UW Medicine
UW SCHOOL OF MEDICINE
DIVISION OF ALLERGY & INFECTIOUS DISEASES

SEATRAC
Seattle Tuberculosis Research Advancement Center

Latent TB Infection Diagnosis

Focus on LTBI
Seattle, Washington
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Special thanks to David Horne

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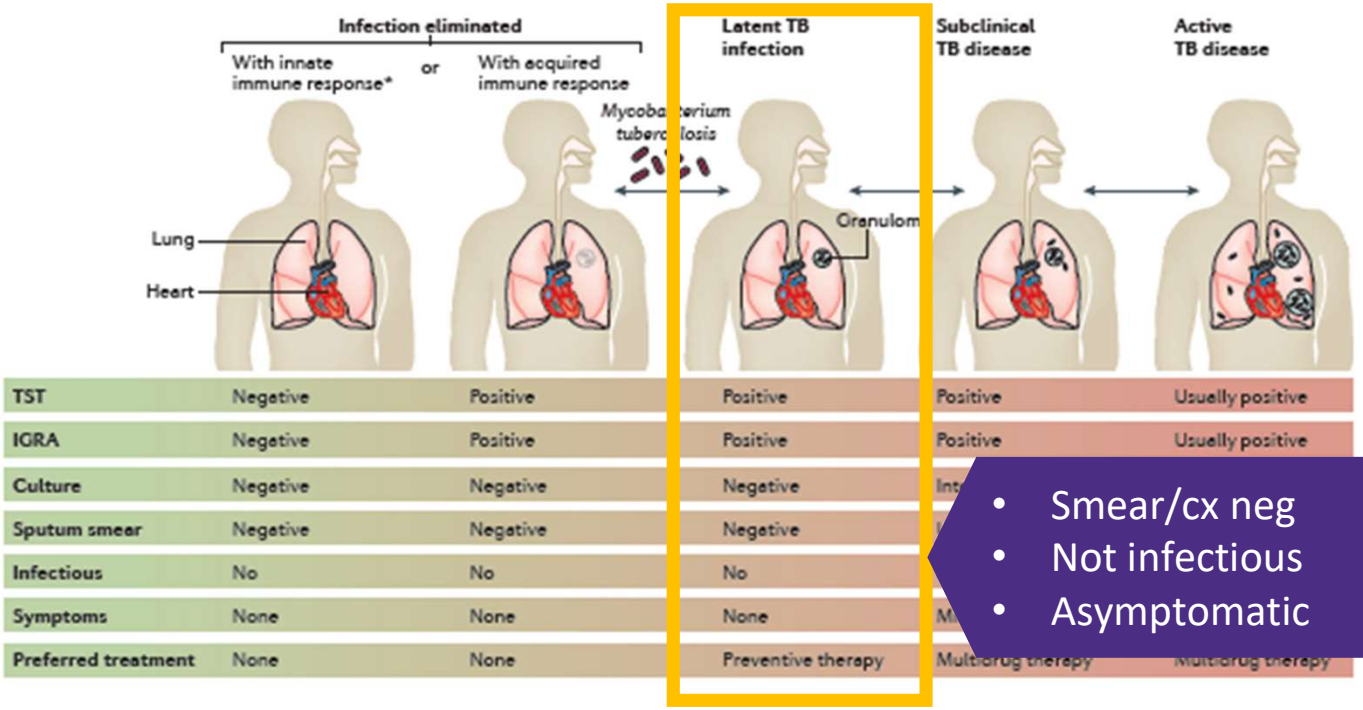
DEPARTMENT OF GLOBAL HEALTH

Latent TB infection: State of persistent immune response to stimulation by *M. tuberculosis* antigens without evidence of clinically active TB (WHO)

WHO, Latent TB Infection, 2018

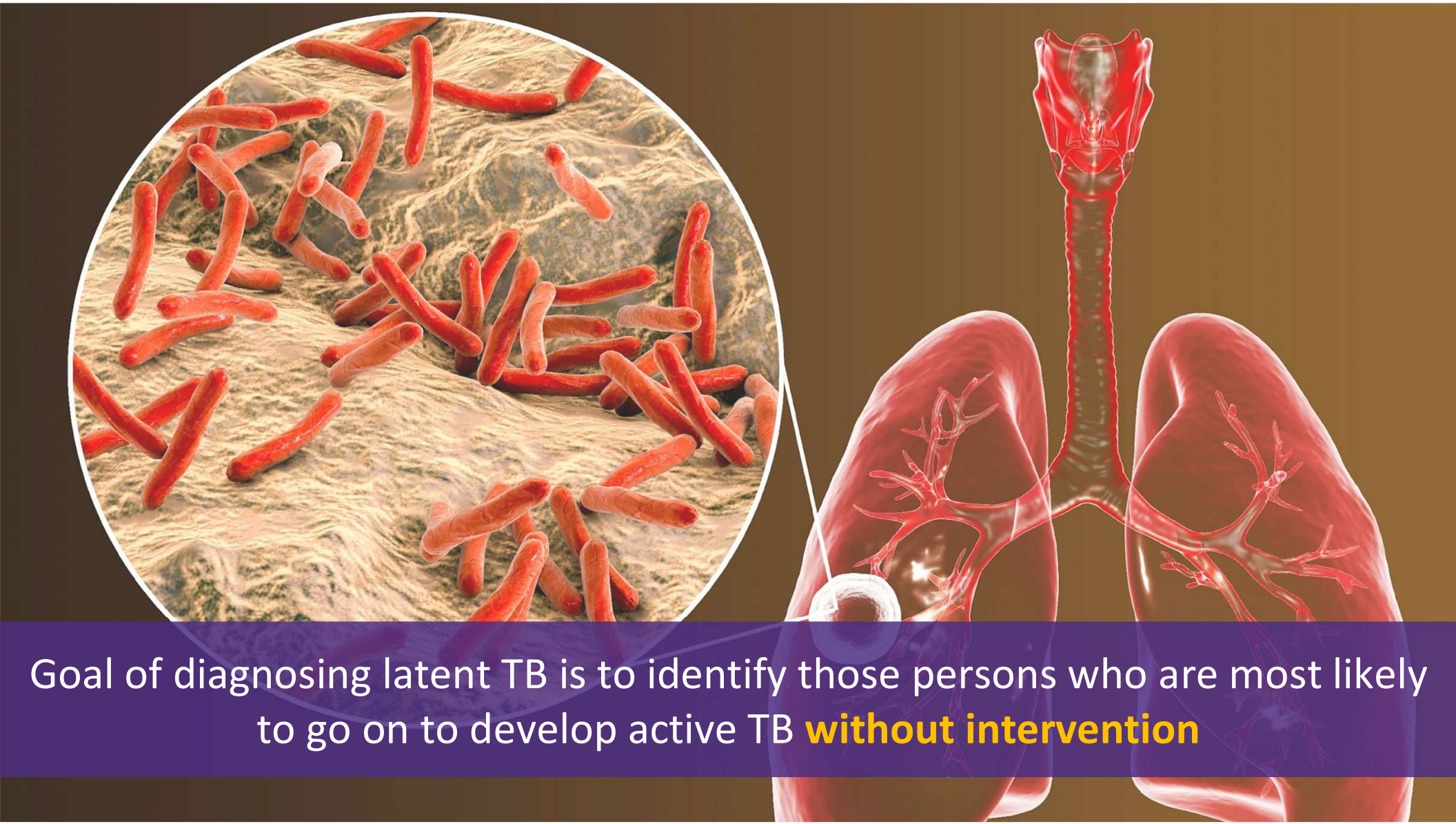
Mtb infection → TB disease



- Smear/cx neg
- Not infectious
- Asymptomatic

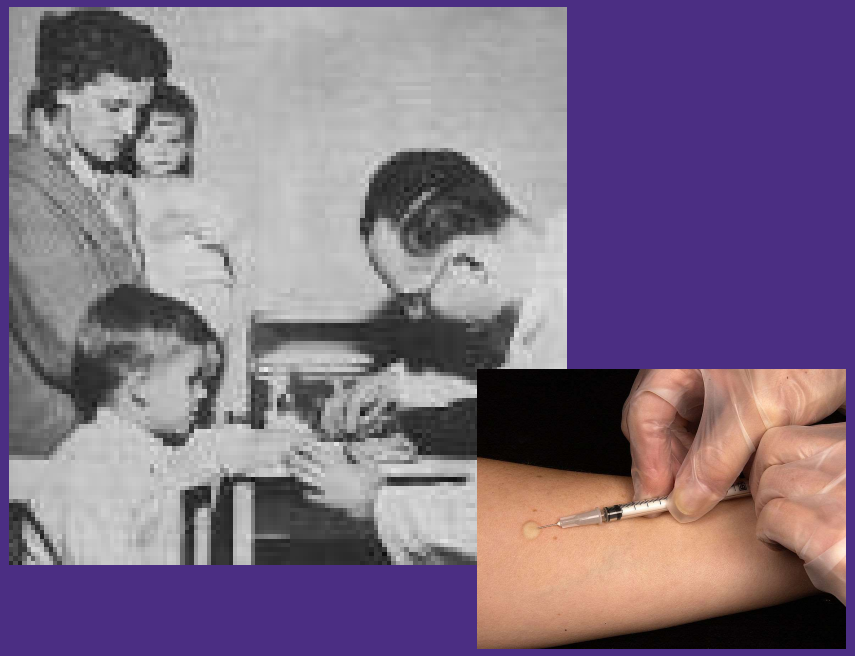
Mtb = *Mycobacterium tuberculosis*

Pai, Nature Reviews, 2016
Drain, Clin Micro Reviews, 2019



Goal of diagnosing latent TB is to identify those persons who are most likely to go on to develop active TB **without intervention**

