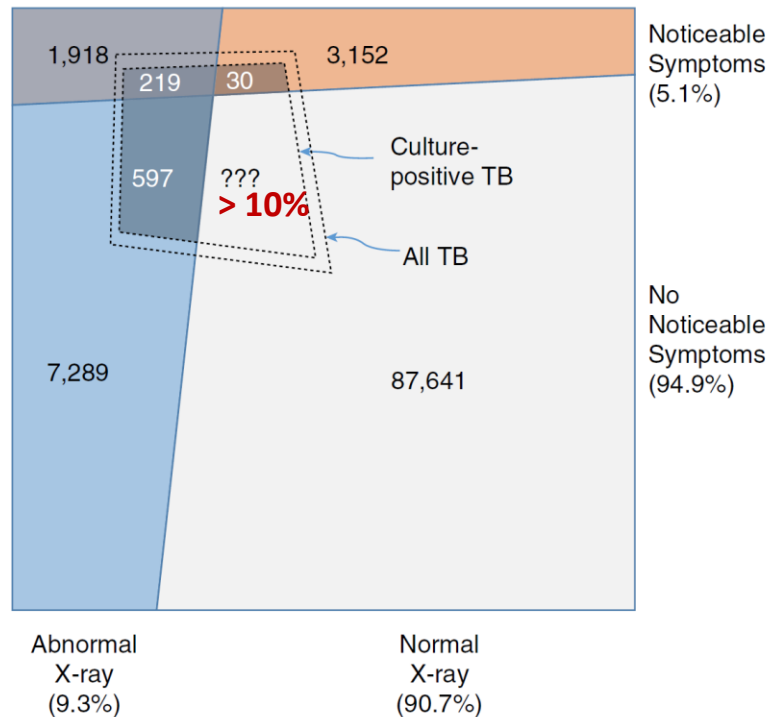
Dowdy DW, Behr M (2022) Lancet Inf Dis

2) Predict incipient TB.

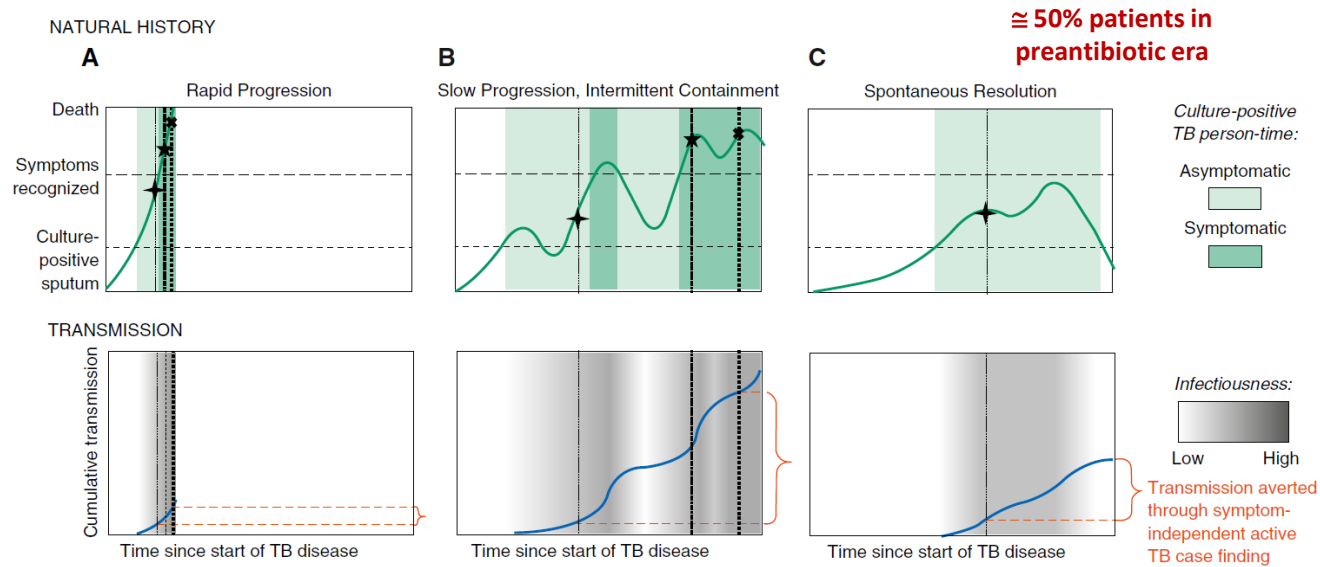
- Identify subjects with increased risk of developing active TB;

Subclinical TB can potentially drive a substantial fraction of transmission on a population level because of its high prevalence and long duration

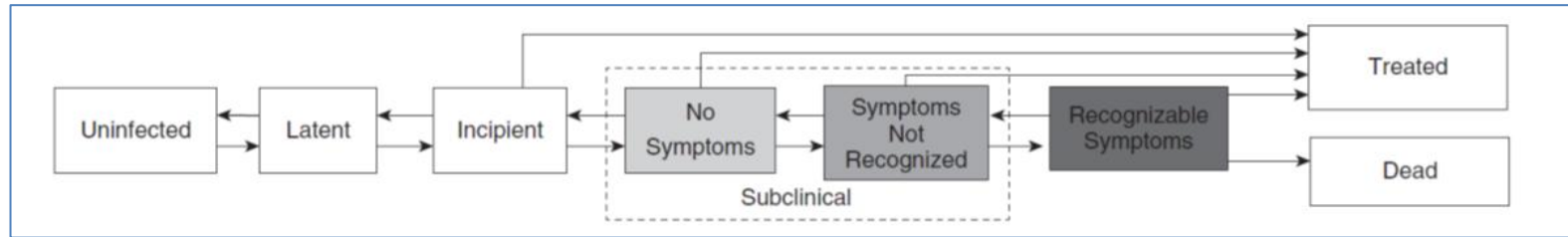
Full population of 100,000 people
(Measured TB prevalence: 846 per 100,000)



- A large proportion of all people with prevalent, bacteriologically positive, and radiographically abnormal TB screen negative for TB symptoms;
- People with no symptoms can still have the high bacillary loads typically associated with transmission;
- In a recent review of 23 national and five subnational prevalence surveys, median 50% of the identified TB cases screened positive based on radiography alone, whereas 7% screened positive based only on symptoms, and the remainder had both symptoms and radiographic abnormalities suggestive of TB;
- At least 7 million to 10 million people are currently living with TB that is not detectable by symptom screen;



Thus, the simplest way to reconcile prevalence and notification data is to conceptualize the natural history of TB as both bidirectional and heterogeneous, such that the populations who account for most infectious person-time may differ from those who comprise most TB notifications



ACTIVE TB PROGRAMS

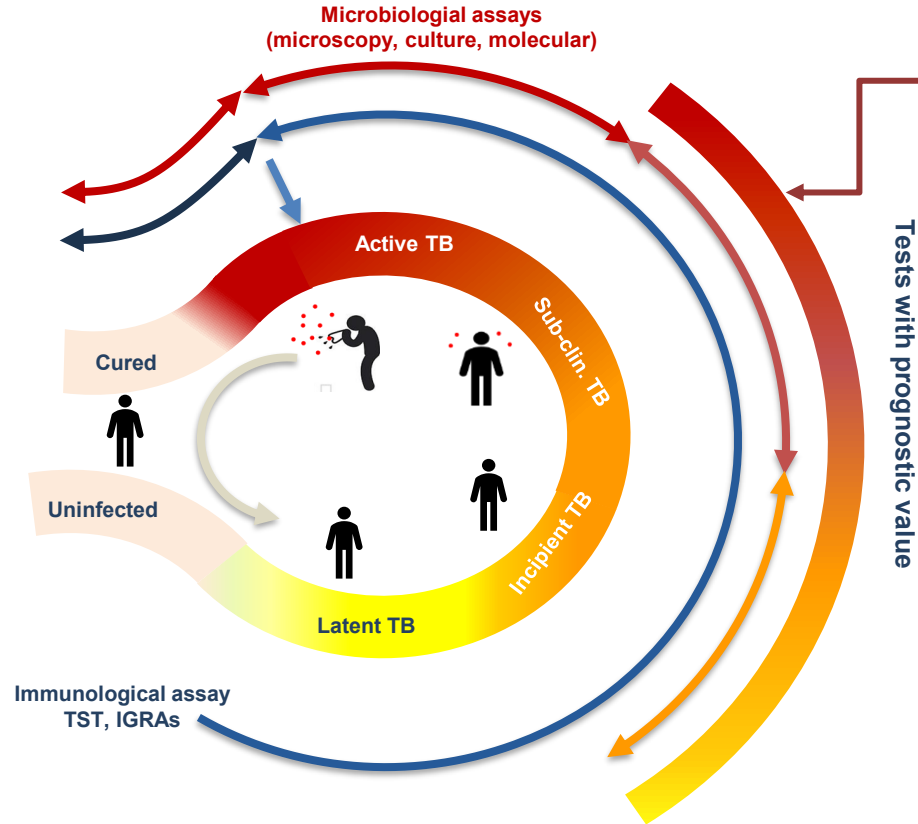
High risk groups;

- Household contacts;
- Recently exposed Mtb infected;

Less invasive specimens

- Tongue swabs; saliva;

Highly efficient molecular tests;

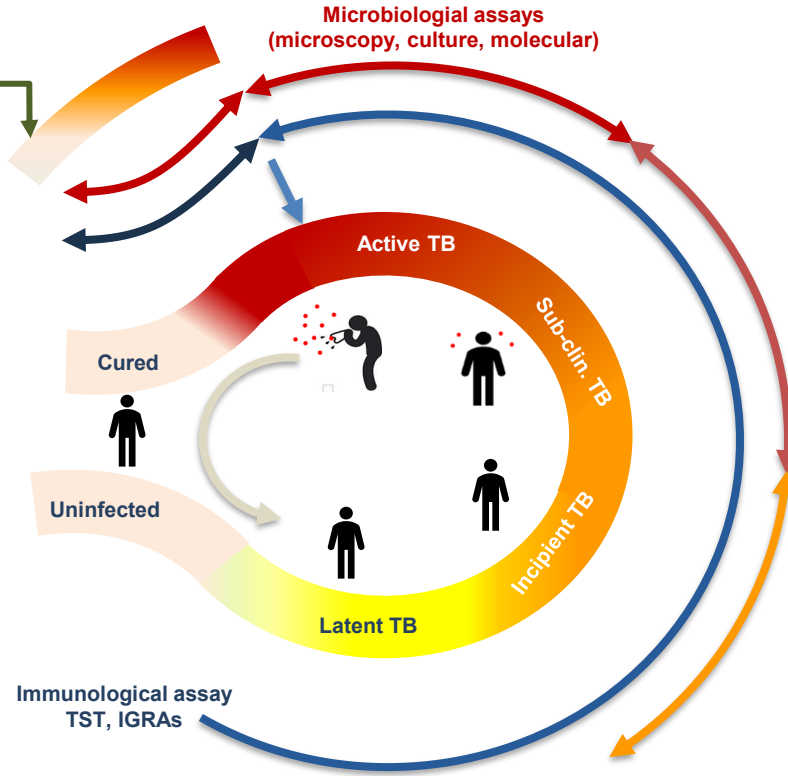


3) Detect subclinical TB

- Many people with bacillary transmissible TB do not show symptoms;
- Main drivers of TB transmission;

4) Monitor TB treatment

- Prompt detection of treatment failure;
- Improved treatments (shorter, etc);
- Assessing new treatment (HDTs)



Monitoring Mtb load

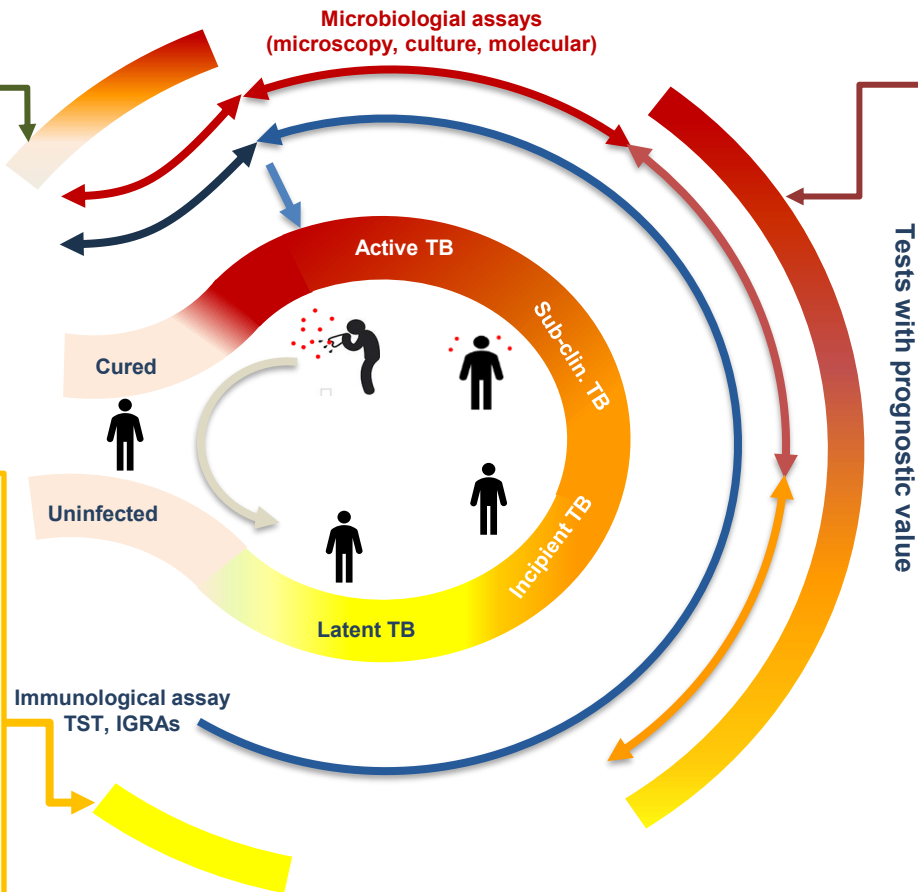
- Culture TTP
- Quantitative analysis of Mtb TB MBLA (rt-RT-PCR, 16S rRNA)
- LAM
- Detection of DCMTB (differentially culturable Mtb)

4) Monitor TB treatment

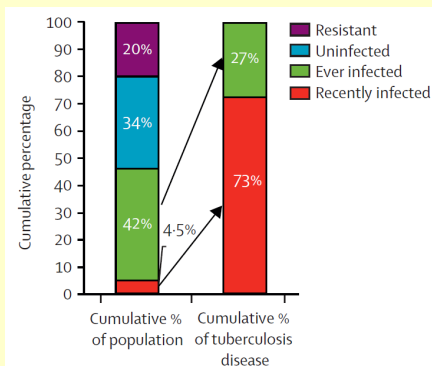
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1) Detect recent *Mtb* infections



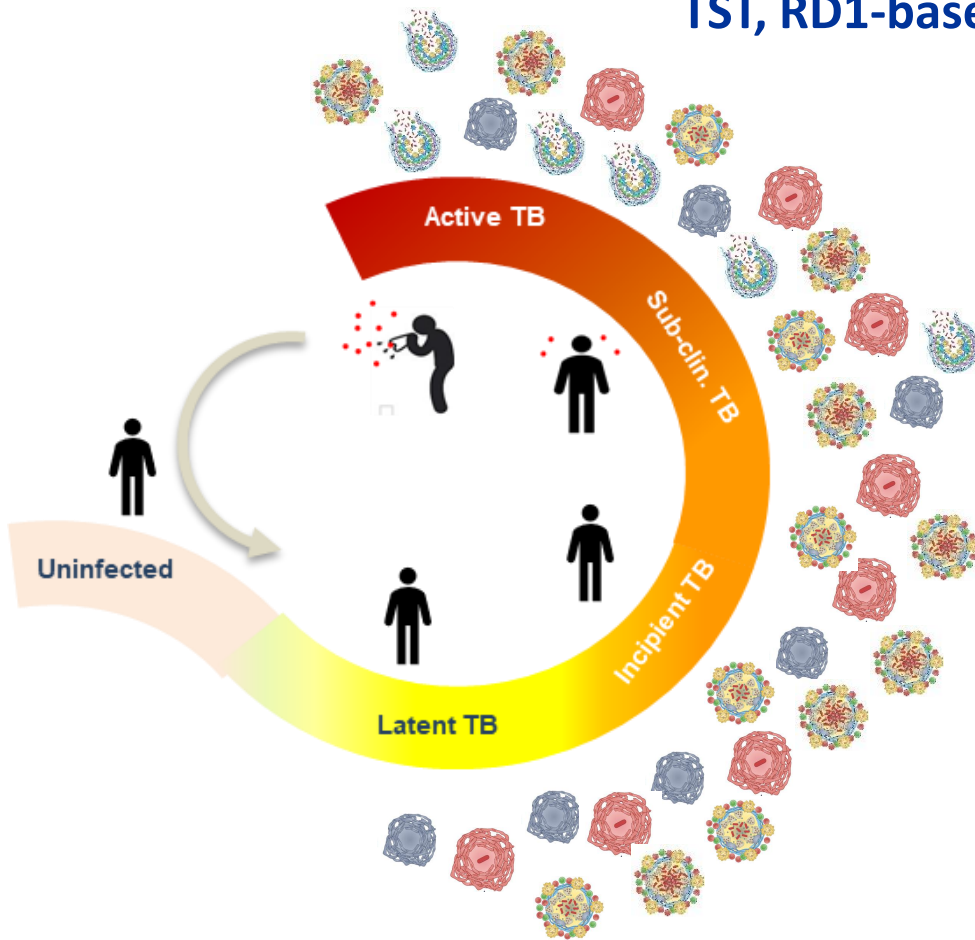
“revised conceptualization”

Dowdy DW, Behr M (2022) Lancet Inf Dis

2) Predict incipient TB.

- Identify subjects with increased risk of developing active TB;

TST, RD1-based IGRAs cannot resolve TB complexity



**Multiparametric
Signatures**
Hypothesis-driven
Agnostic
RNA
Proteins
Metabolites



Mixed parameters



Interrogating Mtb-specific T
cells

Other Mtb antigens
More cyto/chemokines