INTERPRETING INTERFERON GAMMA RELEASING ASSAYS

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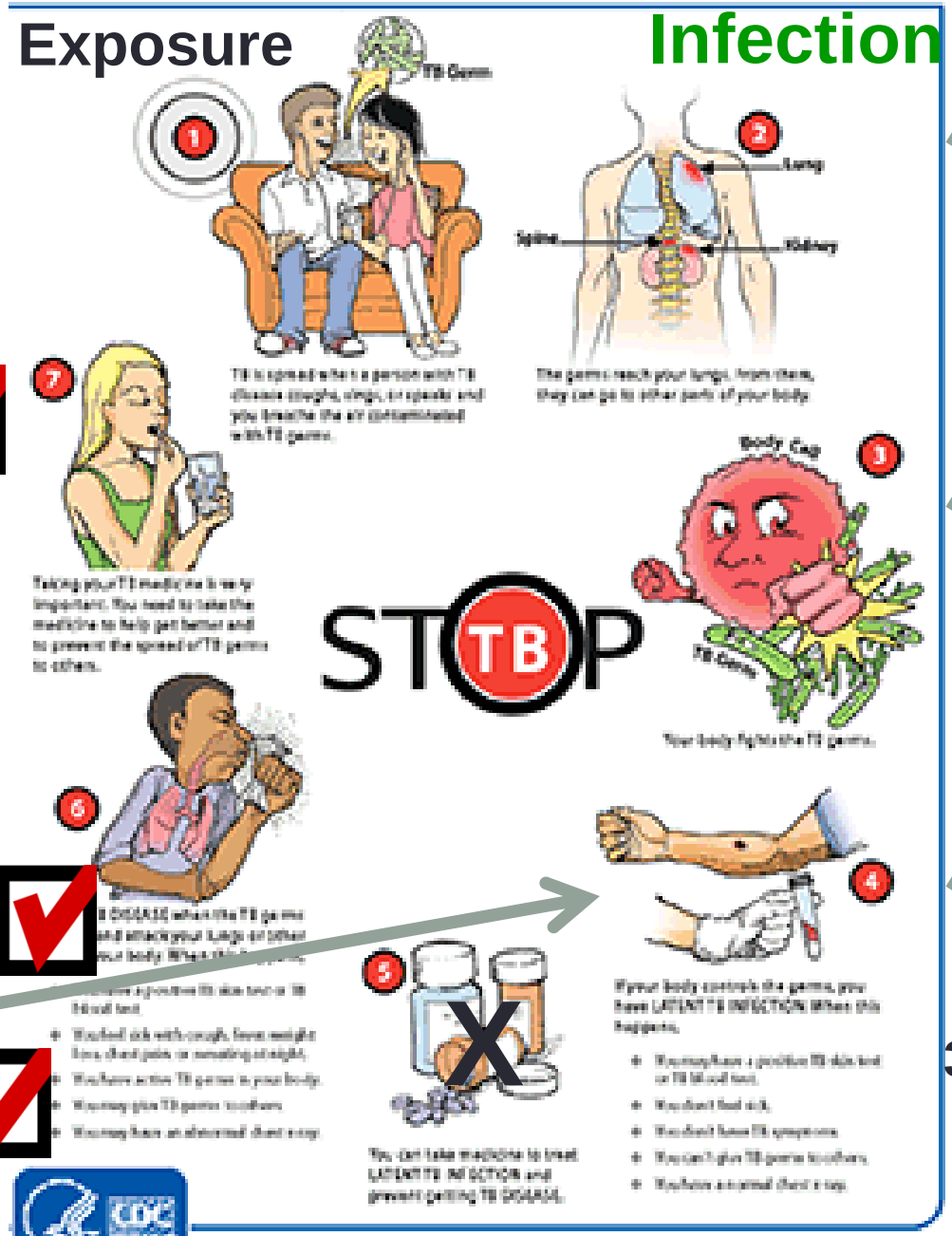


Treated LTBI or Treated TB

Post Primary Disease or Reactivation Tuberculosis

Latent Infection

If treatment is not given

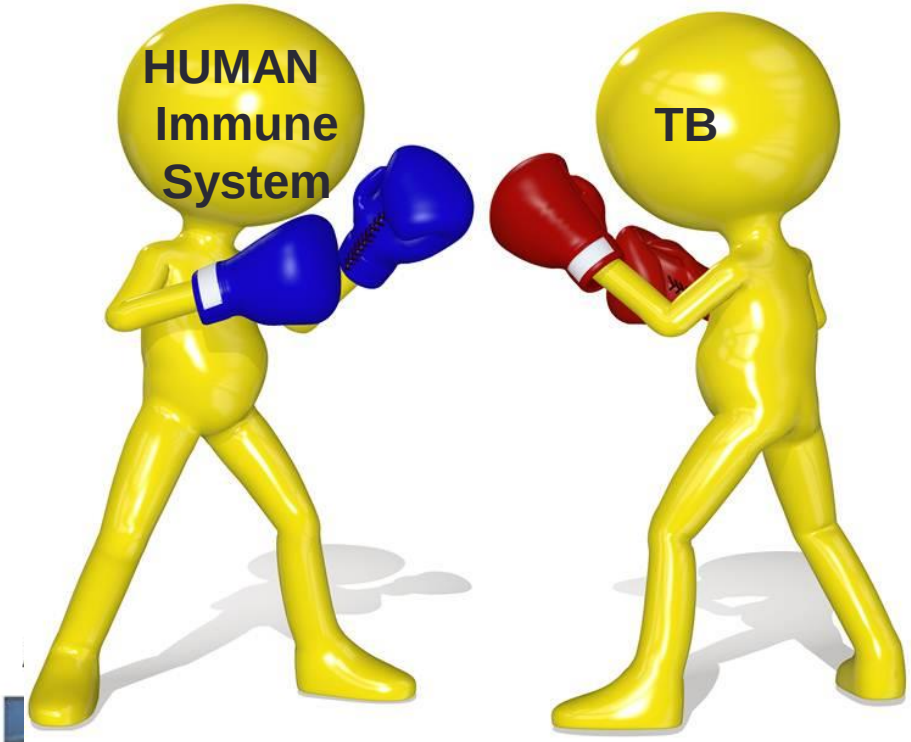
Primary Disease (Tuberculosis) Innate Immunity

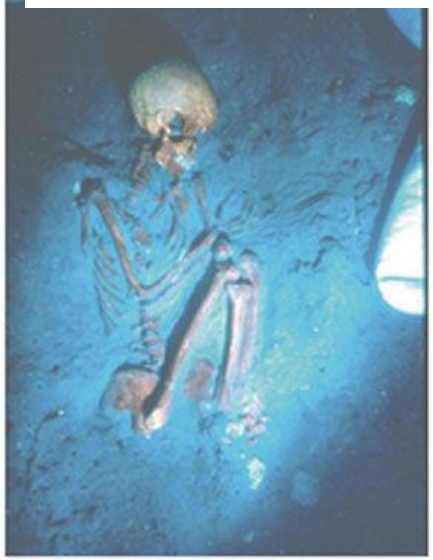
T Cells are being "Primed" & Sensitized to TB Antigens

3-8 weeks Adaptive (cellular) Immunity

MTB vs. Host

- Mtb – the most successful intracellular bacterium
- It uses & evades the immune system, exists protected in the host in latent form, & infects others
  - ~1/3 world's people
- DNA evidence of Mtb in humans 9,000 years ago

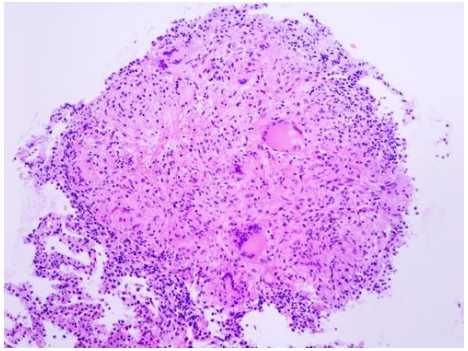


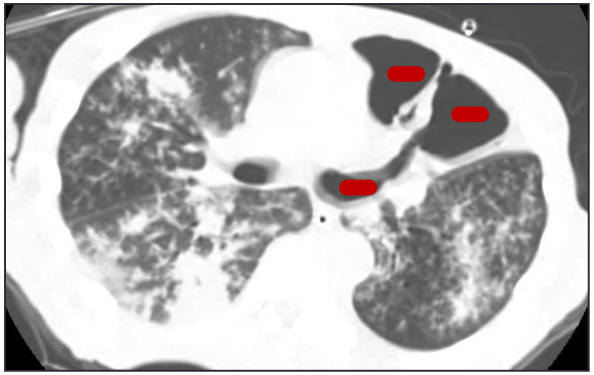


Tel-Aviv University

“Dynamics of a host-pathogen collusion”

- Ehlers S, Schaible UE. (2013) Frontiers in Immunology 3, 1-9
  - “A granuloma is defined as an inflammatory…infiltrate that, while capable of limiting growth of *Mycobacterium tuberculosis*, also provides a survival niche from which the bacteria may disseminate. The tuberculosis lesion is highly dynamic and shaped by both immune response elements and the pathogen…To secure transmission to a new host, *M tuberculosis* has evolved to drive T cell immunity to the point that necrotizing granulomas leak into bronchial cavities to facilitate expectoration of bacilli.”





The Immune Response in TB: Basic Terms (Cell-Mediated Immunity)

- **Antigen -** protein that induces an immune response
- **Amino Acid** – building block of protein
- **Peptides** - two or more amino acids joined by a peptide bond (–NH–CO–) **Used in IGRAs as antigen.**
- **Cytokines -** small proteins that are released by cells and affect the behavior of other cells (signaling)
- **Interferon gamma (IFNγ)** – a cytokine produced by T lymphocytes during the immune response in TB.
  - **This is what is detected & measured by the IGRAs**
- **Tumor Necrosis Factor alpha (**TNFα) – a cytokine produced by T lymphocytes that prevents Mtb dissemination

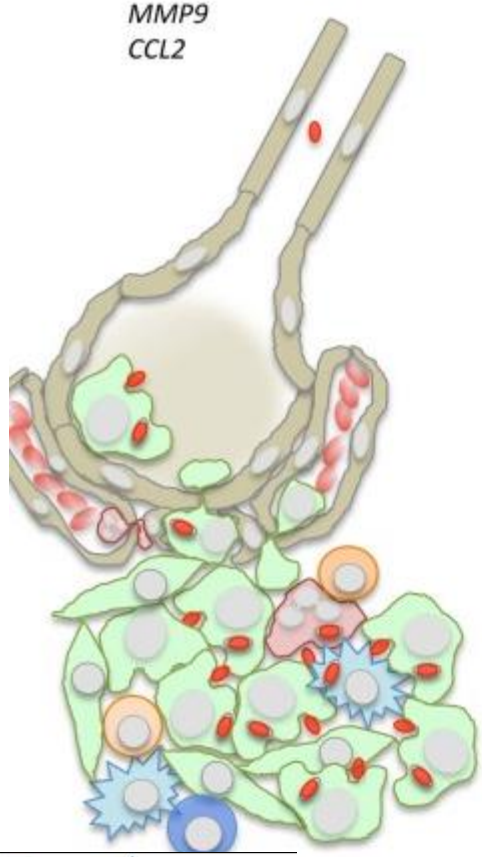
To Understand IGRAs: Understand Immune Response to Mtb

- **Innate immune system** – starts early after infection
  - Does not involve specific immunity
  - Early granuloma forms
  - Overall effect is to promote Mtb replication & dissemination
- **Adaptive immunity –** occurs later
  - Specific, anti-Mtb immunity
  - Its persistence is needed to maintain state of latency (control & containment of Mtb)
  - Memory cells are generated that will recognize Mtb antigens if they encounter them again
  - T Lymphocytes (CD4+ and CD8+) => **IFNγ**, IL-2, TNFα

Primary Tuberculosis

Inflammatory infiltrate
Mtb Replication
Mtb Dissemination
Mtb Growth Control

MMP9
CCL2



Secondary Tuberculosis

Protective Granuloma
Mtb Growth Control
Mtb Elimination
Fibrotic Transformation
Calcification

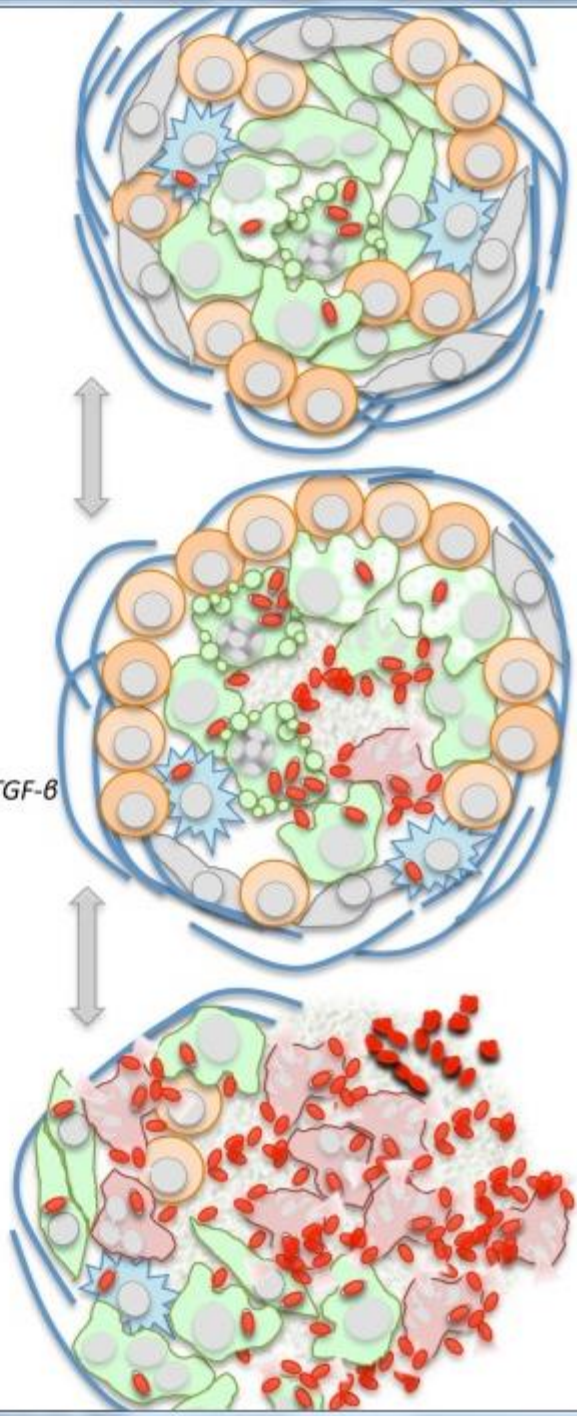
IFN-γ, TNF-α, Granulysin

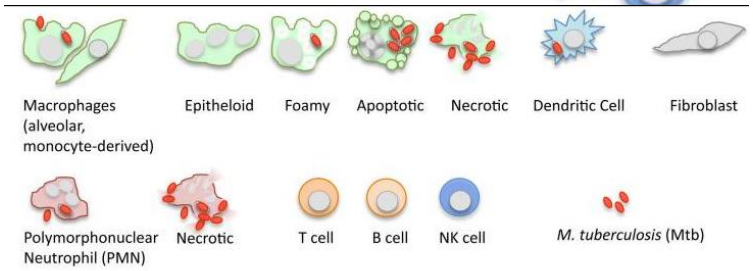
Homeostatic Granuloma
Immune Balance
Latency
Mtb Sequestration
Mtb Dormancy
Mtb Metabolic adaption

T_{reg} cells
IFN-γ/TNF-α vs. IL-4/IL-10/TGF-β

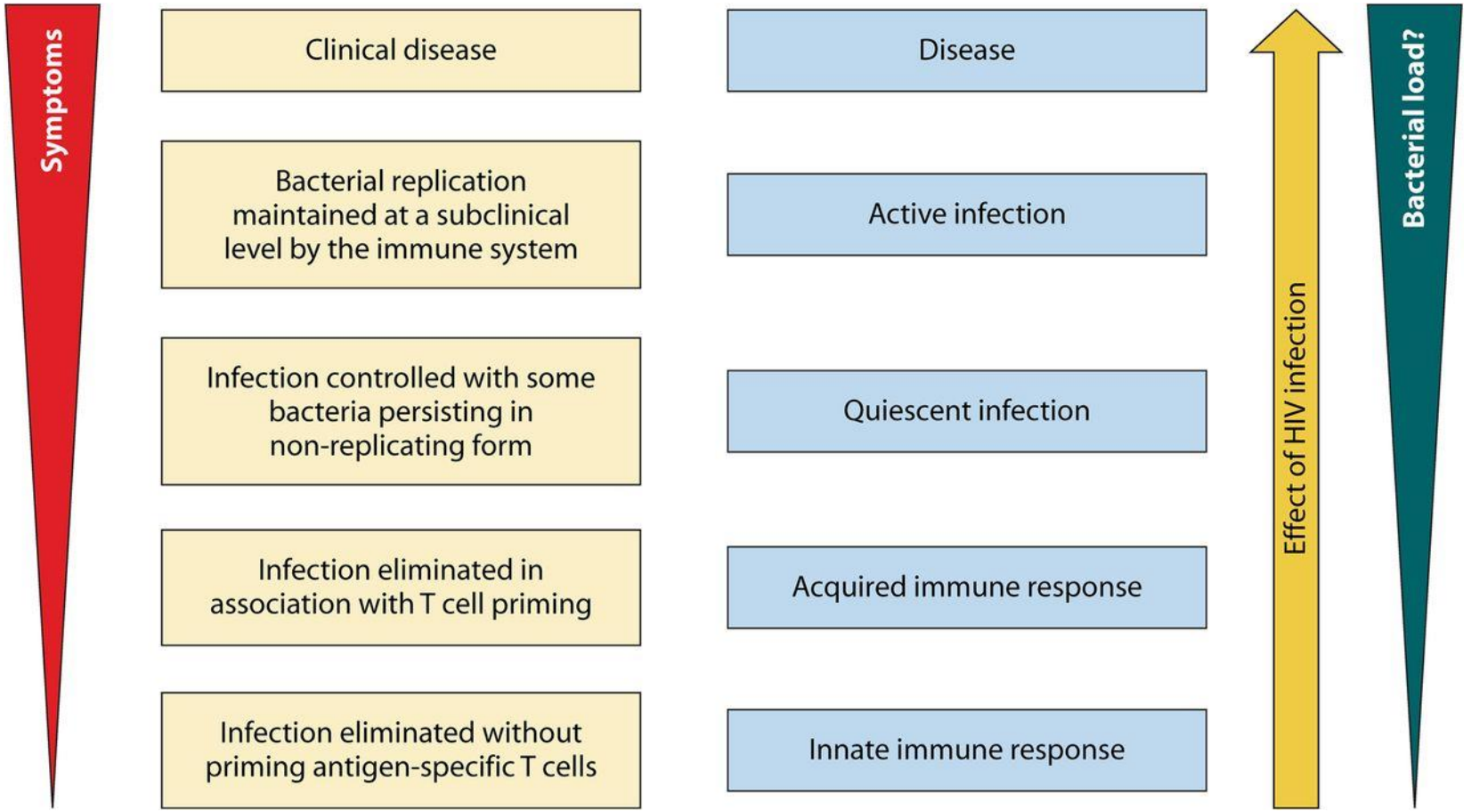
Transmissive Granuloma
Immunopathology
Neutrophil Dominance
Mtb Recrudescence
Mtb Growth

Immuno suppression
TH1/TH2 dysbalance
Superinfection
Hyperimmunity





TB Infection as a Spectrum



Madhukar Pai et al. Clin. Microbiol. Rev. 2014;27:3-20
(original Barry CE et al. Nat Rev. Microbiol 2009, 7, 845-55.)

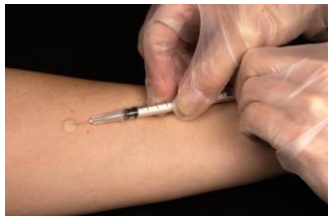
Tests for TB Infection

QuantiFERON®-Gold In-Tube Test (QFT-GIT)

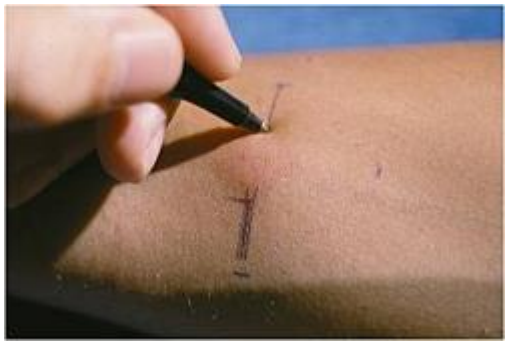
FDA Approved 10/2007

T-SPOT®.*TB* test (T-Spot)

FDA Approved 07/2009



Used for > 1 Century



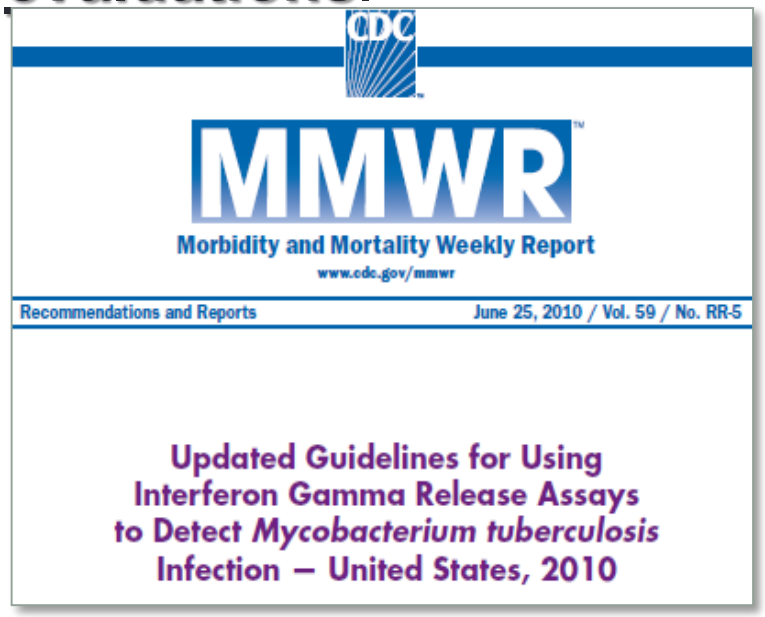
PPD TB Skin Tests & IGRAS

- There is no gold standard for diagnosing LTBI
- These are imperfect tests for diagnosing LTBI
- They indicate a cellular immune response to MTB
- They have a reduced sensitivity (ability to detect LTBI) in immunocompromised persons
- Alone they have a low ability to predict progression to active TB
  - The clinical picture identifies risk factors

- **These tests are indirect. They provide immunologic evidence of host sensitization to TB antigens. They depend on cell-mediated immunity (memory T cell response). They don't distinguish latent infection from treated infection from active disease or from treated active disease.**

PPD Skin Tests and IGRAS: Tests for Detecting Mtb Infection

- Cannot be used to monitor success of treatment
- Intended use is as "indirect tests for *M. tuberculosis* infection (including infection resulting in active disease) when used **in conjunction with risk assessment, radiography, and other medical and diagnostic evaluations**."*

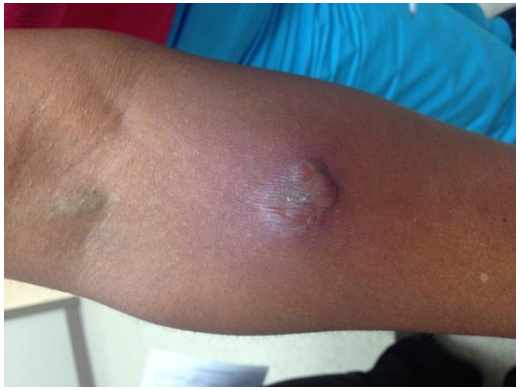


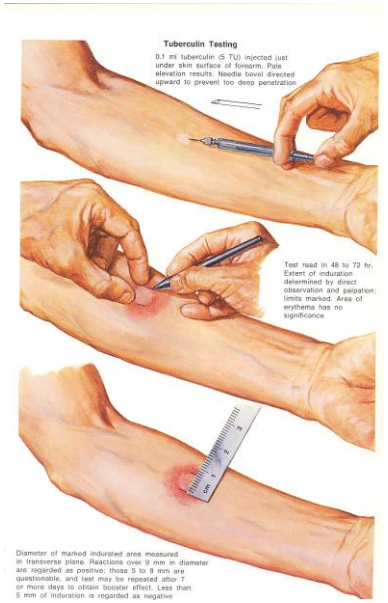
MMWR 2010; 59 (No.RR-5)

Problems with PPD

- Antigens in PPD are also present in BCG vaccine & non-tuberculous mycobacteria => false+ tests
- Proper storage & administration are required
- 2 visits are needed
- Measurement & interpretation of reaction is difficult
- Results are not routinely reported properly or maintained in medical record

Reactions can be severe





IGRAs Were Developed to: Overcome Problems with PPD Skin Test

- Antigens used in the IGRAs are not shared with BCG or most NTMs
- Cross-reacting antigens not completely eliminated, however => possible false + tests
  - M kansasii
  - M szulgai
  - M marinum

  Remember these exceptions for the remainder of this presentation.
- There is no need for a 2nd visit
- Scarring is avoided

IGRAS Test for “Mtb Complex”

- Nucleic amplification tests, PCR tests, and IGRAs detect Mtb Complex.
- Mtb Complex includes
  - Mtb = *Mycobacterium tuberculosis*
  - M bovis
  - M Africanum
  - M mcroti
  - M canetti

Underlying Concept of IGRAs: General

- Mixing blood cells from people who were **never** infected with Mtb with specific Mtb antigens will **not** produce an immune reaction

  - People who were never infected with Mtb have **not** developed adaptive immunity (cell mediated immunity, specific immunity) to Mtb.
  - Their memory cells were **not** sensitized to Mtb and will **not** recognize Mtb antigens

- Mixing blood cells from people who **were** infected with Mtb with antigens that are specific to Mtb **will** cause a reaction

  - Their memory cells were sensitized to Mtb and will recognize Mtb antigens
  - Requires an intact immune system

Underlying Concept of IGRAs: Mtb Antigens & Interferon Gamma (IFNγ)

- **ESAT-6** (early secretory antigenic 6 kDa) and

  CFP-10 (culture filtrate protein 10)*

  are 2 small antigenic proteins that are secreted by Mtb and not by BCG or most NTMs. (Remember those exceptions)

* Encoded by gene in Region of Difference 1 (RDI)

- **Interferon gamma (IFNγ)** is an important cytokine that is secreted during the immune response to Mtb.

- If we mix blood from people who were infected with Mtb with **ESAT-6** and **CFP-10** we will recreate the immune response to Mtb and **IFNγ** level will rise.
  - **TB Antigen** in QFT GIT
  - **Panel A and B** in T-Spot

Labels in QFT GIT and T-Spot

Underlying Concept of IGRAs: Positive and Negative Controls

- If we mix blood with heparin & dextrose we will have a measure of the background (unstimulated) amount of IFNγ
  - **Negative Control**
  - **NIL**

Labels in QFT GIT and T-Spot

- **Mitogens** – potent stimulators of T-cell activation and proliferation independent of antigenic specificity.
- **Phytohemagglutinin** is a useful mitogen to test the adequacy of the immune system.
- If we mix blood with the mitogen phytohemagglutinin the IFNγ level should rise if the immune system is intact.
  - **Positive Control**
  - **Mitogen**

Labels in QFT GIT and T-Spot

Quantiferon Gold-In-Tube (QFT GIT)

- Uses a single mixture (cocktail) of 14 synthetic peptides
  - Represents the entire amino acid sequences of ESAT-6 & CFP-10 ***plus*** parts of TB7.7 (RD4)
- Uses 3 blood collection tubes
  - **Nil** (negative control) contains heparin & dextrose
  - **TB antigen** contains the peptide cocktail
  - **Mitogen** (positive control) contains heparin, dextrose, phytohemaglutinin
- 1 ml. blood is added to each tube (0.8-1.2 ml. is OK)
  - Each tube is shaken 10 times, enough to coat the inside of the tube
  - Purge must be used if butterfly is used to draw blood
  - Blood is incubated for 16-24 hours (can be held for 16 hours)
  - Centrifuged, IFNγ in plasma measured by ELISA, reported as IU/ml

Interpreting Results

- Subtract the nil plasma concentration of IFNγ from the TB antigen and mitogen tubes.
  - Reported as:
  - TB Antigen minus nil and Mitogen minus nil

Interpretation: QFT-GIT: No Borderline or Intermediate Results

Interpretation	NIL	TB Response (TB Antigen) - Nil	Mitogen Response - Nil
Positive	≤8	≥.35 IU/ml & ≥25% of Nil	Any
Negative	≤8	<.35 IU/ml or <25% of Nil	≥0.5
Indeterminate	≤8	<.35 or <25% of Nil	<0.5
Indeterminate	>8	Any	Any

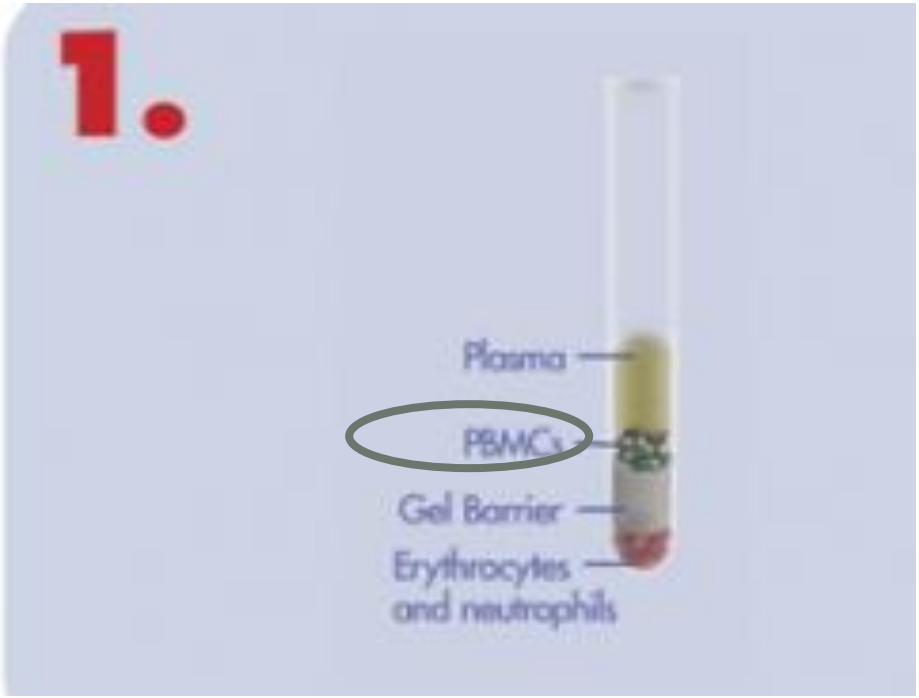
NIL = plasma IFN-gamma concentration in nil tube
TB Response = plasma IFN-gamma concentration in TB Antigen tube ***minus*** Nil level
Mitogen Response = plasma IFN-gamma concentration in Mitogen tube ***minus*** Nil level

T-Spot

- Uses 2 separate mixtures of peptides.
  - 1 represents the entire amino acid sequence of ESAT-6
  - 1 represents that of CFP-10
- Blood is drawn into a standard green top tube by standard phlebotomy technique (no need for purges)
- Blood is sent to a central laboratory
- Uses peripheral blood mononuclear cells (PBMCs), which are incubated with controls & 2 separate mixtures of peptides

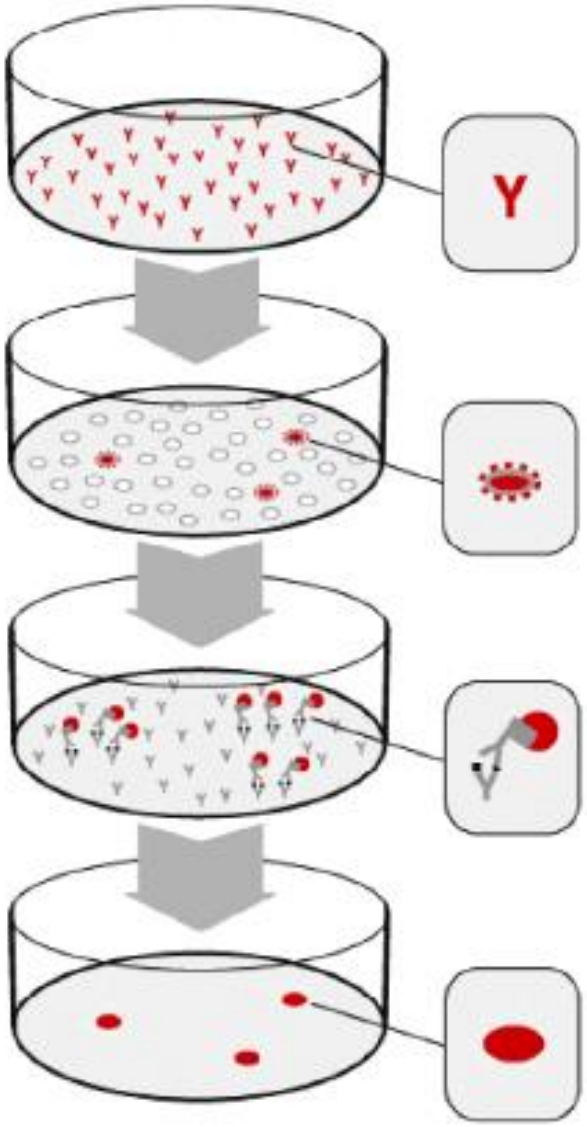
T-Spot Process

- Peripheral blood mononuclear cells (PBMCs) are separated, washed, counted, & innoculated into 4 separate microfilter wells.



T-Spot Process

A. Wells are pre-coated with antibodies to IFNγ. Captures any IFNγ produced later
B. Either ESAT-6 or CFP-10 is added to stimulate PBMCs
C. Incubated. PBMCs & antigen washed away. Conjugated antibody to IFNγ is added & binds to IFNγ secreted by the PBMCs.
D. Substrate added to produce spots.



T-Spot Process

- A spot is formed from a sensitized T cell secreting the cytokine IFNγ after being stimulated by the specific Mtb antigens ESAt-6 or CFP-10.
- T cells that have not been sensitized to Mtb antigens should not secrete much IFNγ.
- Method used is called ELISpot
  - Enzyme-linked immunospot assay
  - Detects # of cells, visualized as spots, that secrete IFNγ
- TB response = greatest # of spots counted from blood stimulated with ESAT-6 or CFP-10 ***minus*** # spots from Nil.
- Mitogen response = # spots counted from blood stimulated with mitogen ***minus*** # spots from Nil.

T-Spot Interpretation of Results: TB Response is for Panel A **or** Panel B

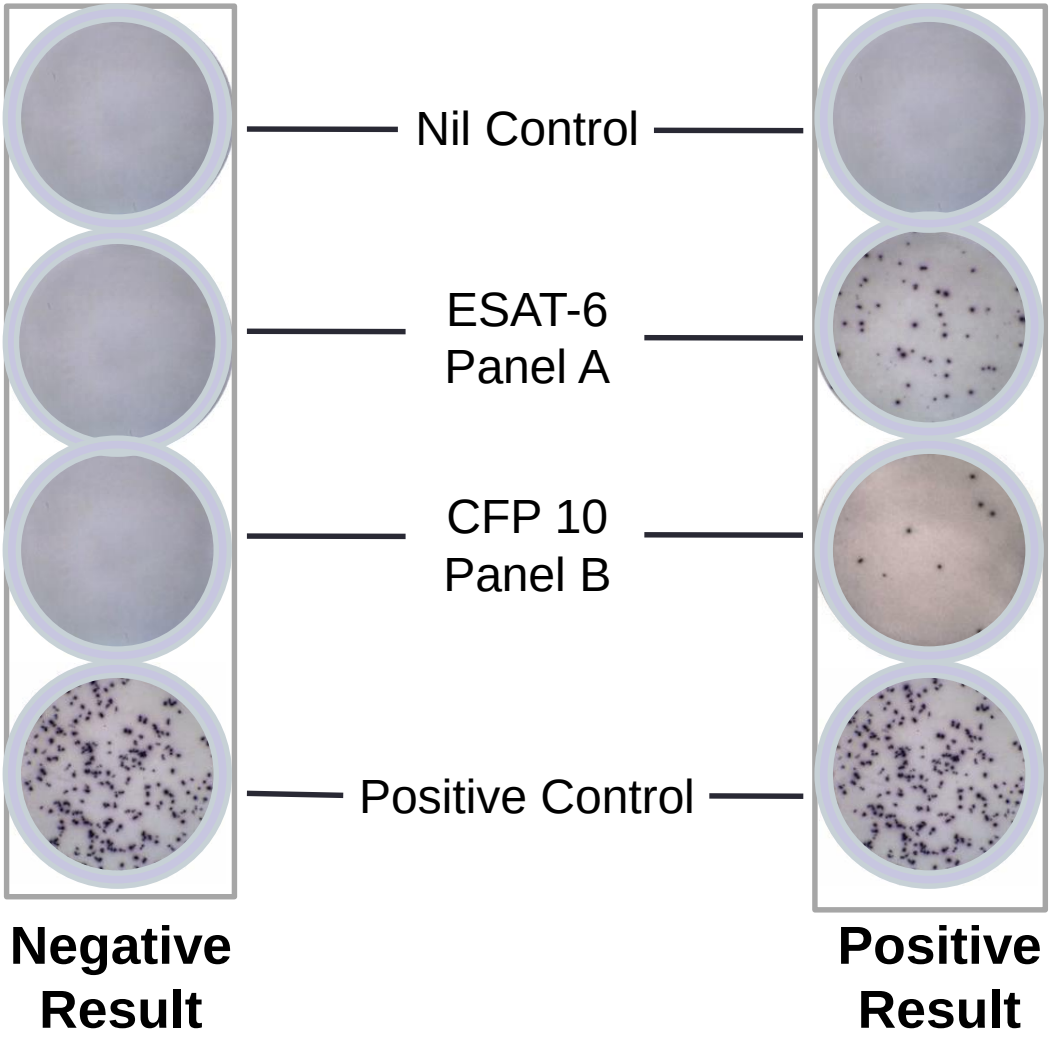
Interpretation	Nil	TB Response (TB Antigen)	Mitogen Response
Positive	≤10 spots	≥8 spots	Any result
Borderline – repeat test	≤10 spots	5, 6, or 7 spots	Any result
Negative	≤10 spots	≤4 spots	≥20 spots
Invalid – repeat test	>10 spots	Any result	Any result
Invalid – repeat test	≤10 spots	<5 spots	<20 spots

Nil = # spots

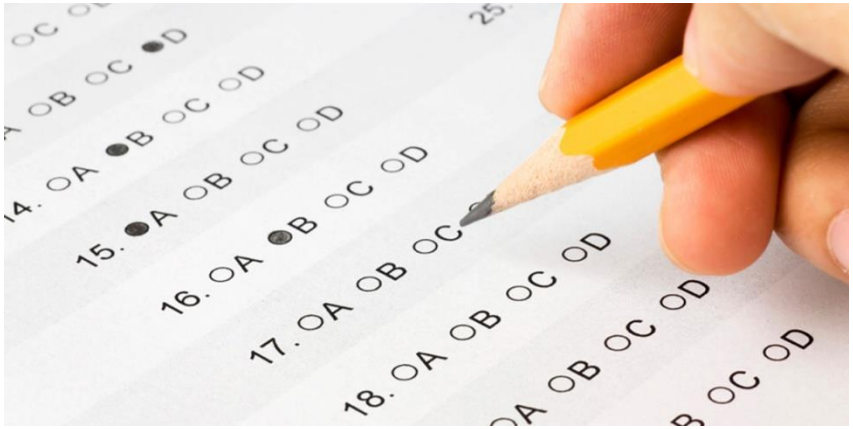
TB Response = the greatest # spots (ESAT-6 or CFP-10) ***minus*** Nil spots

Mitogen Response = # spots ***minus*** Nil spots

Interpretation of T-Spot Results




TEST


15. ●A ○B ○C ○D
16. ○A ●B ○C ○D
17. ○A ○B ○C
18. ○A ○B ○C ○D

Case 1: Panic!

- A 50 year old woman who has lived in Detroit her whole life had a QFT-GIT done, which was reported “positive”
- She has NO risk factors for being infected with TB
- She has NO risk factors for progressing to TB after infection
- Her only medical problem is hypertension
- She has no TB symptoms
- The blood was drawn in a facility that does not do phlebotomy often for QFTs
- Her primary care doctor ordered a chest x-ray that is normal

Results for Case 1

Nil	Mitogen - nil	TB Antigen - nil
2 IU/ml	>10 IU/ml	0.36 IU/ml

Remember: To be valid the nil result should be <8, so this is a valid test

Remember: Cut point for QFT is 0.35. ≥ .35 is "positive"

The official report:
Positive

- What should you do now?

A. Treat for LTBI.
B. Reassure her that treatment for LTBI is not indicated without symptoms of TB
C. Inform her the test is not valid
D. Repeat the QFT

Case 2: Do I really have to be evaluated for this?

- A 45 year old woman, life long resident of Detroit, was recently diagnosed HIV/AIDS. Her father had TB when she was an adolescent. She is being treated with Triumeq, which has a significant drug drug interaction with Rifampin and Rifapentine. She is doing well and her CD4+ count is normal. She is not very compliant with medications and even stopped the Triumeq for 2 months.
- She has no TB symptoms. Hypertension is out of control because she forgets to take her medicine.
- Chest x-ray is normal.

Results for Case 2

Nil	Mitogen - nil	TB Antigen - nil
0 IU/ml	0.3 IU/ml	0.53 IU/ml

Remember: To be valid the mitogen result should be ≥ 0.5 for a negative or an indeterminate result with a normal nil. Otherwise any result over 0 is valid.

Remember: Cut point for QFT is 0.35. ≥ .35 is "positive". There is no borderline result.

The official report: **Positive**

- What should you do now?

A. Consider this to be a borderline result because it is <1
B. Treat with INH 300 mg. daily for 9 months
C. Do a T-Spot
D. Treat with INH 900 mg. 2 days a week by DOT for 9 months

Case 3: No, I don't want to deal with this.

- This 38 year old African man from Somalia is a contact to an active case of TB. His wife has highly infectious pulmonary TB
- He has no risk factors for progressing to TB after infection
- He is asymptomatic and has a normal chest x-ray
- There are 2 children at home. One has a + T-Spot and is receiving INH and Rifapentine for LTBI.
- He delays getting his T-Spot, but finally agrees to it.

Results for Case 3

Nil	0 IU/ml
Panel A	6 spots
Panel B	7 spots
Mitogen	>10 spots

Remember: The reported spots in Panel A and B is the # of spots minus the nil

Borderline is 5, 6, or 7 spots in **EITHER** panel A or B.

The official report: **Borderline**

- What should you do now?

A. Begin treatment with INH 900 mg and Rifapentine 900 mg weekly for 12 doses by DOT
B. This is not a positive test. Wait another month and repeat it.
C. Reassure the patient that he does not need treatment
D. Obtain a QFT

Case 4: I will take your advice

- This is a 35 year old woman, born in the United States. Her parents are from India and she has traveled frequently to India, including trips for many months.
- She is a health care worker and requires TB testing annually. She cares for psychiatric patients. For the past 5 years she has had negative results on yearlyT B testing.
- She has not been to India or other high burden area since 6 months prior to her last TB test.
- She has had no contact with anyone with TB or symptoms of TB.
- She has no risk for progressing to TB after infection.
- She has no TB symptoms and chest x-ray is normal.

Results for Case 4

Nil	0 spots
Panel A	9 spots
Panel B	4 spots
Mitogen	>10 spots

Remember:

Positive is 8 spots or more in **EITHER** panel A or B.

The official report: **Positive**

- What should you do now?

A. Begin treatment for LTBI with Rifampin 600 mg daily for 4 months
B. Obtain a QFT
C. Reassure her that treatment for LTBI is not indicated
D. Consider this a false + test, perhaps due to M marinum

Star Pupils

UNITE TO END TB