Diagnosing LTBI: TST & IGRA

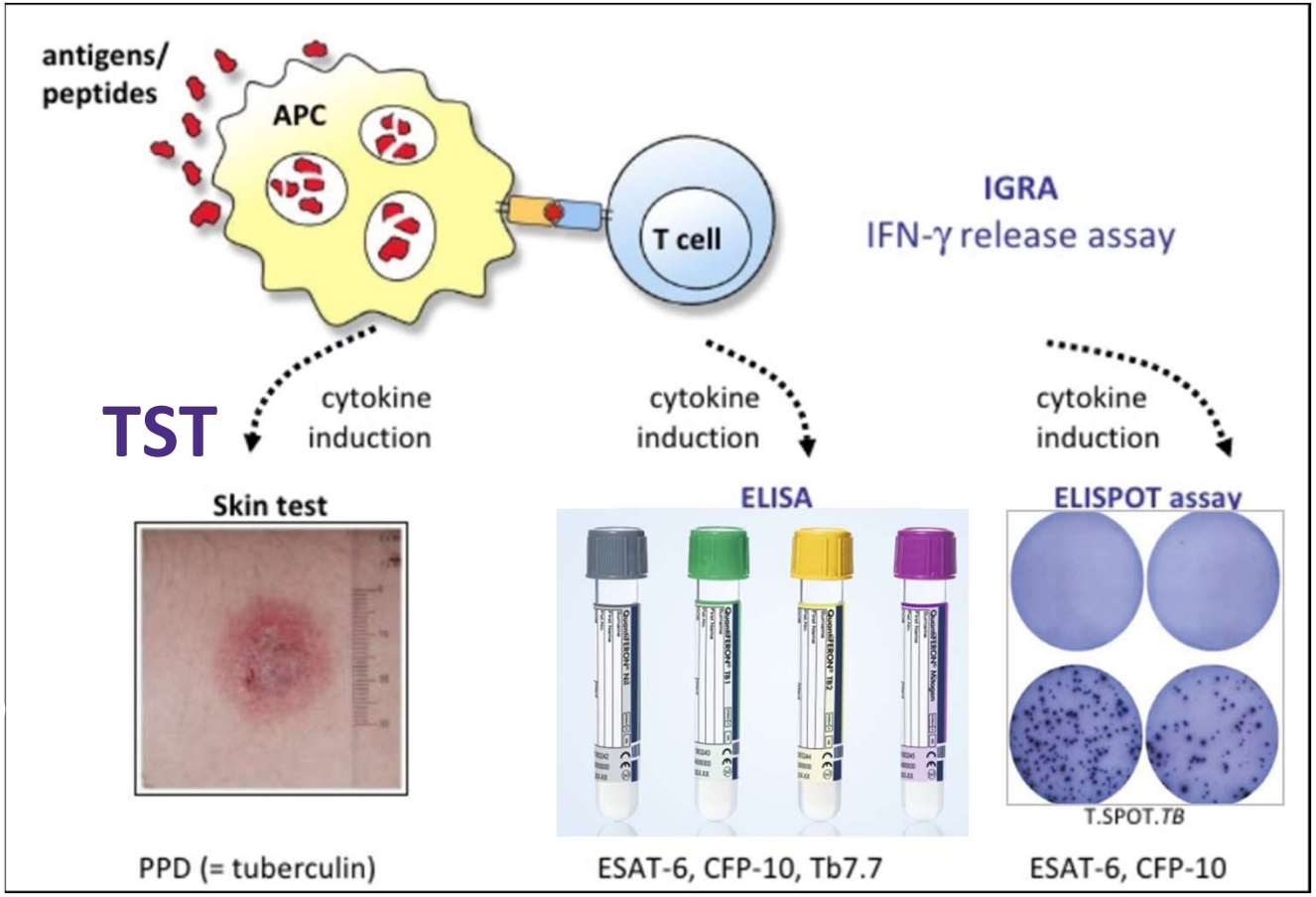


Tuberculin Skin Test (TST)

Interferon Gamma Release Assays (IGRAs)

Both are indirect tests of latent TB infection
(they assess whether or not T cells have had prior exposure to TB antigens)

Diagnosing LTBI: TST & IGRA

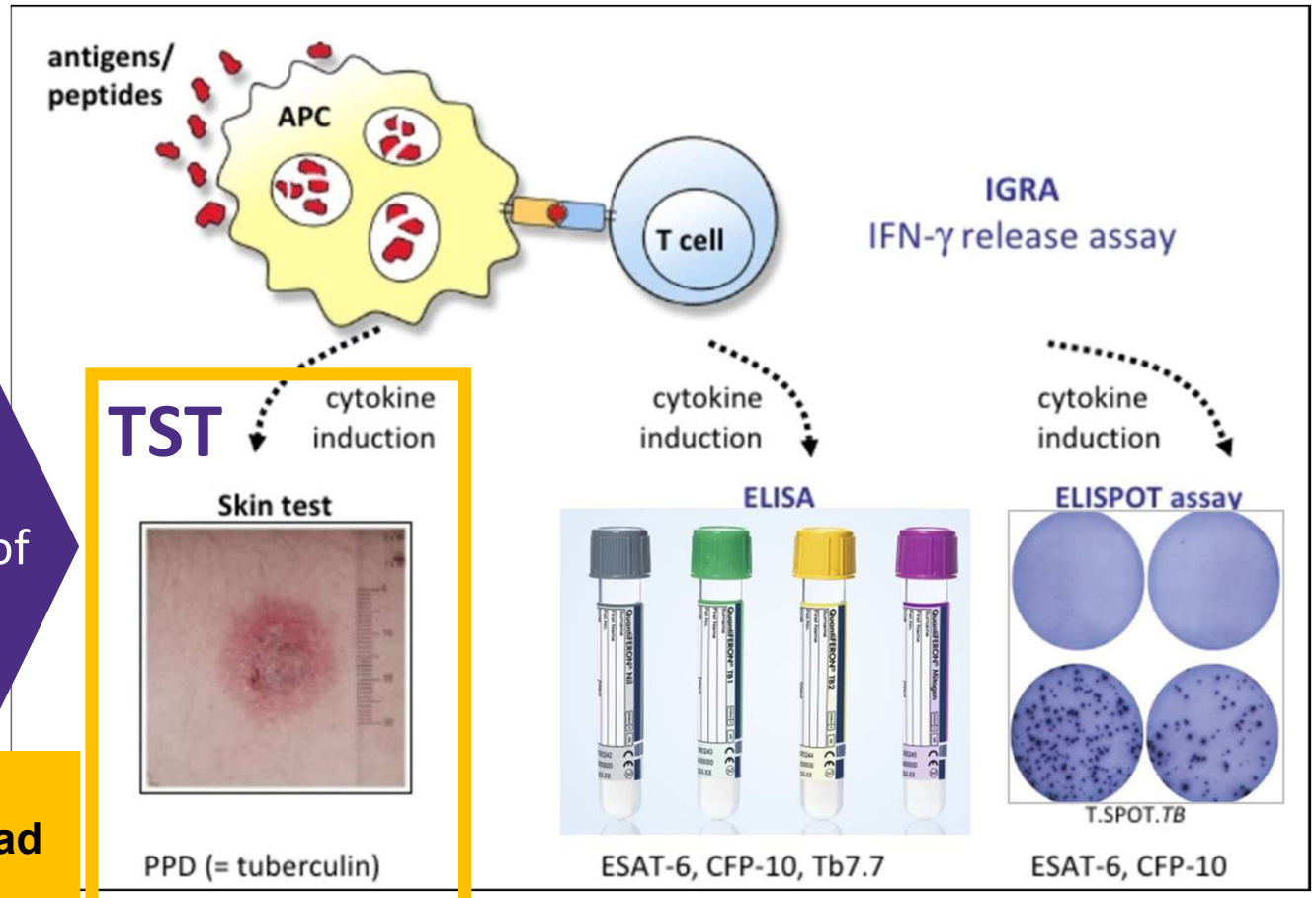


Horne, D.

Diagnosing LTBI: TST & IGRA

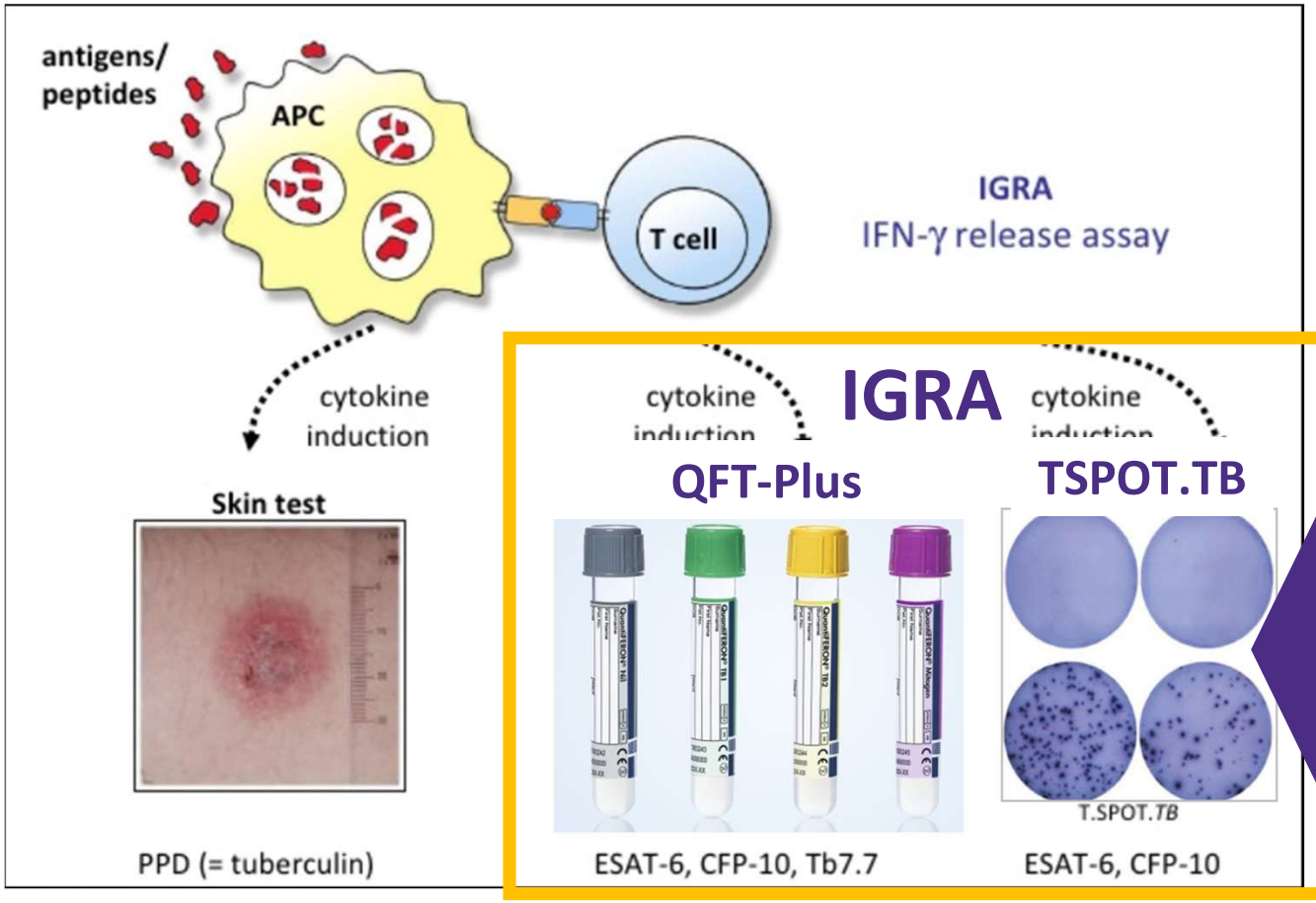
Conceived in late 1890s and includes the use of protein purified derivative (PPD)

PPD: mixture of antigens with broad cross reactivity



Horne, D.

Diagnosing LTBI: TST & IGRA



Use TB specific antigens, which means there is no cross reactivity with BCG

Horne, D.

TST: PPD tuberculin solution is injected **intradermally** on the flat inside surface of the lower arm

CDC

Diagnosing LTBI: TST

Exam: At 48 to 72 hours, **measure induration not erythema**

Results: Risk based cutoffs (≥5mm, ≥10mm, or ≥15 mm)

Timing of TST: Give MMR, oral polio, or varicella vaccination same day or wait 6 weeks

TST: Thresholds Based on Risk

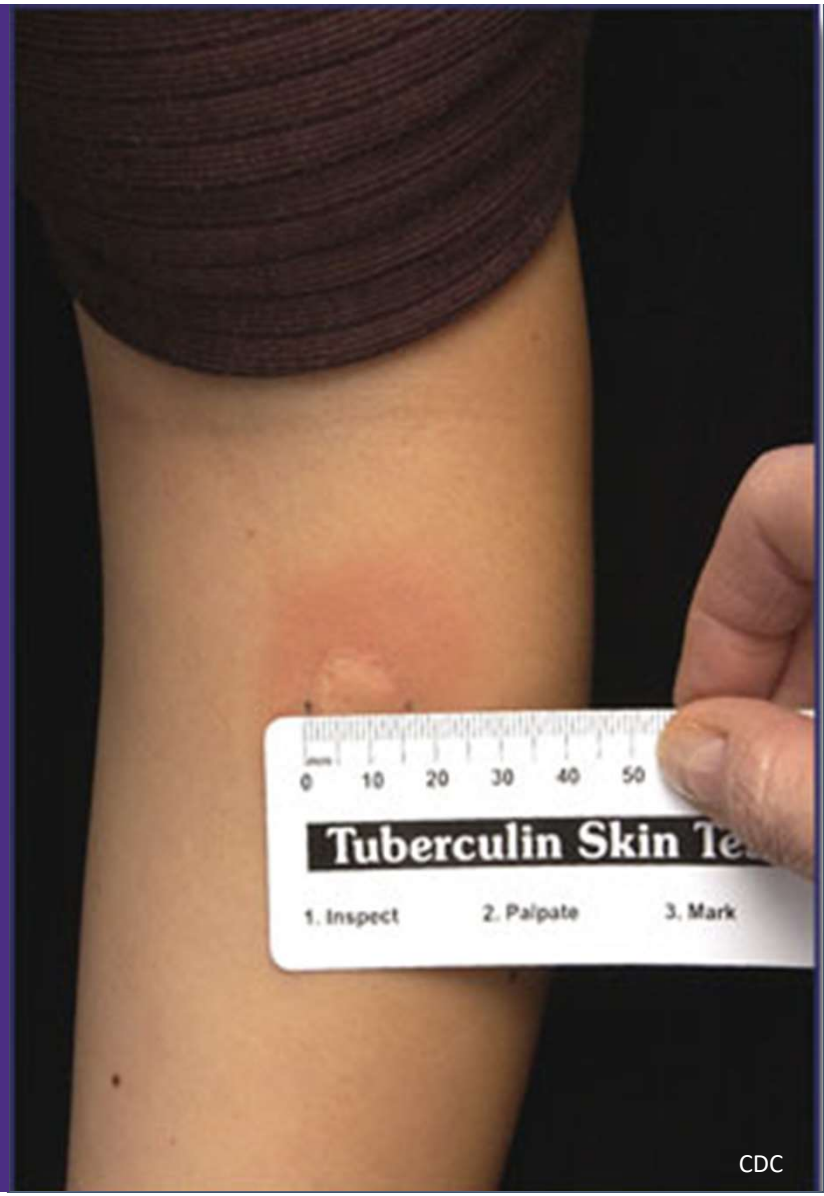
≥5 mm	≥10 mm	≥15 mm
• HIV • close contact of infectious TB • fibrotic changes on CXR consistent with old TB • severely immunosuppressed (e.g., organ transplant, TNFα blockade, prednisone ≥15 mg/day)	• Recent immigrants (<5 yrs) from high prevalence countries • Residents/employees of high-risk congregate settings • TB lab personnel • Medical conditions at elevated risk* • IVDU • Children < 4 years of age (screened if there are risk factors)	• all others: no known risk factors for TB (editorial: why screening?)

* silicosis, DM, chronic renal insufficiency, leukemia/lymphoma, head/neck/lung cancer, weight loss of >10% of ideal body weight, gastrectomy/jejunoileal bypass

TST: False positives and negatives

False positive: BCG, non-tuberculosis mycobacteria

False negative: anergy with immunosuppression, early in exposure window



TST Limitations: False positives

Which of the following does not cause a false-positive TST?

A) Infection with non-tuberculous mycobacteria

B) Pleural TB

C) Previous BCG vaccination

Case Study

AB is a 50 year old nurse. 10 years ago, their TST was negative. Starts a new job at Hospital X, TST 0 mm. 3 months later, retested as part of TB screening program in the unit. TST now 11 mm

Ms. A has <u>definitely</u> been exposed to a TB case in the last 3 months?

A) True

B) False

TST: Booster phenomenon

Ms. AB may have been exposed and infected with M.tb sometime in the 3 months OR this may represent a **booster phenomenon**

Positive TST after prior negative TST without TB exposure

- Due to recall of waned cell-mediated immunity
- Maximal if interval 1-5 weeks although may persist for >1 year
- More common in elderly, BCG-vaccinated, sensitization due to NTM

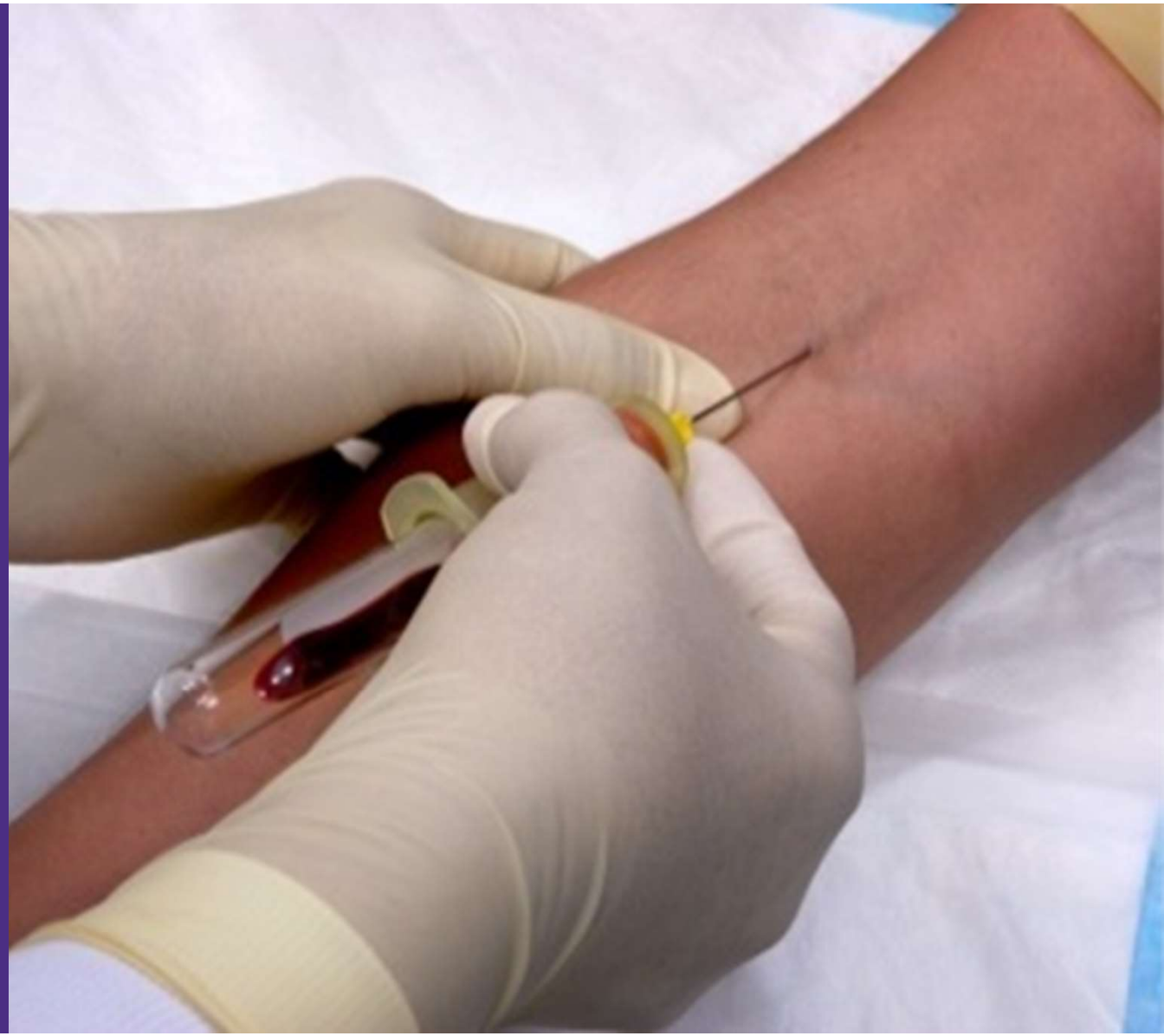
IF boosted TST reflects true LTBI→ risk of progression lower than w/ new conversion

Two-step testing: Differentiate "boosted reaction" vs. recent infection. For annual TST screening programs, the initial test (if negative) should have 2nd TST 1-3 weeks later (typically done at baseline)

IGRA may be "boosted" by TST administration

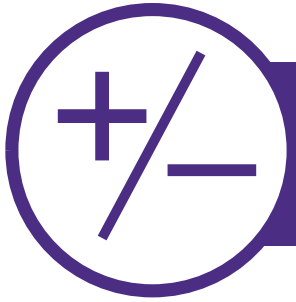
- May increase IFN response enough to go from negative to positive
- IGRA boosting occurred at 7 days, but not 3 days, post-TST (van Zyl-Smit, AJRCCM 2009)

IGRA: Blood is collected in specialized tubes



Diagnosing LTBI: IGRA (QFT-Plus)

Plasma collected for ELISA, measure IFN-γ

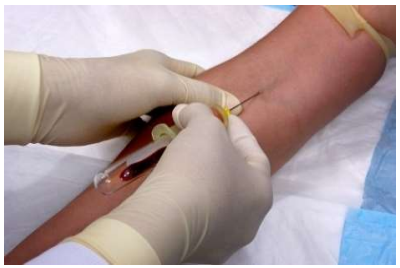


QFT-Plus+: TB ag-nil ≥0.35 IU/mL

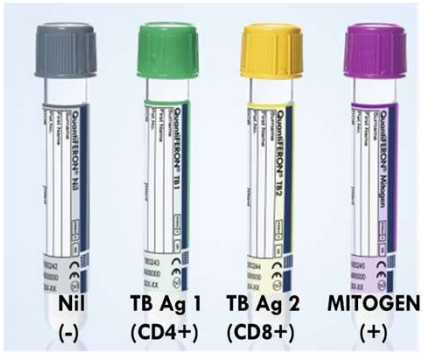
Unlike TST, IGRA uses one cut-off irrespective of immunosuppressive status or TB risk

Diagnosing LTBI: IGRA (QFT-Plus)

Plasma collected for ELISA, measure IFN-γ



Collect 1mL of blood in 4 tubes ***or Standard lithium heparin tube–> 16 hours to transfer to 4 tubes***



Collect plasma for ELISA

Measure IFN-γ

RESULT

Nil	TB1	TB2	Mitogen	TB1-Nil	TB2-Nil	Mitogen-Nil	Result
0.19	1.39	1.46	6.85	1.20	1.27	6.66	POSITIVE

Diagnosing LTBI: IGRA (QFT-Plus)

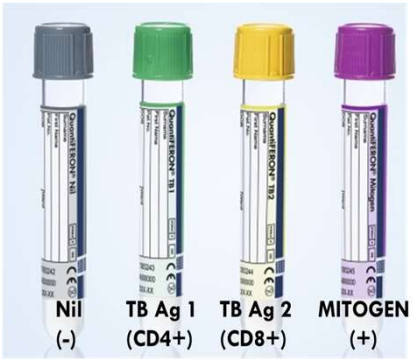


Photo: Qiagen

QFT-Plus components	
NIL	Negative control (background)
TB 1	TB antigens: ESAT-6, CFP10 →CD4 response
TB 2	TB antigens: ESAT-6, CFP10 → CD4 & CD8 response
Mitogen	Positive control

Why CD8+ antigens? May incite stronger response in recent infection and remain relatively intact in in immunocompromised and children

Lancioni AJRCCM 2012

Nil	TB1	TB2	Mitogen	TB1-Nil	TB2-Nil	Mitogen-Nil	Result
0.19	1.39	1.46	6.85	1.20	1.27	6.66	POSITIVE

Subtract:
TB1-Nil,
TB2- Nil,
Mitogen-Nil (background)

QFT: False positives and negatives

False positive: non-tuberculosis mycobacteria (though less cross-reactivity than TST)

NO cross reactivity with BCG

False negative:
"indeterminate" test results with immunosuppression, early in exposure window

QFT-Plus interpretation

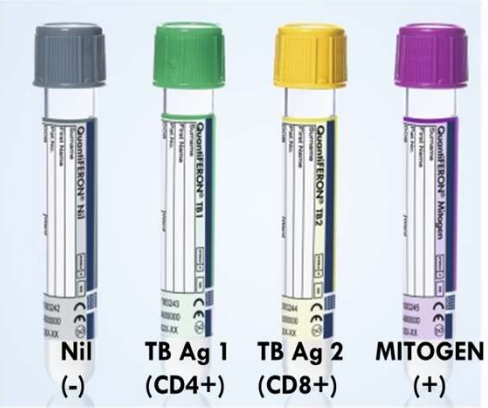


Photo: Qiagen

Nil (IU/ml)	TB1 minus Nil (IU/ml)	TB2 minus Nil (IU/ml)	Mitogen minus Nil (IU/ml)*	QFT-Plus Result	Report/interpretation
≤8.0	≥0.35 and ≥25% of Nil	Any	Any	Positive†	*M. tuberculosis* infection likely
	Any	≥0.35 and ≥25% of Nil			
	<0.35 or ≥0.35 and <25% of Nil	<0.35 or ≥0.35 and <25% of Nil	≥0.50	Negative	*M. tuberculosis* infection NOT likely
	<0.35 or ≥0.35 and <25% of Nil	<0.35 or ≥0.35 and <25% of Nil	<0.50	Indeterminate‡	Likelihood of *M. tuberculosis* infection cannot be determined
>8.0§	Any				

RESULT

Nil	TB1	TB2	Mitogen	TB1-Nil	TB2-Nil	Mitogen-Nil	Result
0.19	1.39	1.46	6.85	1.20	1.27	6.66	POSITIVE

Subtract TB1-Nil, TB2- Nil, Mitogen-Nil (background)

Either TB1-Nil or TB2-Nil considered positive

QFT-Plus interpretation: Positive test

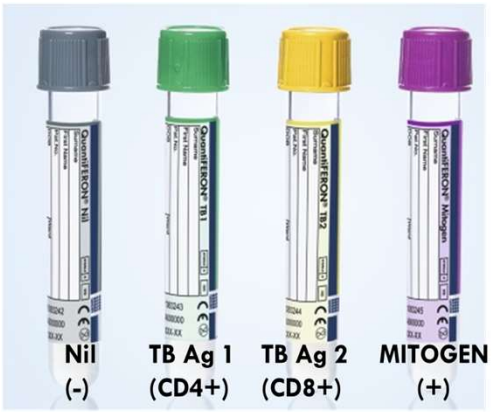


Photo: Qiagen

	NIL	TB1-Nil	TB2-Nil	MIT-Nil
Positive	-	+	+/-	+
Positive	-	+/-	+	+
Negative	-	-	-	+
Indeterminate	+	-	-	+
Indeterminate	-	-	-	-

Positive test: Either TB1-Nil or TB2-Nil positive

QFT-Plus interpretation: Negative test

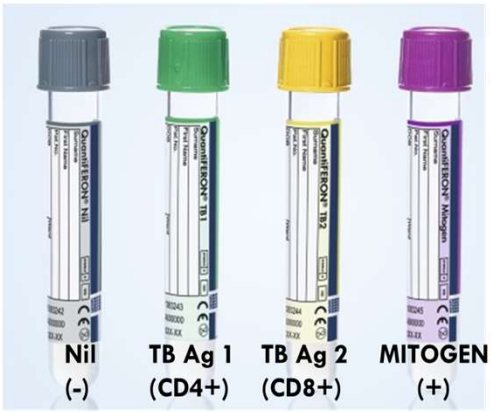


Photo: Qiagen

	NIL	TB1-Nil	TB2-Nil	MIT-Nil
Positive	-	+	+/-	+
Positive	-	+/-	+	+
Negative	-	-	-	+
Indeterminate	+	-	-	+
Indeterminate	-	-	-	-

Negative test: <u>Both</u> TB1-Nil or TB2-Nil negative

QFT-Plus interpretation: Indeterminate test

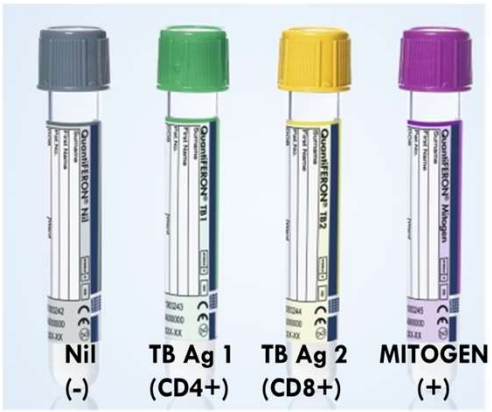


Photo: Qiagen

	NIL	TB1-Nil	TB2-Nil	MIT-Nil
Positive	-	+	+/-	+
Positive	-	+/-	+	+
Negative	-	-	-	+
Indeterminate	↑	-	-	+
Indeterminate	-	-	-	↓

Indeterminate result:

- **Low Mitogen response** (weak immune response to a strong stimulant or technical issues)
- **High Nil response** (background level of IFN-gamma)

IGRA Indeterminate results

- **Indeterminate result tells you that MTB infection data cannot be obtained from the IGRA test**
  - Low lymphocyte count
  - Low lymphocyte activation potential
- Optimally, an improvement over the TST in which “anergy” cannot be diagnosed
- Repeat test with valid result (pos/neg) in 68% (Banach IJTLD 2011)

IGRA vs. TST

IGRA	TST
specific Mtb antigens (no BCG cross-reactivity)	PPD (BCG cross-reactivity)
1 visit	2 visits
Phlebotomy (in-vitro)	intracutaneous injection (in-vivo)
stimulate within hours	injected = done
results possible in 1 day	results in 2–3 days
complex laboratory test	point-of-care test
built in negative/positive controls	no controls
one cutoff regardless of risk	risk-based cutoffs

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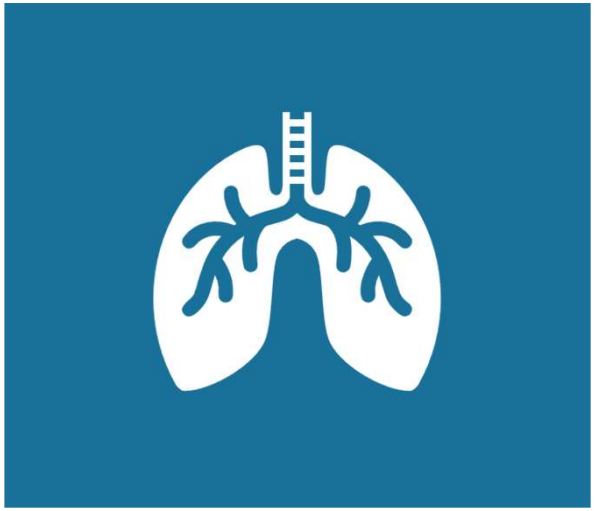
IGRA vs. TST: Important limitations



Both tests are an indirect measure of LTBI

Require: Mtb infection and functioning T cells for positive result

They can have cross-reactivity with NTM

TST only: cross-reactivity with BCG

2-8 week delay after exposure until test positive

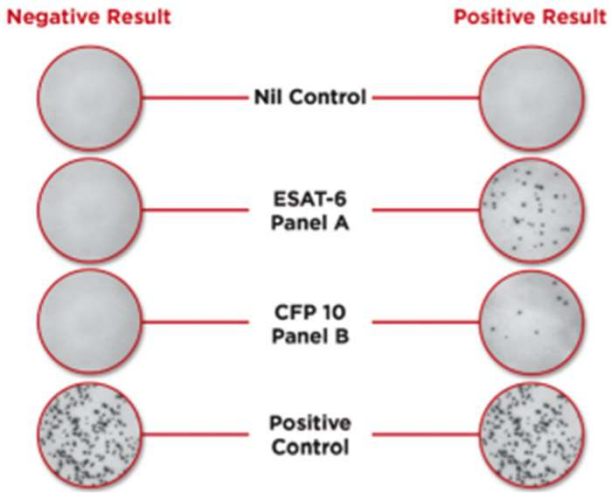
Neither test can be used to assess treatment response

IGRA vs. TST: Important limitations

Neither test should be used to diagnose active TB disease*
or
LTBI treatment response

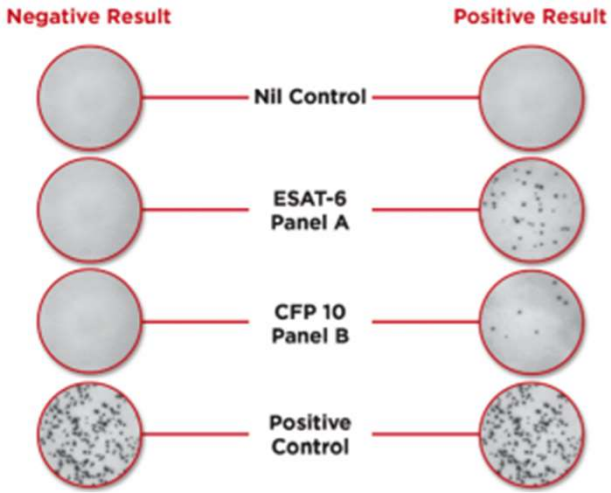
* Exception may be used to help make diagnosis in pediatric TB

IGRA: TSPOT



Interpretation Criteria for T-SPOT.TB Test (T-Spot)			
Interpretation	**Nil***	**TB Response†**	**Mitogen§ (Positive Control)**
Positive¶	≤10 spots	≥8 spots	Any number of spots
Borderline**	≤10 spots	5, 6, or 7 spots	Any number of spots
Negative††	≤10 spots	≤4 spots	≥ 20 spots
Indeterminate**	>10 spots	Any	Any number of spots
	≤10 spots	<5 spots	< 20 spots

IGRA: TSPOT positive result



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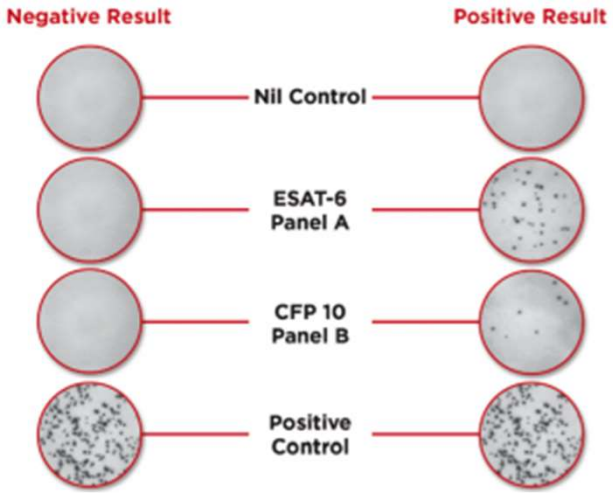
Positive test:
Either ESAT-6 or CFP 10 ≥8 spots

IGRA: TSPOT negative result



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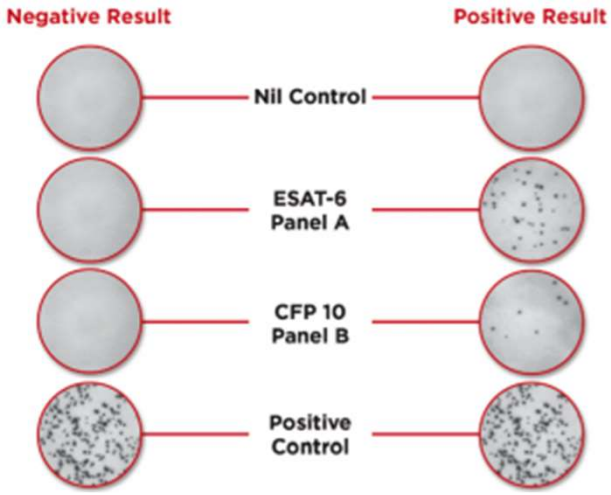
Negative test:
Both ESAT-6 or CFP 10 ≤4 spots

IGRA: TSPOT indeterminate result



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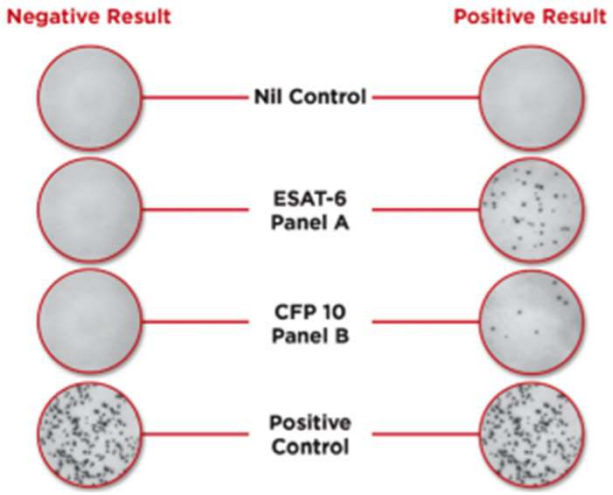
Indeterminate result: Either

- **Low Mitogen response (weak immune response to a strong stimulant or technical issues), OR**
- **High Nil (background level of IFN-gamma)**

IGRA: TSPOT borderline result



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Borderline result: used in some settings

- **TB response in between a positive or negative response**

LTBI Diagnosis Guidelines

Clinical Infectious Diseases

IDSA GUIDELINE

Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children

David M. Lewinsohn,[1,a] Michael K. Leonard,[2,a] Philip A. LoBue,[3,a] David L. Cohn,[4] Charles L. Daley,[5] Ed Desmond,[6] Joseph Keane,[7] Deborah A. Lewinsohn,[1] Ann M. Loeffler,[8] Gerald H. Mazurek,[3] Richard J. O'Brien,[9] Madhukar Pai,[10] Luca Richeldi,[11] Max Salfinger,[12] Thomas M. Shinnick,[3] Timothy R. Sterling,[13] David M. Warshauer,[14] and Gail L. Woods[15]

RISK OF TB INFECTION **X** RISK OF PROGRESSION **=** RISK OF TB DISEASE

LTBI Diagnosis Guidelines: Summary

Group	Testing Strategy	Considerations
Likely to be Infected **High** Risk of Progression (TST ≥ 5mM)	**Adults** **Acceptable**: IGRA OR TST Consider dual testing where a positive result from either result would be considered **positive** **Children ≤ 5 years of age** **Preferred**: TST **Acceptable**: IGRA OR TST Consider dual testing where a positive result from either would be considered **positive**[1]	Prevalence of BCG vaccination Expertise of staff and/or laboratory Test availability Patient perceptions Staff perceptions Programmatic concerns
Likely to be Infected **Low** to **Intermediate** Risk of Progression (TST ≥ 10mM)	**Preferred**: IGRA where available **Acceptable**: IGRA or TST	
Unlikely to be Infected (TST > 15mM)	**Testing for LTBI is not recommended** **If necessary:** **Preferred:** IGRA where available. **Acceptable**: Either IGRA OR TST **For serial testing**: **Acceptable**: Either IGRA OR TST Consider repeat or dual testing where a negative result from either would be considered **negative**[2]	

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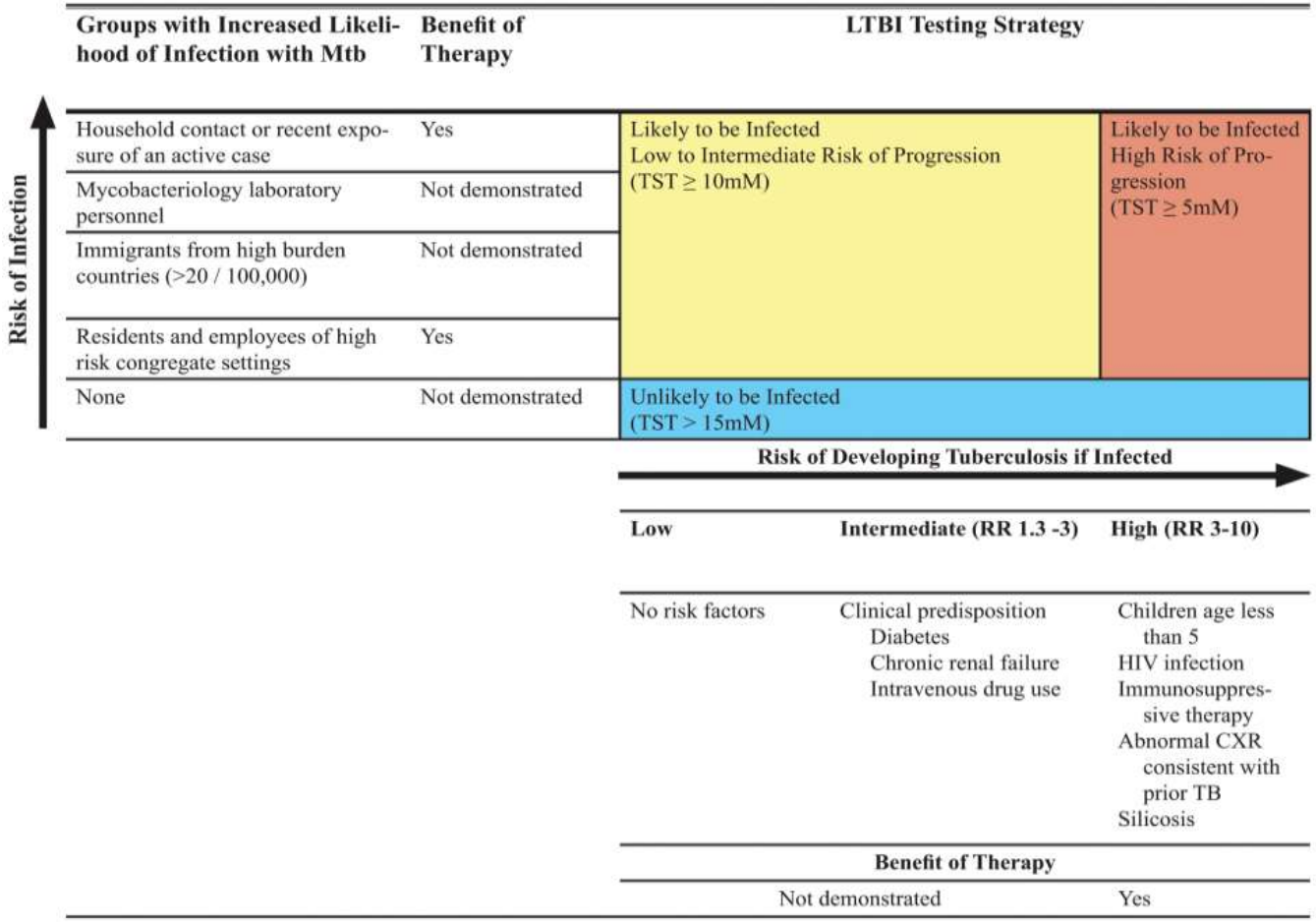
Screening Guidelines (age >5 yrs)



Groups with Increased Likelihood of Infection with Mtb	Benefit of Therapy	LTBI Testing Strategy	
Household contact or recent exposure of an active case	Yes	Likely to be Infected Low to Intermediate Risk of Progression (TST ≥ 10mM)	Likely to be Infected High Risk of Progression (TST ≥ 5mM)
Mycobacteriology laboratory personnel	Not demonstrated		
Immigrants from high burden countries (>20 / 100,000)	Not demonstrated		
Residents and employees of high risk congregate settings	Yes		
None	Not demonstrated	Unlikely to be Infected (TST > 15mM)	

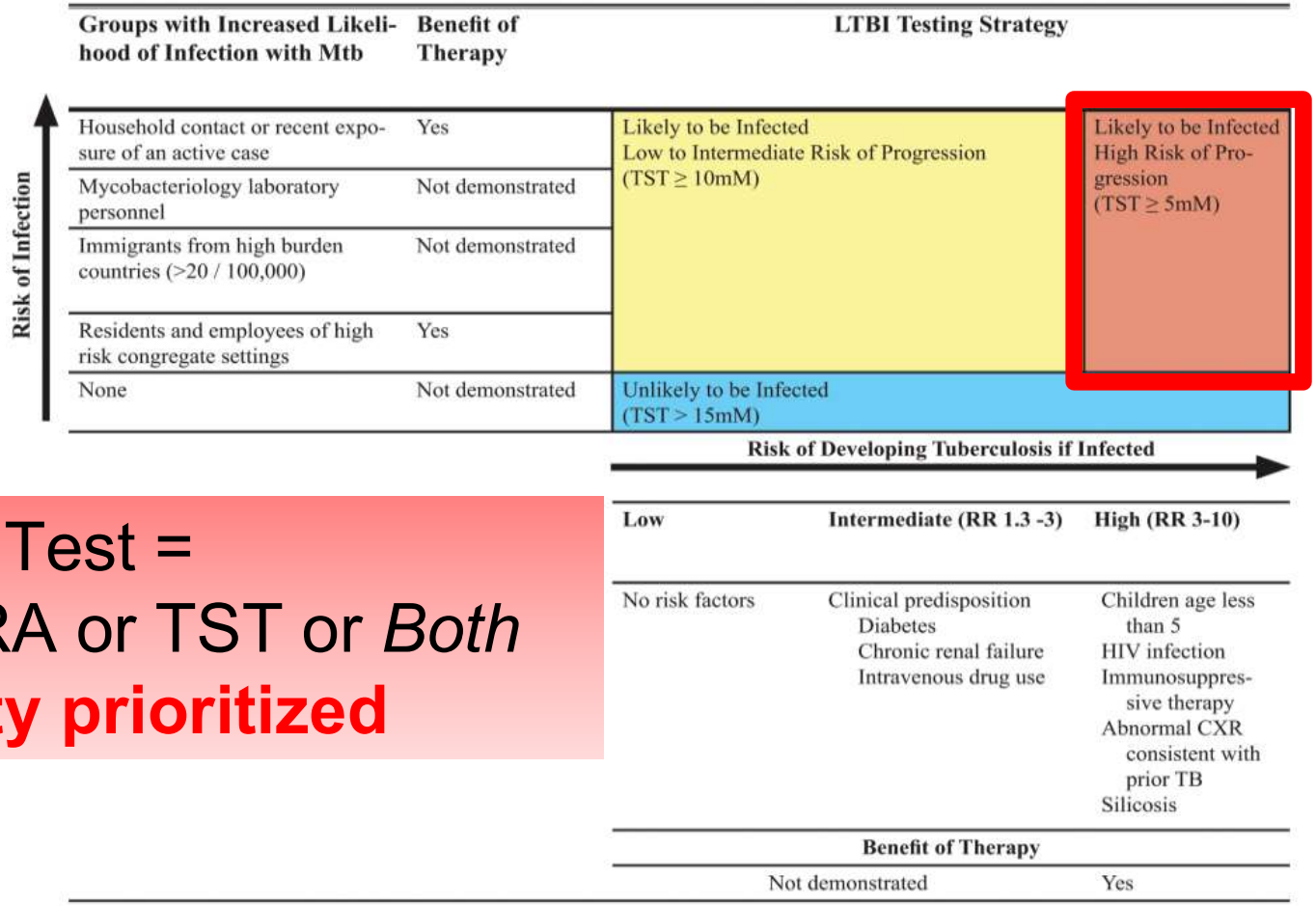
Risk of Infection

Risk of Developing Tuberculosis if Infected

Low	Intermediate (RR 1.3 -3)	High (RR 3-10)
No risk factors	Clinical predisposition Diabetes Chronic renal failure Intravenous drug use	Children age less than 5 HIV infection Immunosuppressive therapy Abnormal CXR consistent with prior TB Silicosis

Benefit of Therapy	
Not demonstrated	Yes

Screening Guidelines (age >5 yrs)



Groups with Increased Likelihood of Infection with Mtb	Benefit of Therapy	LTBI Testing Strategy
Household contact or recent exposure of an active case	Yes	Likely to be Infected Low to Intermediate Risk of Progression (TST ≥ 10mM)
Mycobacteriology laboratory personnel	Not demonstrated	
Immigrants from high burden countries (>20 / 100,000)	Not demonstrated	
Residents and employees of high risk congregate settings	Yes	
None	Not demonstrated	Unlikely to be Infected (TST > 15mM)

Risk of Infection

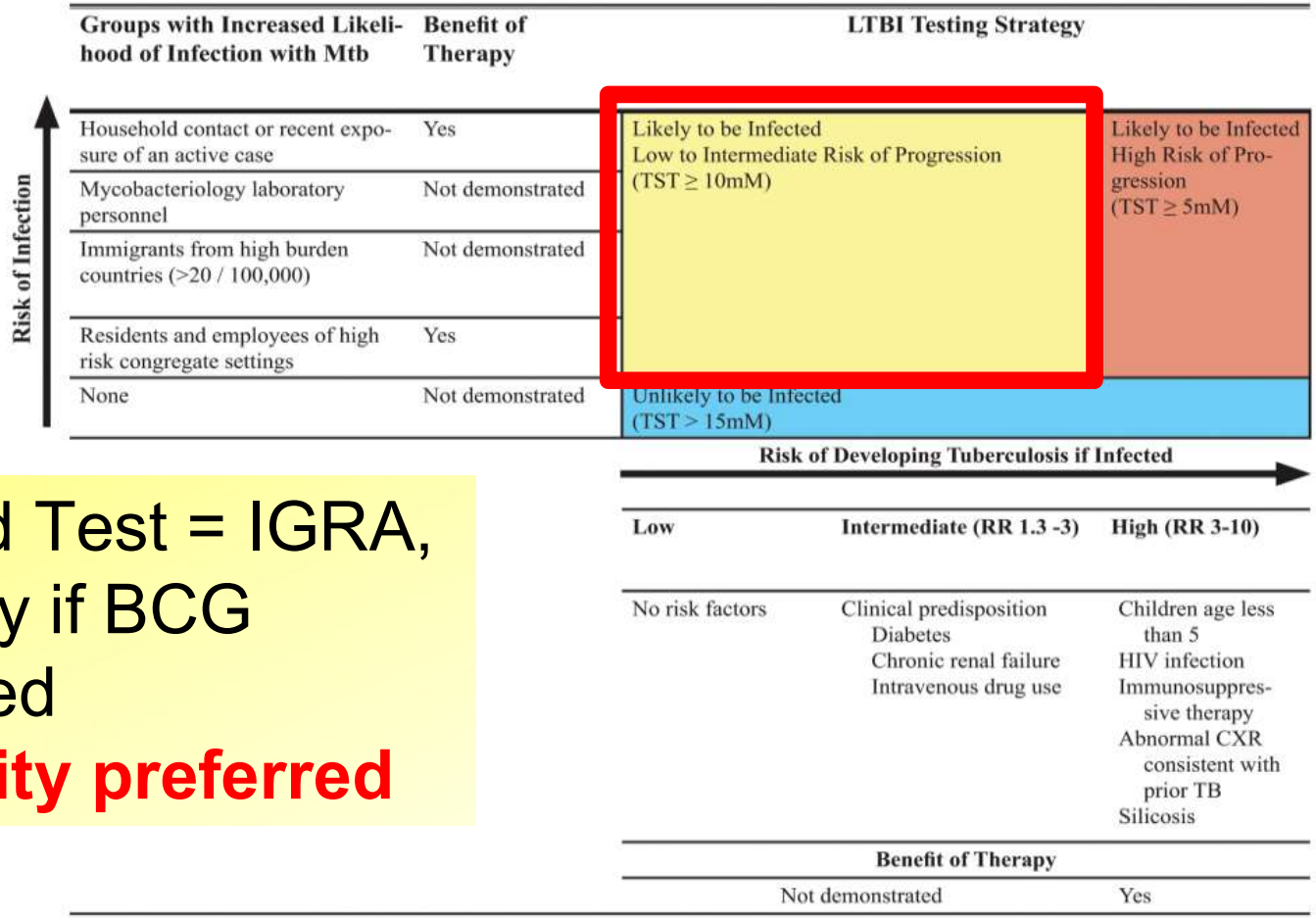
Likely to be Infected
High Risk of Progression
(TST ≥ 5mM)

Risk of Developing Tuberculosis if Infected

Low	Intermediate (RR 1.3 -3)	High (RR 3-10)
No risk factors	Clinical predisposition Diabetes Chronic renal failure Intravenous drug use	Children age less than 5 HIV infection Immunosuppressive therapy Abnormal CXR consistent with prior TB Silicosis
Benefit of Therapy		
Not demonstrated		Yes

Preferred Test =
either IGRA or TST or *Both*
Sensitivity prioritized

Screening Guidelines (age >5 yrs)



Groups with Increased Likelihood of Infection with Mtb	Benefit of Therapy	LTBI Testing Strategy
Household contact or recent exposure of an active case	Yes	Likely to be Infected Low to Intermediate Risk of Progression (TST ≥ 10mM)
Mycobacteriology laboratory personnel	Not demonstrated	
Immigrants from high burden countries (>20 / 100,000)	Not demonstrated	
Residents and employees of high risk congregate settings	Yes	Likely to be Infected High Risk of Progression (TST ≥ 5mM)
None	Not demonstrated	Unlikely to be Infected (TST > 15mM)

Risk of Infection

Risk of Developing Tuberculosis if Infected

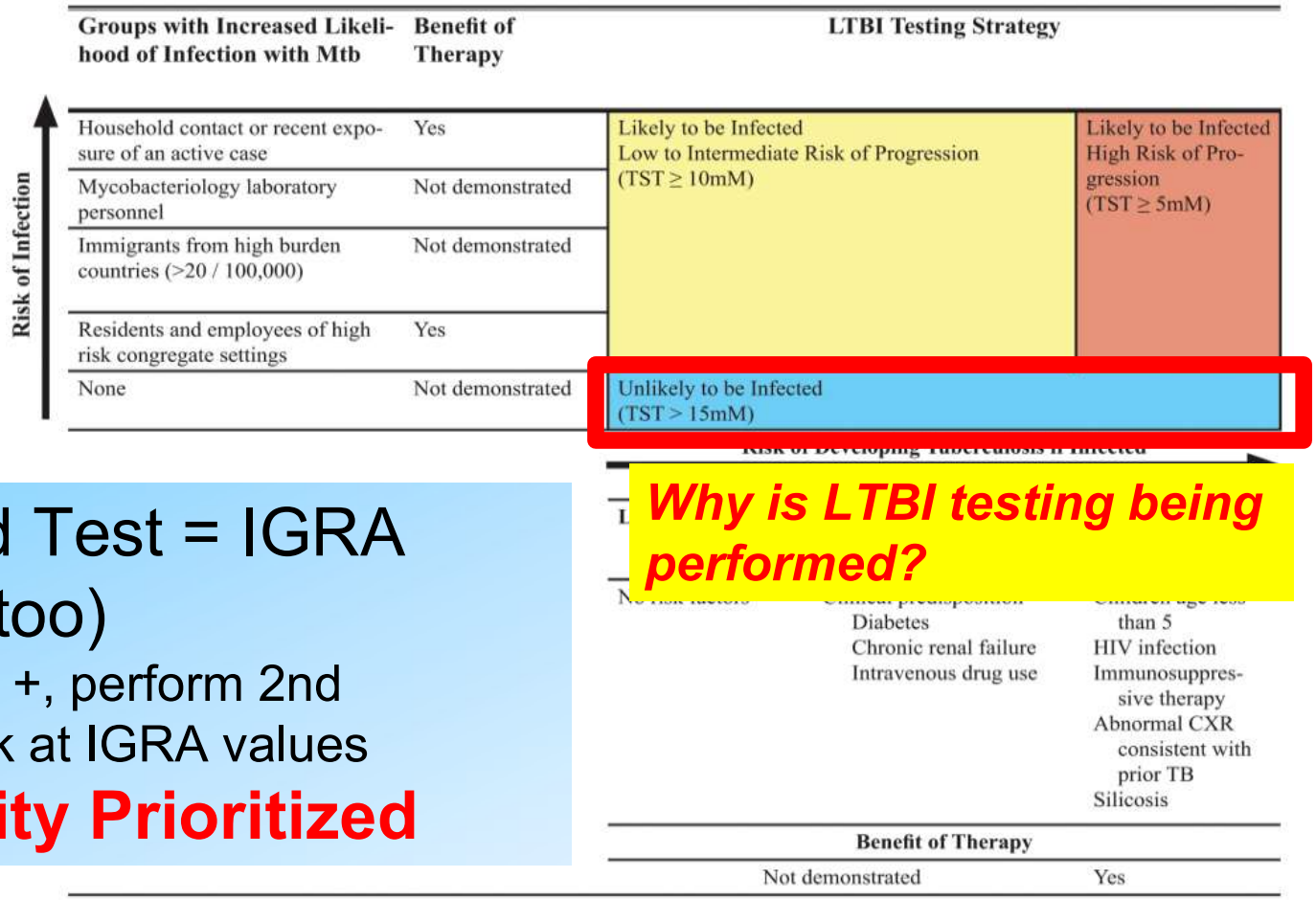
Low	Intermediate (RR 1.3 -3)	High (RR 3-10)
No risk factors	Clinical predisposition Diabetes Chronic renal failure Intravenous drug use	Children age less than 5 HIV infection Immunosuppressive therapy Abnormal CXR consistent with prior TB Silicosis

Benefit of Therapy	
Not demonstrated	Yes

Preferred Test = IGRA, especially if BCG vaccinated

Specificity preferred

Screening Guidelines (age >5 yrs)



Groups with Increased Likelihood of Infection with Mtb	Benefit of Therapy	LTBI Testing Strategy	
Household contact or recent exposure of an active case	Yes	Likely to be Infected Low to Intermediate Risk of Progression (TST ≥ 10mM)	Likely to be Infected High Risk of Progression (TST ≥ 5mM)
Mycobacteriology laboratory personnel	Not demonstrated		
Immigrants from high burden countries (>20 / 100,000)	Not demonstrated		
Residents and employees of high risk congregate settings	Yes		
None	Not demonstrated	Unlikely to be Infected (TST > 15mM)	

Risk of Infection

Risk of Developing Tuberculosis if Infected

Diabetes
Chronic renal failure
Intravenous drug use

than 5
HIV infection
Immunosuppressive therapy
Abnormal CXR consistent with prior TB
Silicosis

Benefit of Therapy

Not demonstrated | Yes

Why is LTBI testing being performed?

Preferred Test = IGRA
(TST ok too)

- if 1st test +, perform 2nd
- