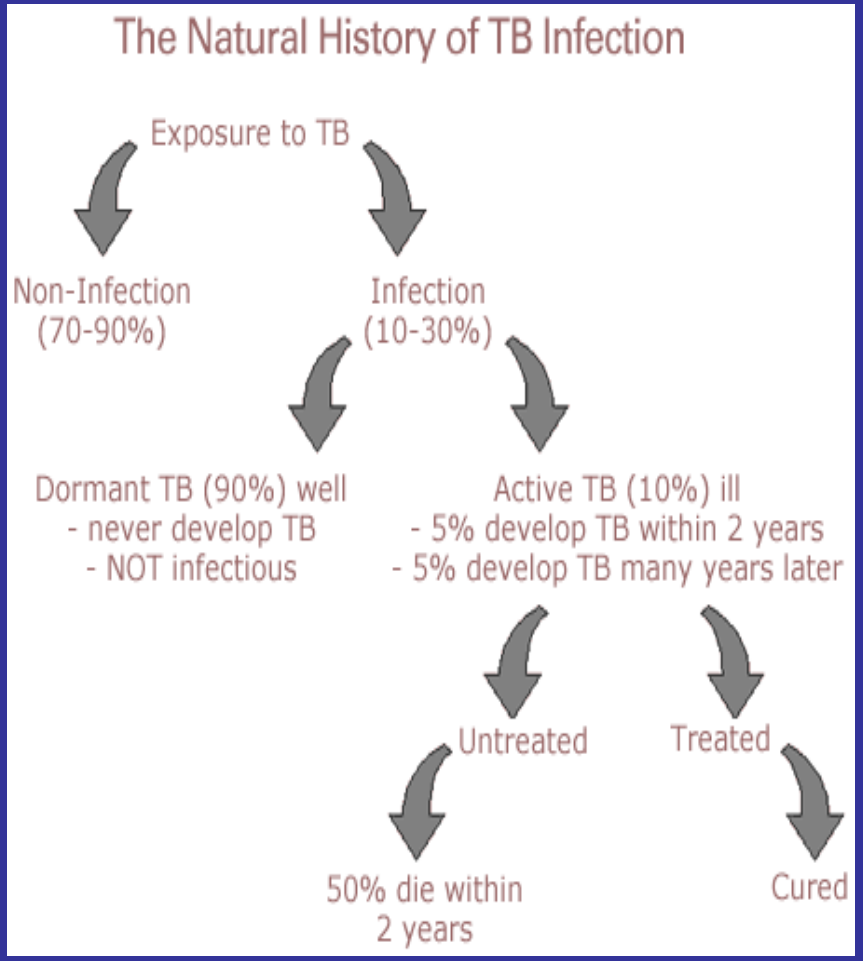
Mycobacterium

- **Gram positive obligate aerobic rods**
- ***Mycobacterium tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti* all cause tuberculosis**
- ***M. tuberculosis* is pathogenic for humans and *M. bovis* for animals**
- **Acid-fast bacilli**

Pathogenesis

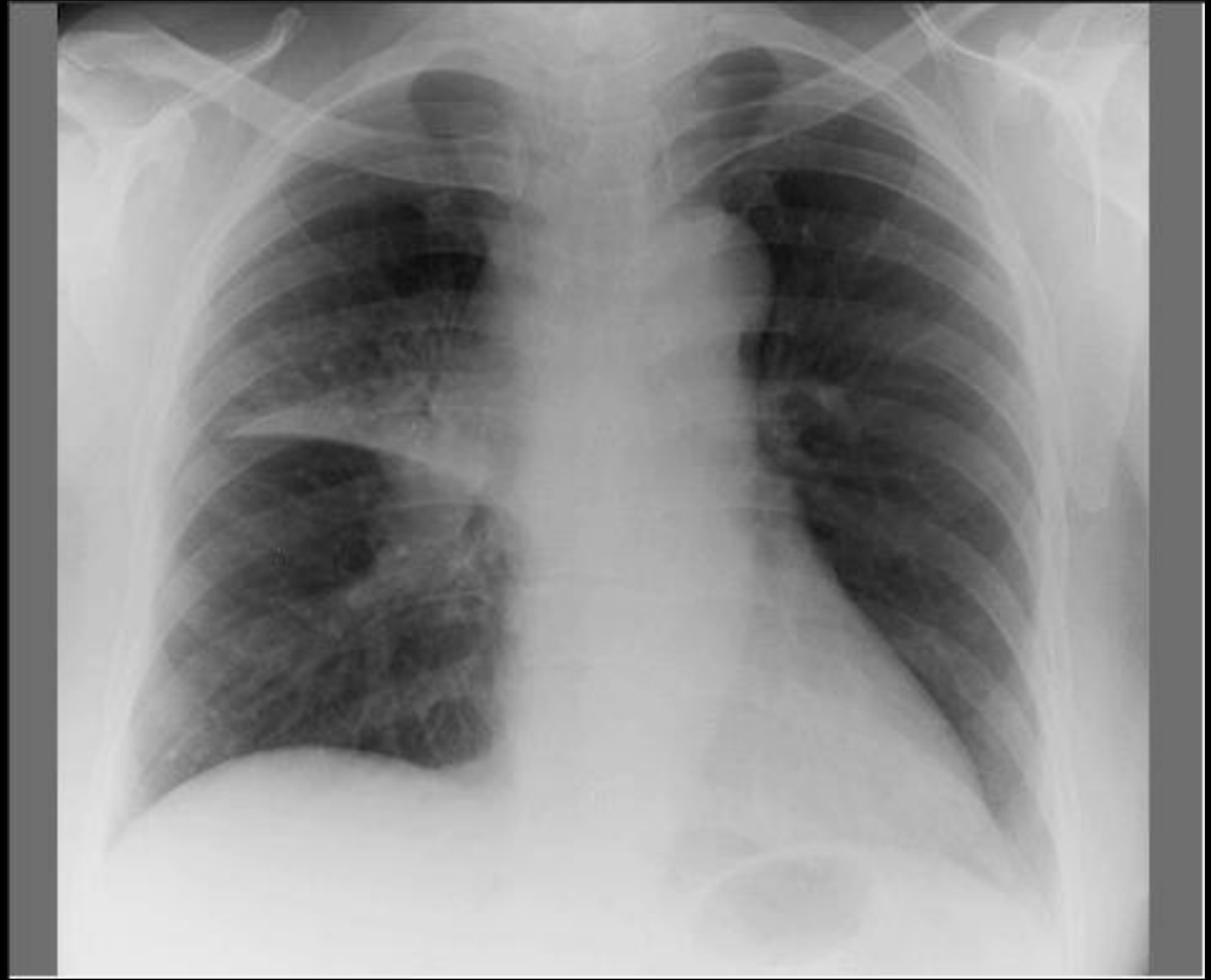
Outcomes after exposure:

1. Clearance of organism
2. Primary Disease
3. Latent Infection
4. Reactivation Disease

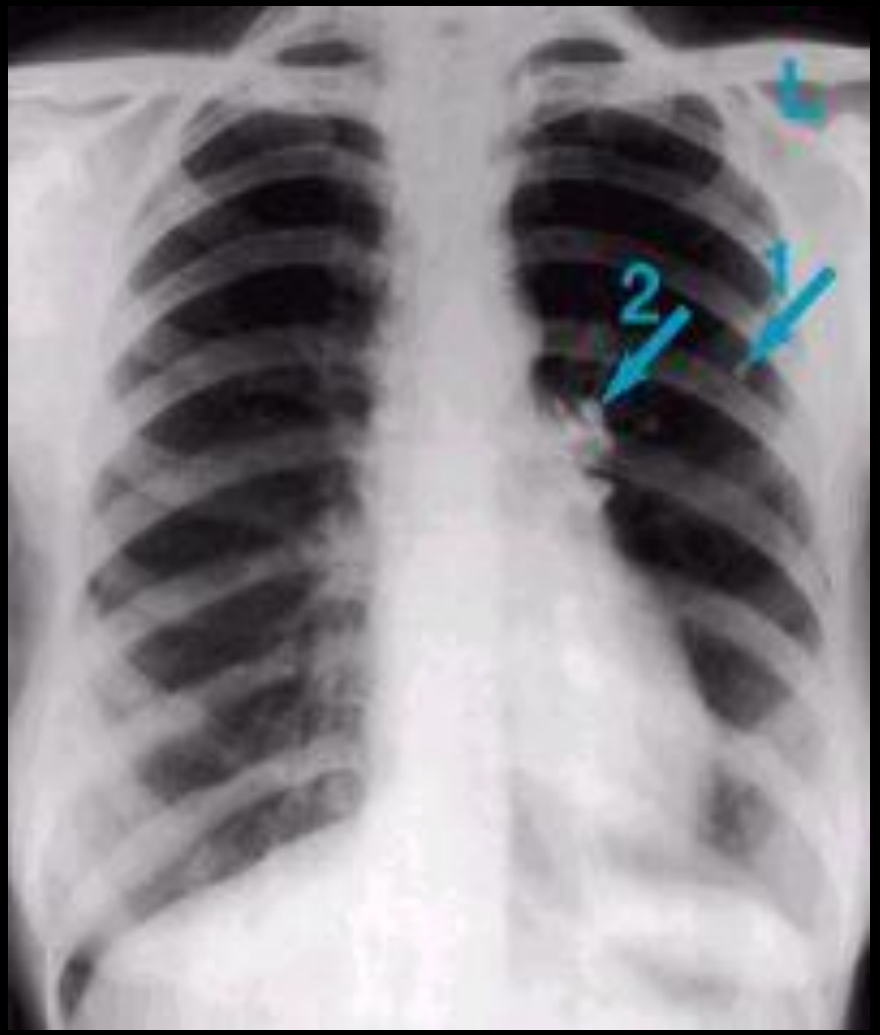


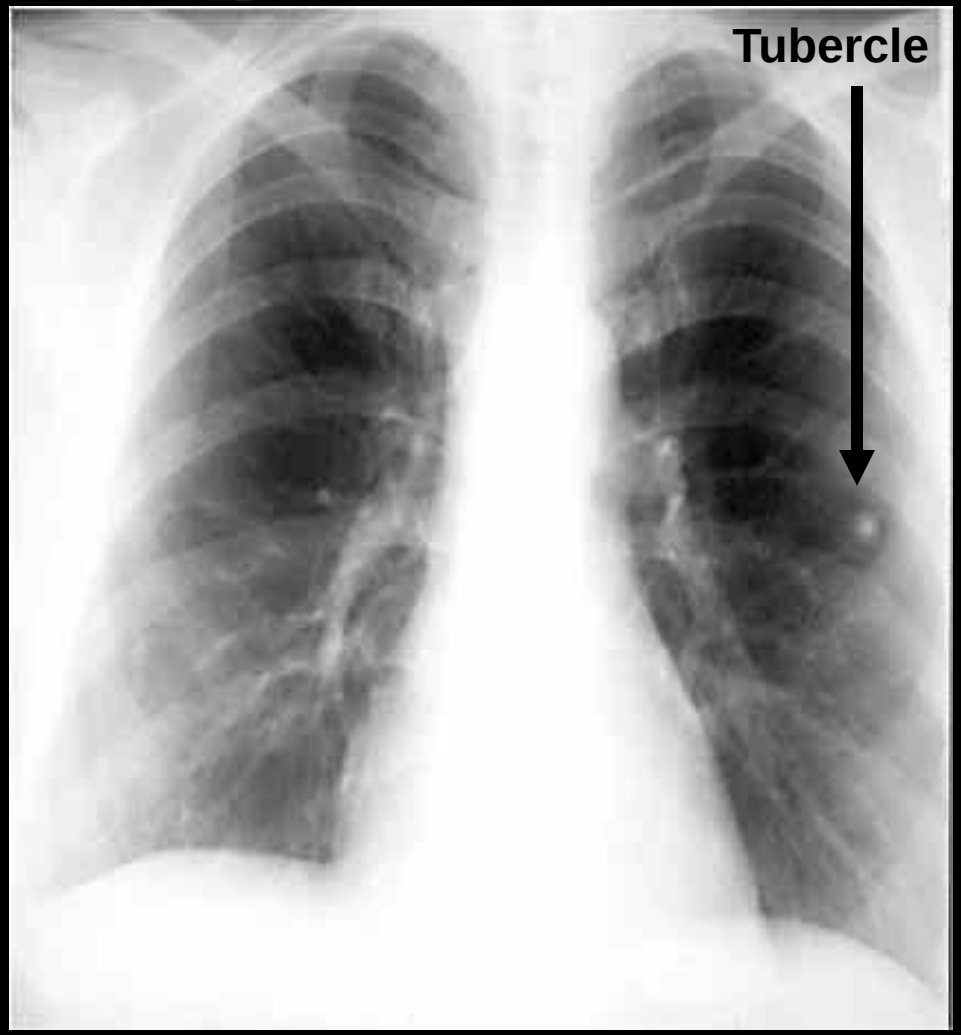
Primary Disease

- Tubercle
- Lymphadenopathy
- Ghon Complex
- Caseating Necrosis



Ghon Complex





Extrapulmonary Tuberculosis

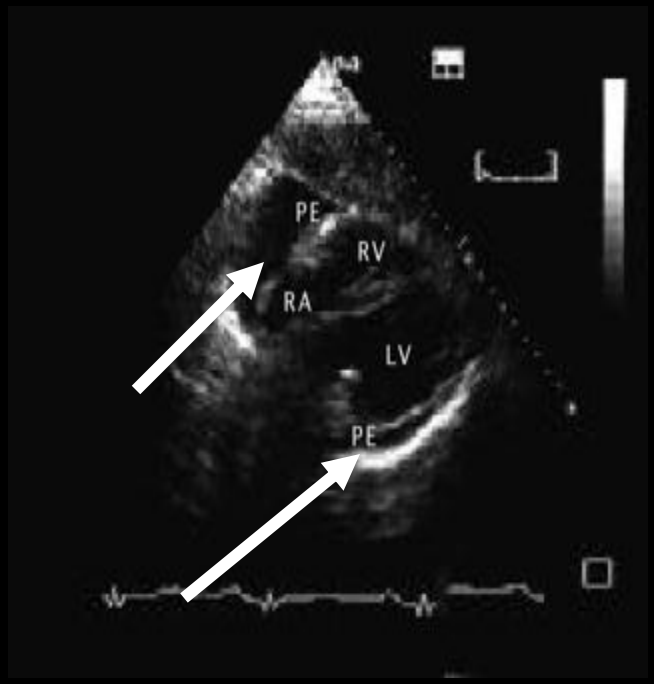
Pott's Disease

MRI imaging modality of choice to detect spinal cord compression or cauda equina

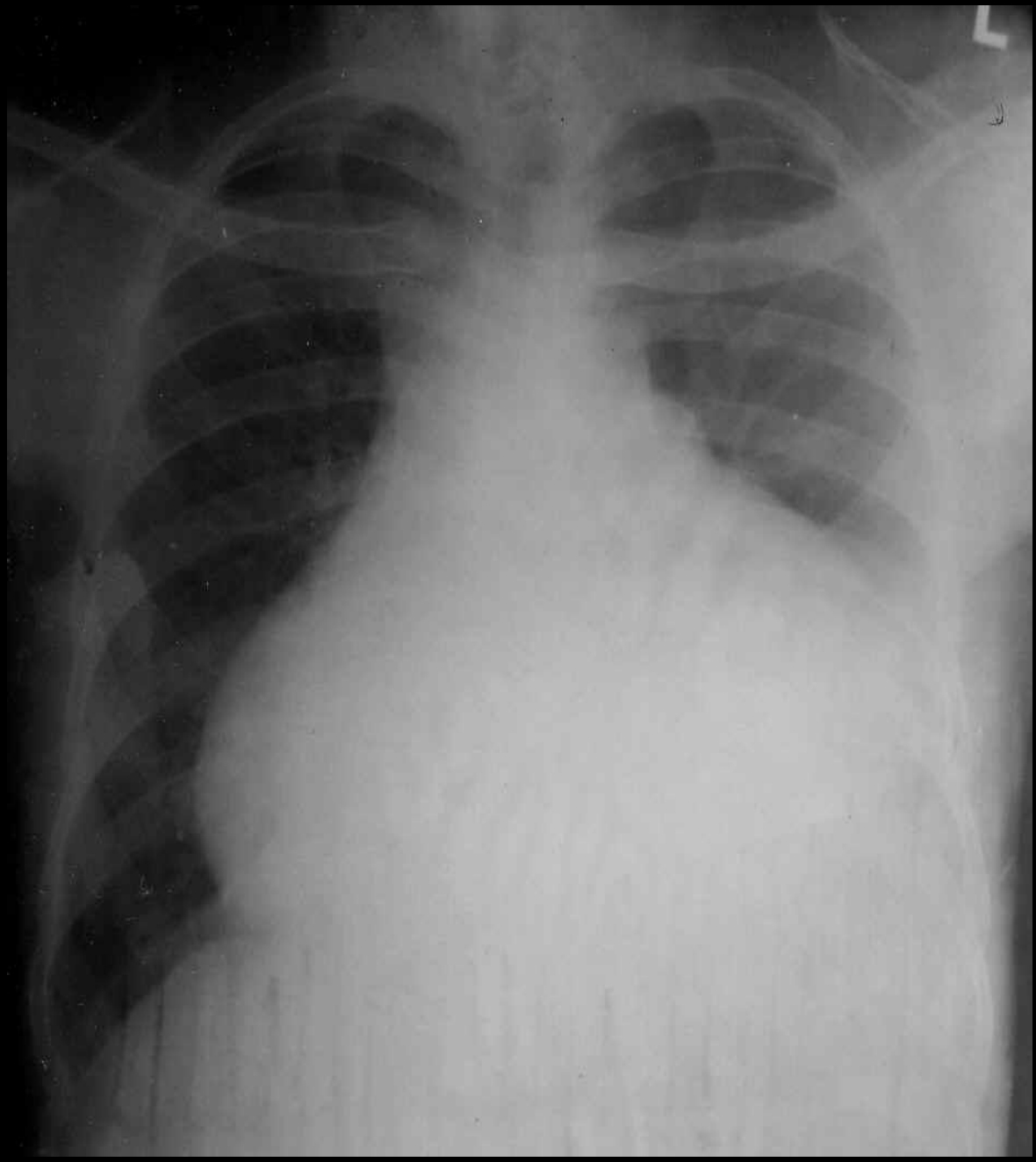
Genitourinary Tuberculosis

- Direct infection or amyloidosis
- Granulomas in glomeruli heal or caseate
- Dysuria and hematuria
- **IVP** helpful
- Upper and lower GU tract

Tuberculous pericarditis



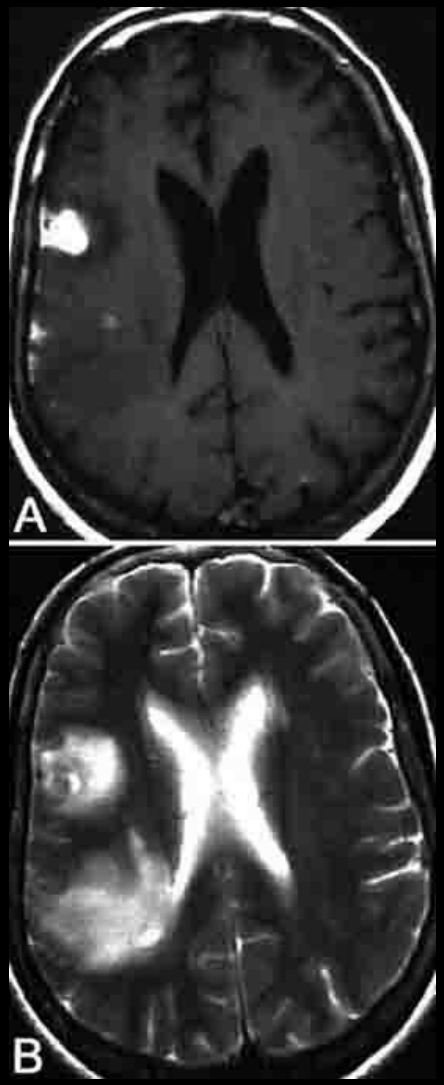
Echo is the imaging modality of choice for definitive diagnosis



Tuberculous Meningitis and Tuberculoma

TB Meningitis CSF:

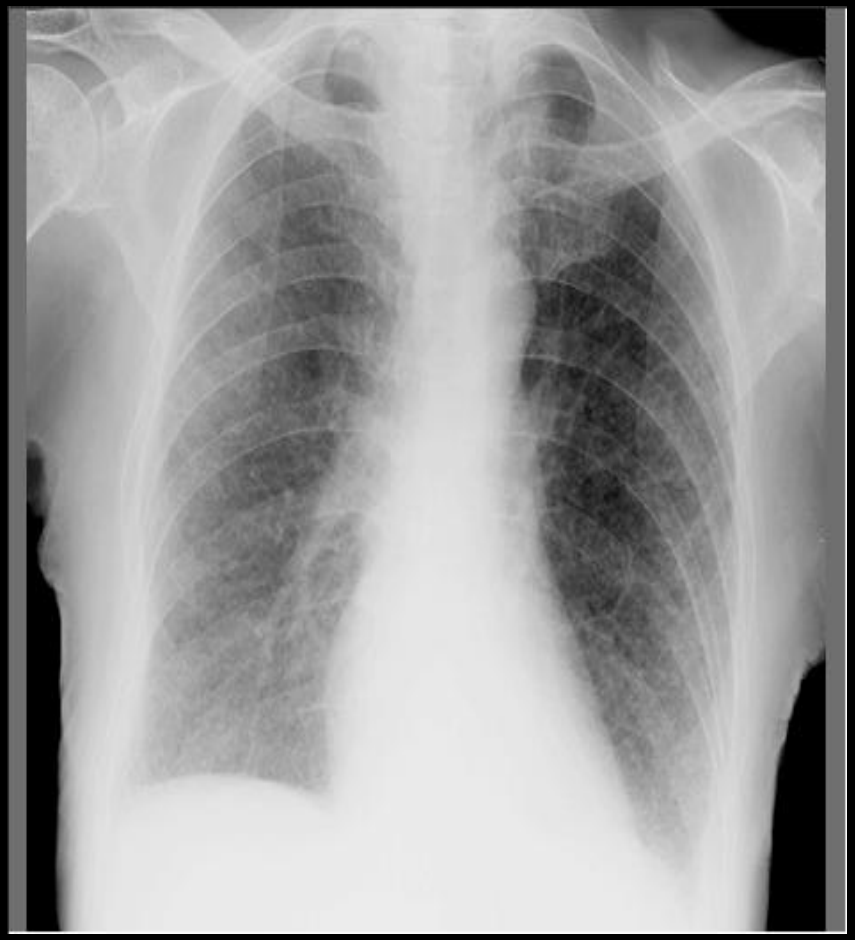
- Lymphocytic pleocytosis
- ↓ glucose
- ↑ protein
- CT or MRI: may show hydrocephalus

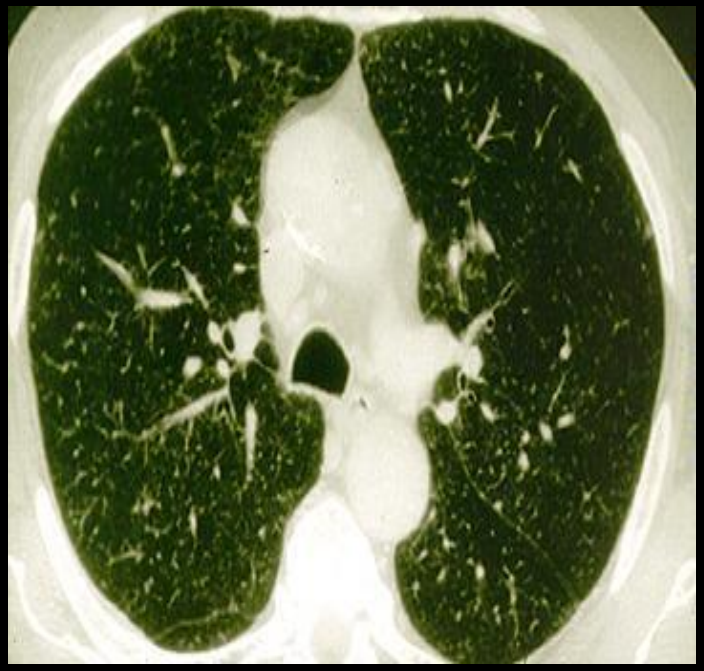


- Tuberculoma
- Seizures and focal signs
- CT or MRI: contrast-enhanced ring lesions

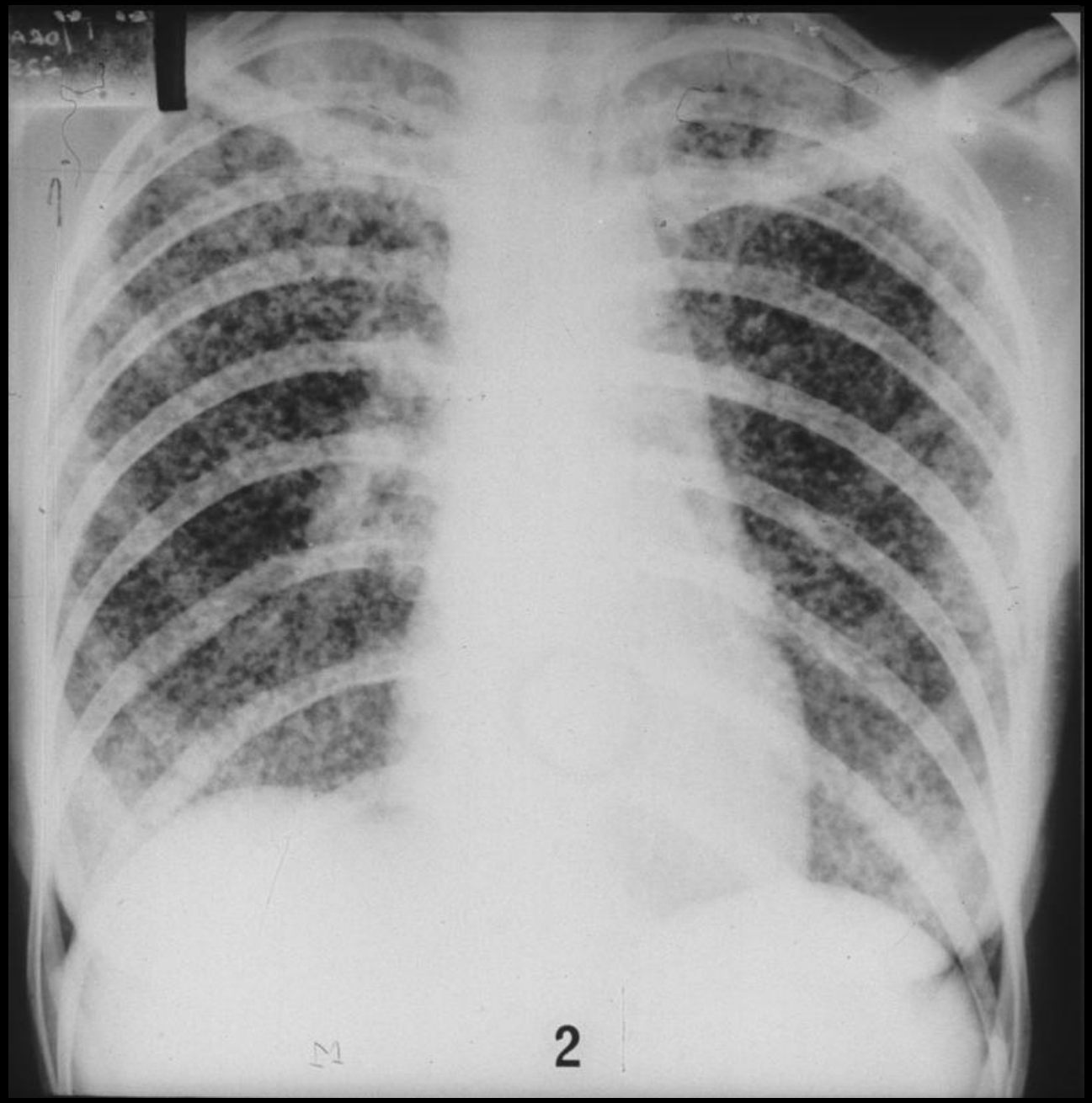
Miliary Tuberculosis

- Hematogenously disseminated TB
- Lesions are yellowish granulomas resembling millet seeds
- CXR: reticulonodular infiltrate





Miliary TB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Latent TB

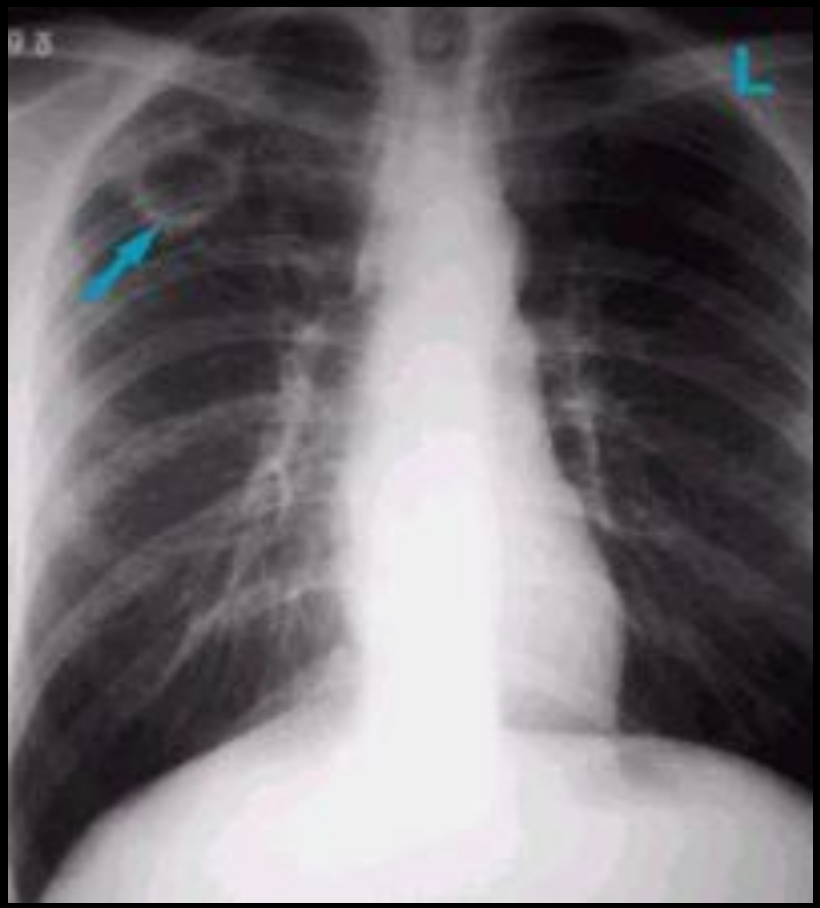
- Infection with ***no*** evidence of active TB in a patient with a ***positive*** tuberculin skin test:
- Negative CXR
- Negative sputum cultures
- Asymptomatic

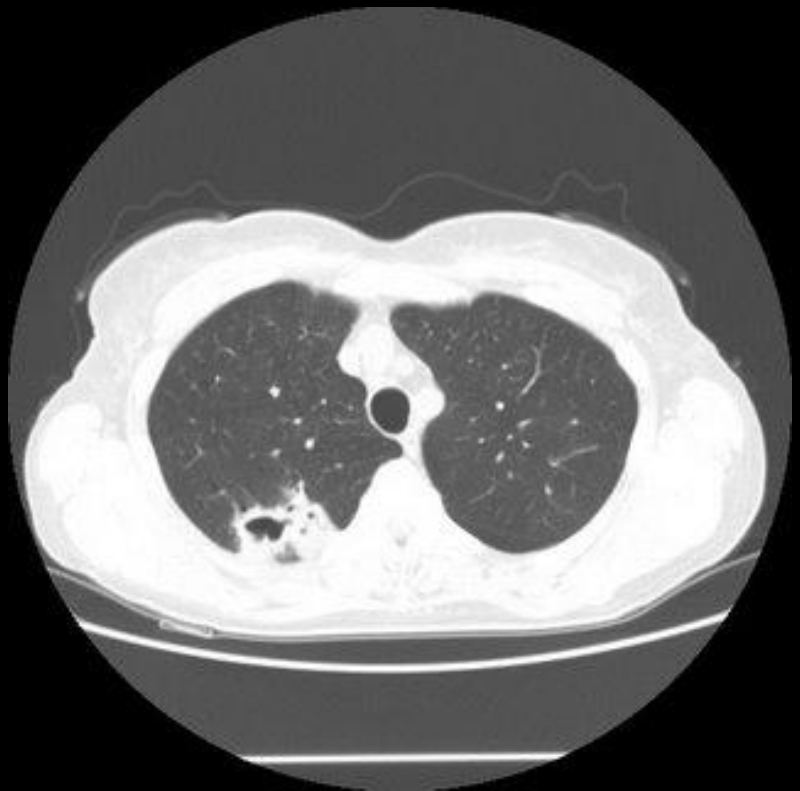
NOT infectious!

Reactivation Disease

- Previously sensitized host
- Occurs in lung apices
- Cavitary lesions
- Immunosuppression
- Exogenous reinfection
- CT scan > sensitive than plain CXR

Symptoms: cough, weight loss, fever, night sweats, chest pain, hemoptysis.



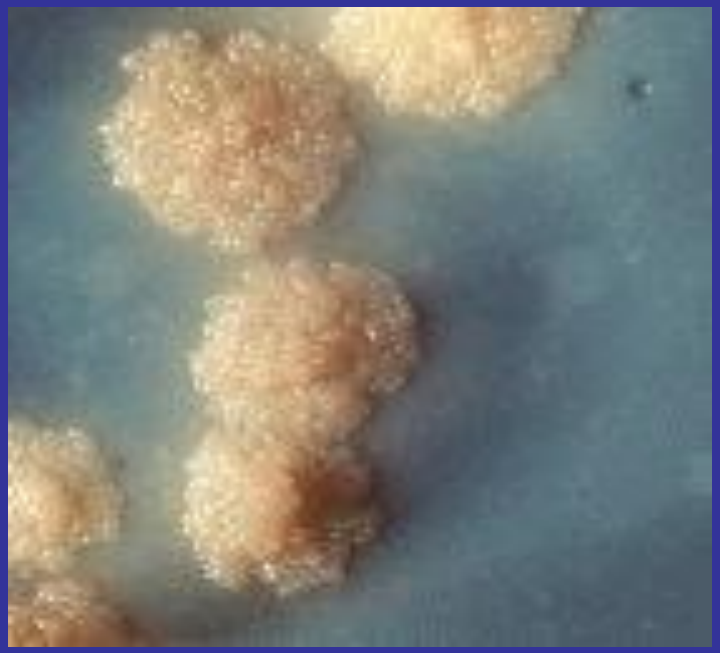


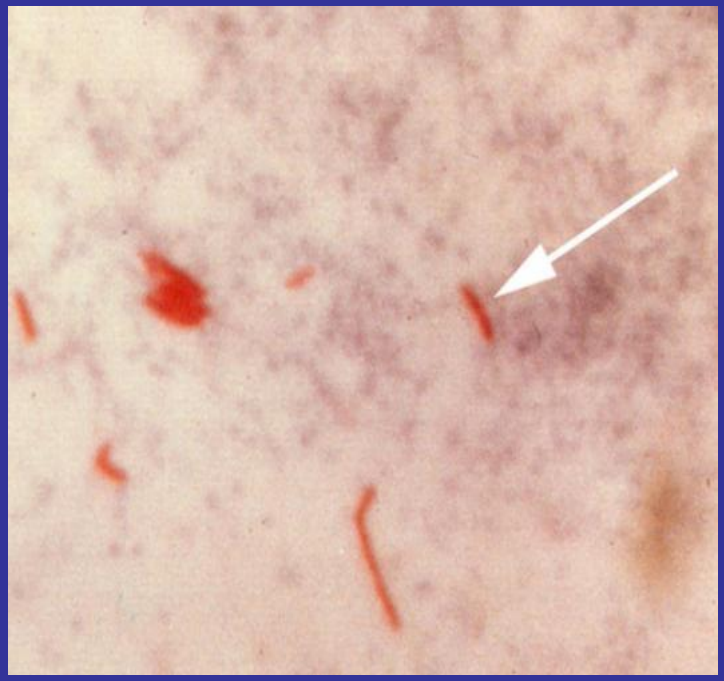
Cavitary Lesions


R

Diagnosing TB

- Tuberculin Skin Test
- CXR
- CT
- MRI
- Culture: GOLD STANDARD
- Sputum AFB smear
- Gastric aspiration





Toolbox for Diagnosis of TB Disease

History

Physical Exam

Sputa

Cultures

PCR

CXR

Pathology

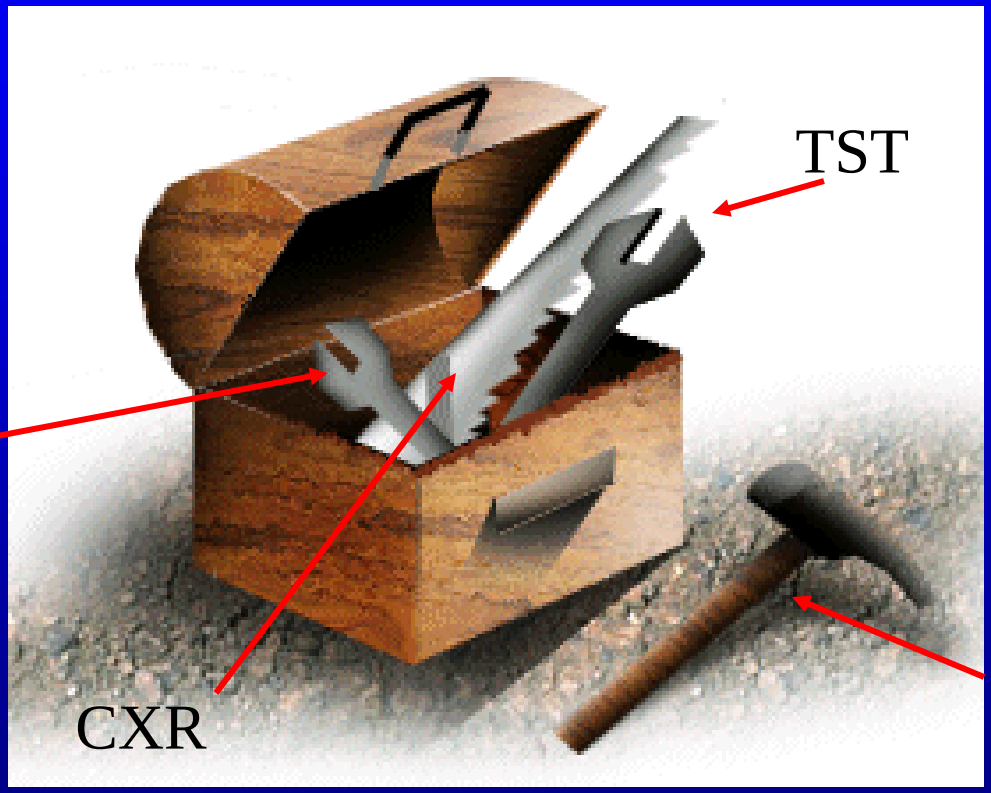
TST

IGRAs

Response to therapy

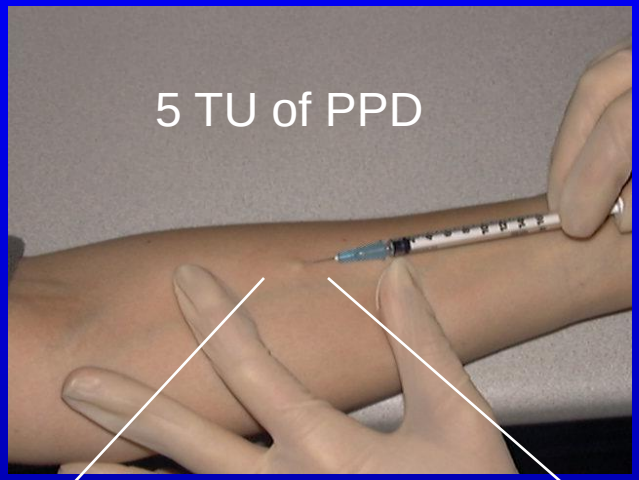
Gold Standard = Culture

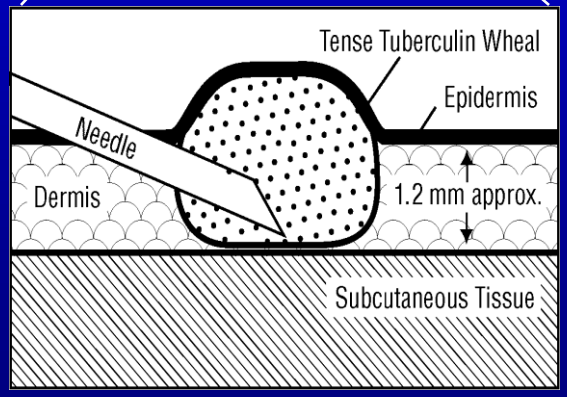
Toolbox for Diagnosis of Latent TB



No Gold Standard!

Tuberculin Skin Testing
Mantoux Method





48 to 72 hours

Interpretation depends on person's risk factors

Screening for TB
Skin Test

Figure 2. Correct measure of reaction to the tuberculin skin test.

PPD

(Purified Protein Derivative)

- “Reaction” is induration (palpable swelling), not color (erythema)
- Swelling will go away
- Is safe during pregnancy
- Is not harmful or infectious
- Once positive, will always remain positive

BCG and TST (1)

- General teaching is that reactivity from BCG wanes after a few years and is unlikely to persist > 10 years, but may be boosted by PPD.
- Study done in Switzerland* suggests that false positives due to BCG may be much more common than we thought:
  - 40% of 5000 HCW had positive TST
  - Prior BCG strongest risk factor for positive TST among those less than age 40 with TSTs $\leq$18 mm (was not as strong a risk factor for those > 40 years old and those with TSTs $\geq$ 20 mm)

*CID 2005; 40:211 – 217.

BCG and TST (2)

- Review of studies that compared TST responses to BCG during and after infancy
- Vaccination during infancy estimated to cause false-positive TST in 6.3% overall, but only 1% of those tested more than 10 years after vaccination
- Vaccination at 2 years of age or older estimated to cause false-positive TST in 40% of persons overall, 20% of those tested 10 years or more after vaccination

Farhat M et al, Int J Tuberc Lung Dis 2006; 10: 1192-204

Definitions

- “Positive PPD”: a tuberculin skin test (TST) that is indurated:
  - ≥5 mm: HIV+, recent contact of TB case, CXR c/w old TB, organ transplant or other immunosuppression
  - ≥10 mm: everybody else (in California)
- Latent TB Infection (LTBI): TB infection without evidence of clinically active disease (+PPD, but no symptoms); CXR usually normal, or may be abnormal, but sputa negative
- TB Disease: active tuberculous infection of any organ

TST: False negatives / False positives

False negatives

- Technical factors
  - Application
  - Reading
  - Improper storage of PPD
- Biological factors
  - Poor nutrition
  - Infection
  - Immunosuppressive drugs
  - Malignancy
  - Age
  - Stress

False positives

- Infection with nontuberculous mycobacteria
- BCG vaccination

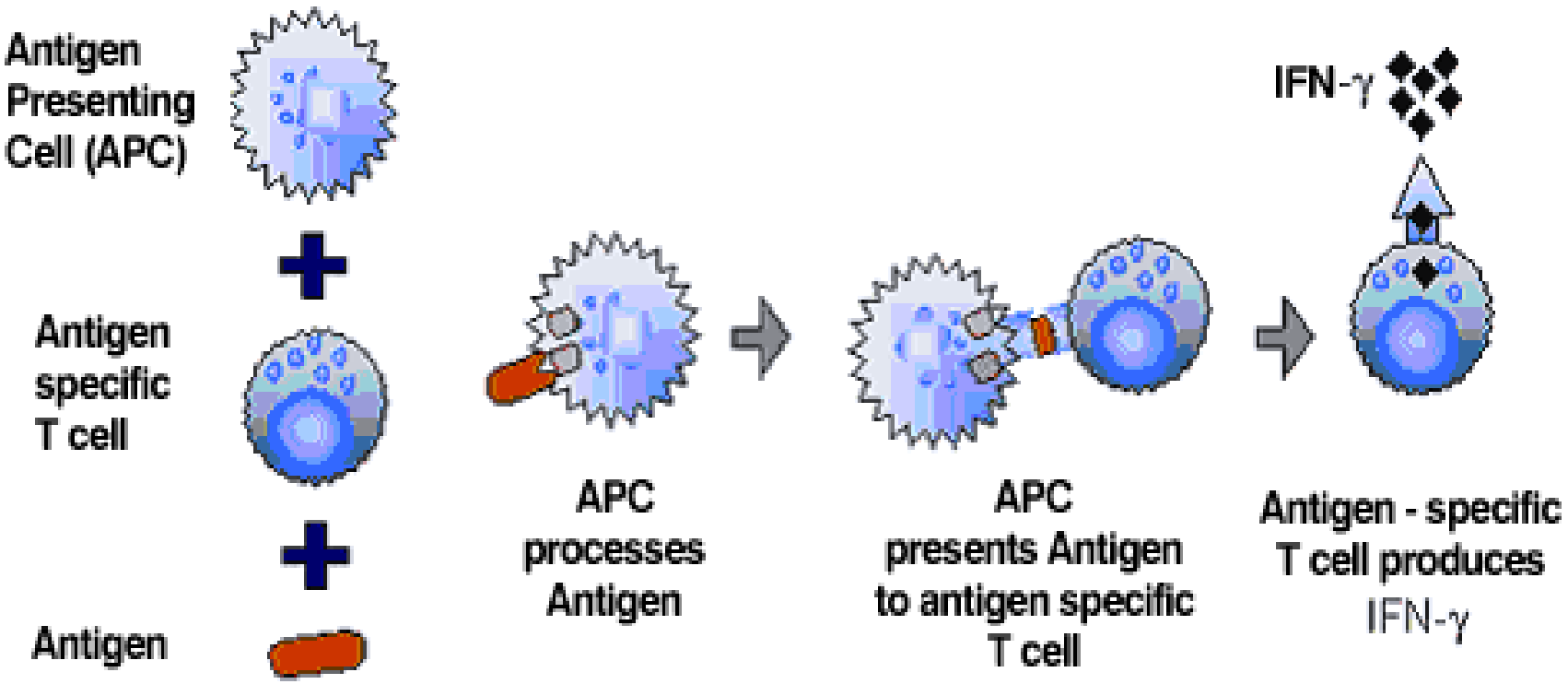
What is Quanti-FERON®-TB Gold

- Blood assay for *M. tuberculosis* > Interferon γ release assay
- *In vitro* test using whole blood specimen for the diagnosis of TB infection, whether latent or active
- Does not distinguish between latent TB infection or TB disease

Quanti-FERON®-TB Gold – Scientific Basis

- Individuals infected with *M. tuberculosis* complex organisms have lymphocytes in their blood that recognize mycobacterial antigens
- This recognition process involves the generation of interferon-γ, a specific cytokine for cell mediated immune response
- The detection and subsequent quantification of IFN-γ is the basis of this test
- The test uses synthetic peptide antigens (ESAT-6, CFP-10) that simulate mycobacterial proteins to generate the immune response

Interferon Gamma Release



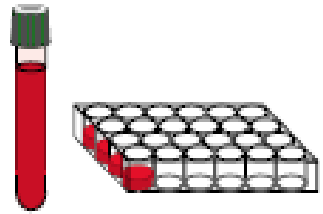
Species Specificity of ESAT-6 and CFP-10

Tuberculosis complex	Antigens	
	ESAT	CFP
M tuberculosis	+	+
M africanum	+	+
M bovis	+	+
BCG substrain		
gothenburg	-	-
moreau	-	-
tice	-	-
tokyo	-	-
danish	-	-
glaxo	-	-
montreal	-	-
pasteur	-	-

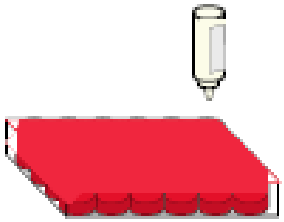
Environmental strains	Antigens	
	ESAT	CFP
M abcessus	-	-
M avium	-	-
M branderi	-	-
M celatum	-	-
M chelonae	-	-
M fortuitum	-	-
M gordonii	-	-
M intracellulare	-	-
M kansasii	+	+
M malmoense	-	-
M marinum	+	+
M oenavense	-	-
M scrofulaceum	-	-
M smegmatis	-	-
M szulgai	+	+
M terrae	-	-
M xenopi	-	-

QFT Assay

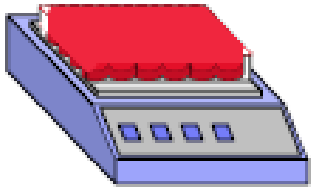
Stage One – Blood Stimulation and Harvesting



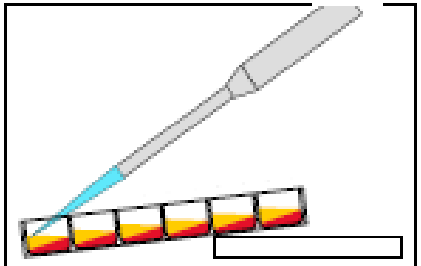
Dispense 1 mL of subject's heparinized whole blood into 4 wells of a 24-well culture plate.



Add 3 drops of the appropriate stimulating antigen to each well.

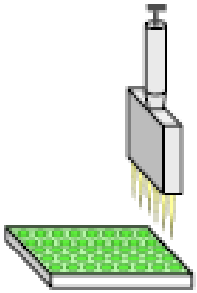


Shake covered plate for 1-2 min. Incubate for 16-24 hrs at 37°C (humidified).

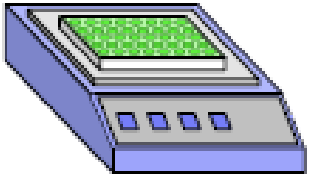


Harvest at least 200 µL plasma from each well. Store in racked microtubes or uncoated microplates.

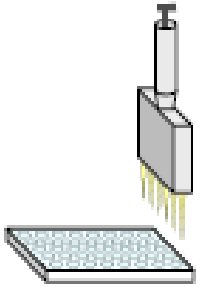
Stage Two – Human IFN-γ ELISA



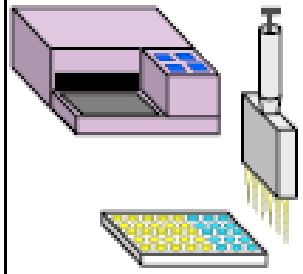
Add 50 µL of conjugate solution to each well. Add 50 µL of plasma or standard.



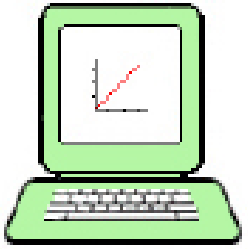
Shake covered plate for 1 min. Incubate for 120 minutes at Room Temperature.



Wash plate ≥6 times. Add 100 µL of substrate. Incubate 30 min. at Room Temperature.



Add 50 µL of stop solution. Read absorbance within 5 min at 450nm (620-650nm ref).



Calculate Results using standard analysis programs (QFT-Gold Analysis Software available soon).

Results and Interpretation

RESULT	INTERPRETATION
POSITIVE	**ESAT-6 and/or CFP-10 responsiveness detected** ***M. tuberculosis* infection likely**
NEGATIVE	**No ESAT-6 or CFP-10 responsiveness detected** ***M. tuberculosis* unlikely**
INDETERMINATE	**MTB infection status cannot be determined as a result of impaired immunity and/or incorrect performance of the test**

QFT-G Sensitivity Estimates

Reference	Population	+ IFN-γ (n)	+ TST (n)
Mori; 2004	Untreated Cult+TB; Japan	89% (118)	66% (76)
Kang; 2005	Pulmonary TB; Korea	81% (54)	78% (54)
CDC; Unpub.	Untreated Cult+TB; US	81% (41)	81% (41)
Ravn; 2005	Active TB; Denmark	85% (48)	Not done
Lee, 2006	Active TB, Korea	70% (61)	67% (58)
Menzies* 2007	Meta-analysis (9 studies)	80% (393)	74% (394)

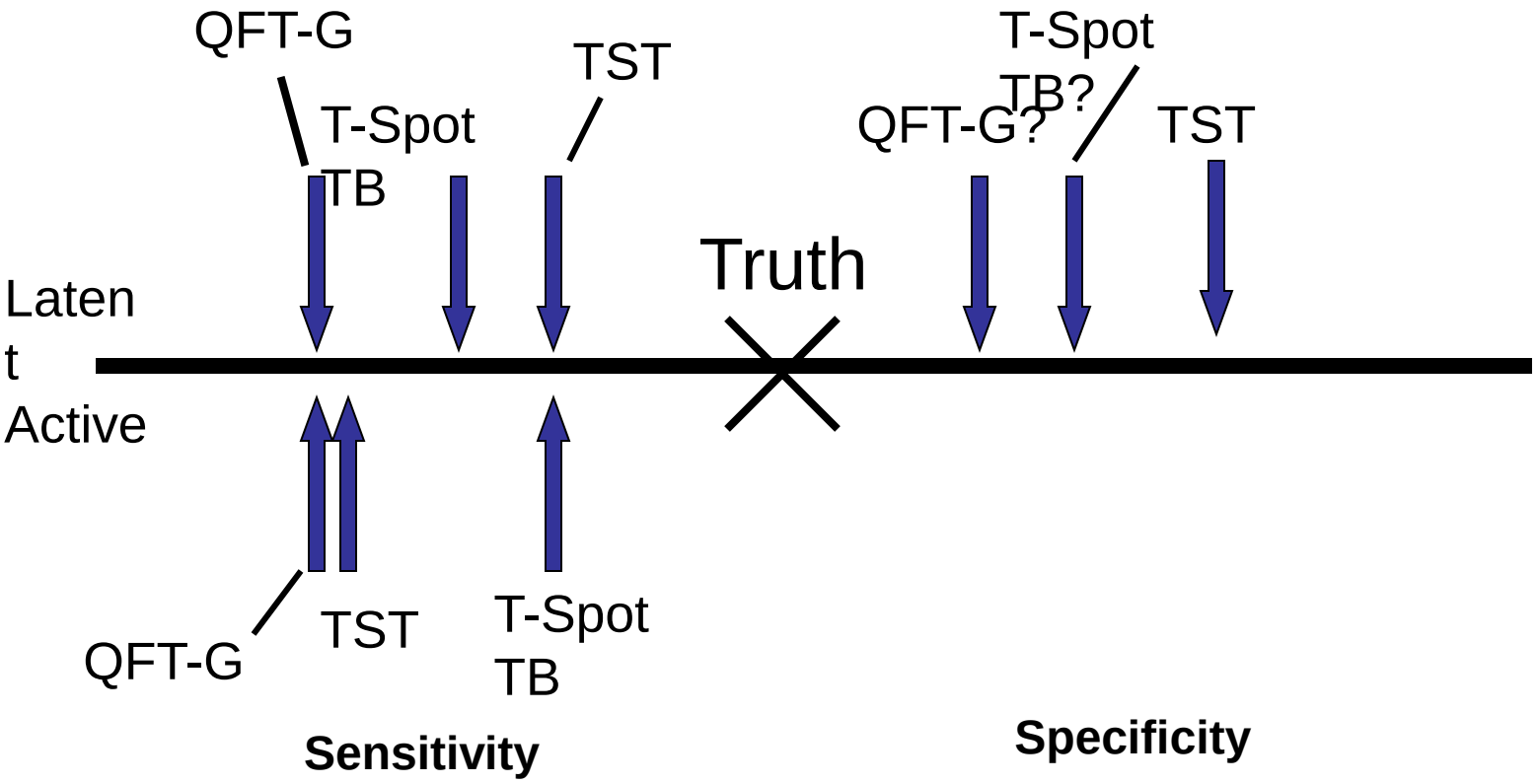
*Menzies, D. et al, Annals of Int Med 2007;146 (5): 340-354

QFT-G Specificity Estimates

Reference	Population	+ IFN-γ (n)	+ TST (n)
Mori; 2004	Nursing Students; Japan	2% (213)	65% (113)
Kang; 2005	Med Students; Korea	4% (99)	51% (99)
CDC; Unpub.	Navy recruits; US	.2% (532)	.9% (532)
Menzies* 2007	Meta-analysis (9 studies)	3% (711)	+BCG: 44% (516) No BCG: 2% (156)

*Menzies, D. et al, Annals of Int Med 2007;146 (5): 340-354

Diagnosis of TB: The Truth*?



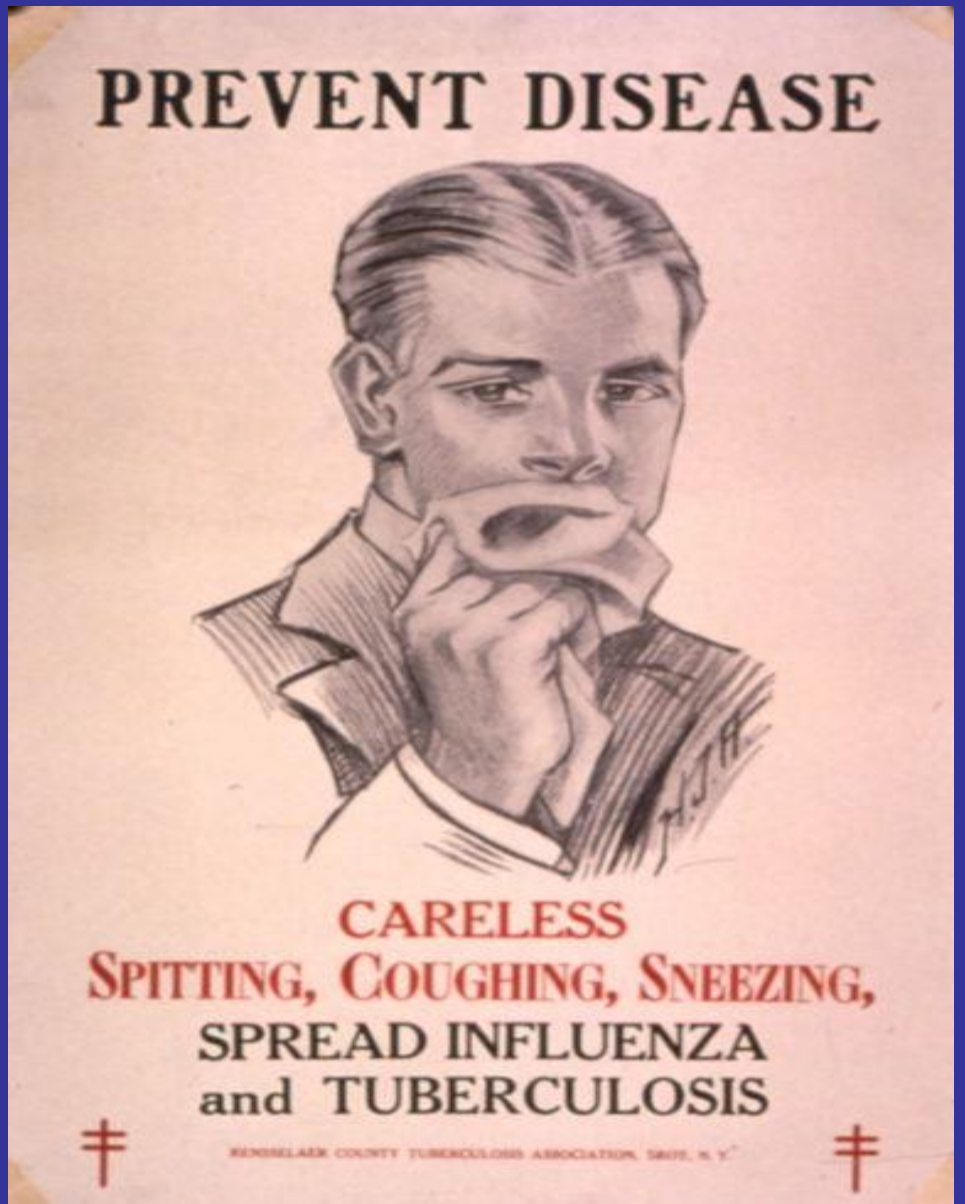
* My opinion only, based on impression of available data

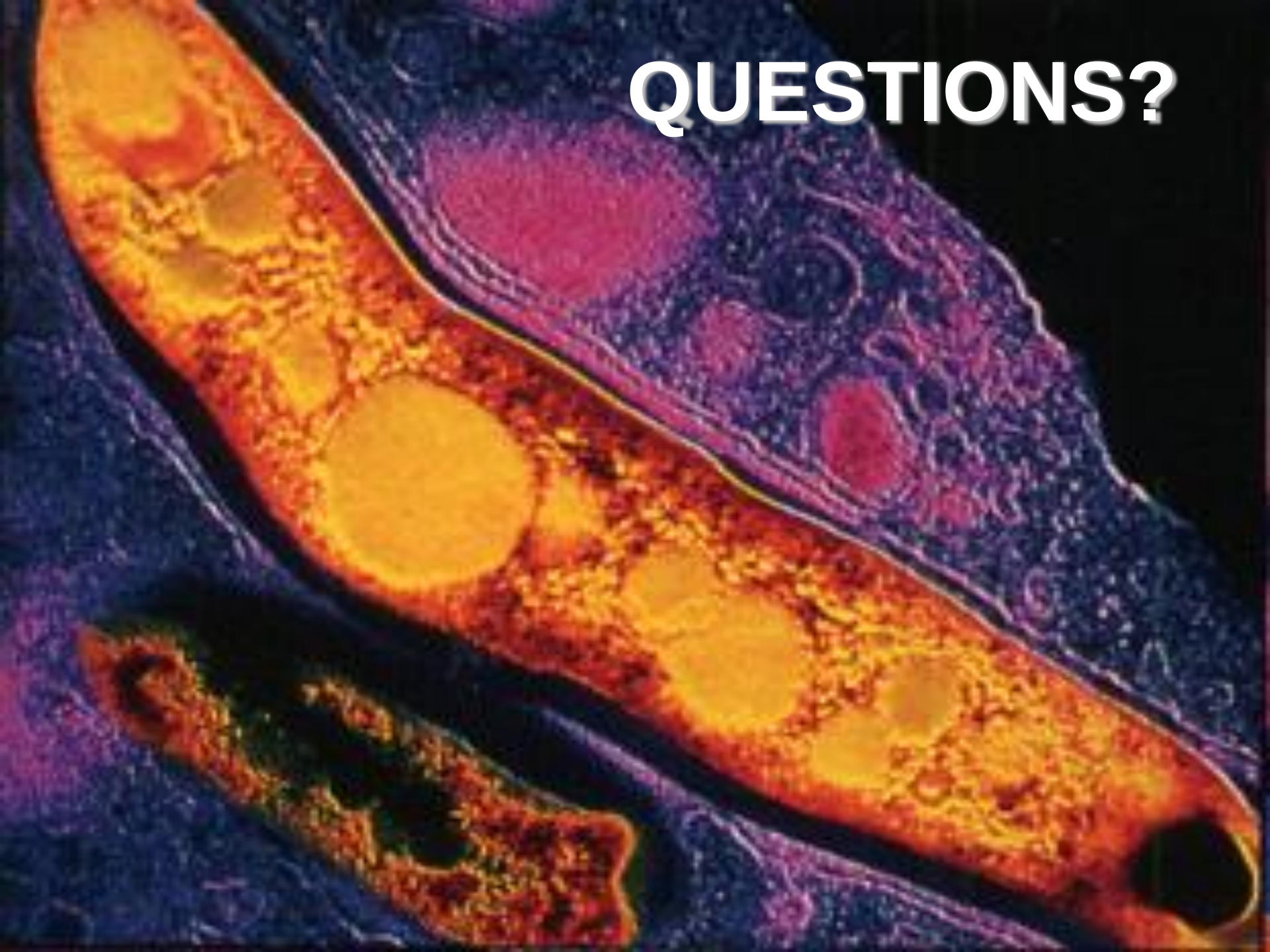
Treatment of TB

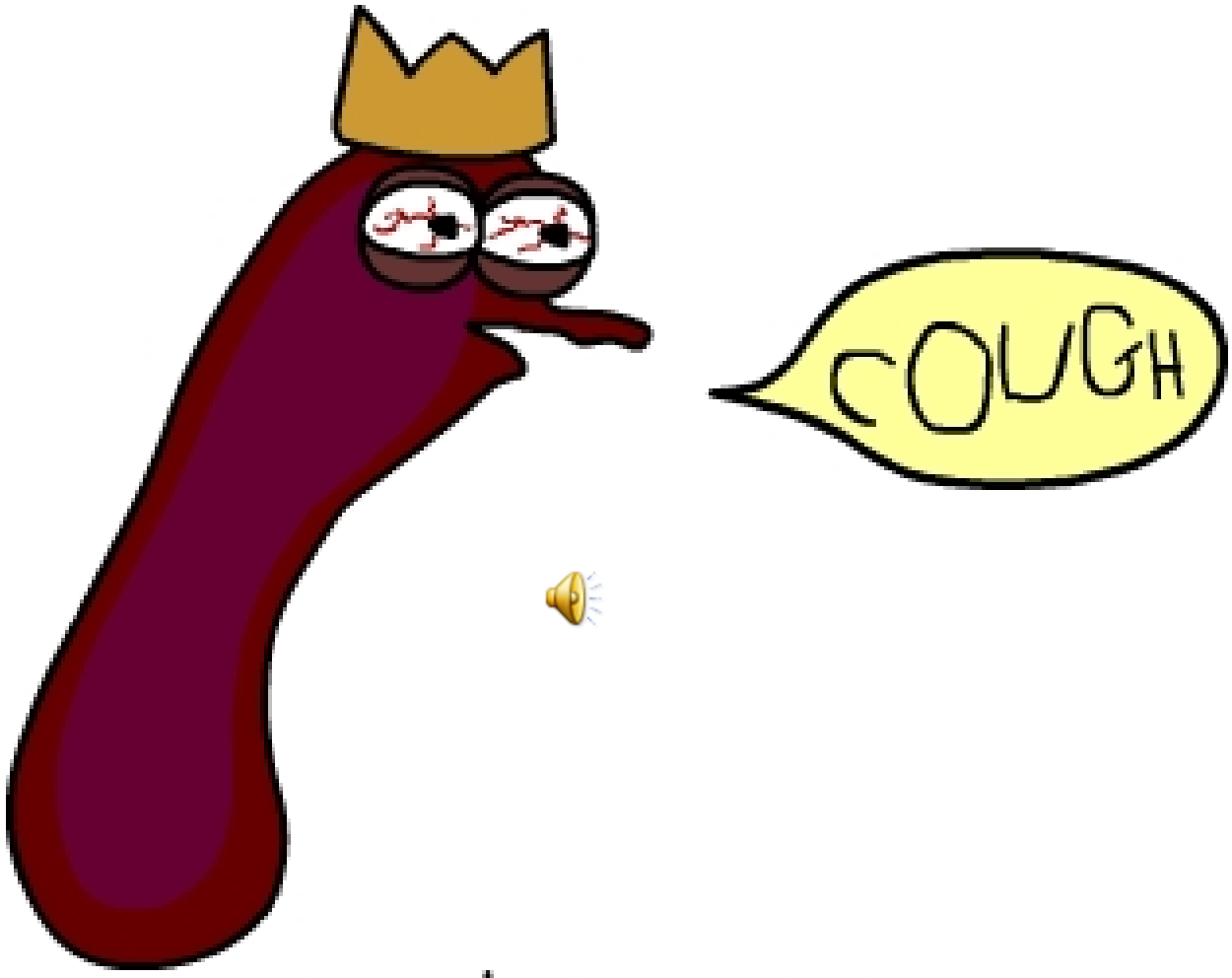
- **Initial phase:** 2 months
  - Rifampin, Isoniazid, Pyrazinamide, Ethambutol
- **Continuation phase:** 4 or 7 months
  - Isoniazid and Rifampin
- **Latent TB:** 6 months
  - Isoniazid
- **Directly Observed Therapy**
- **MDR-TB vs. XDR-TB**

Prevention

- Isolate and treat
- BCG Vaccine
- Treat Latent TB




QUESTIONS?



mycobacterium tuberculosis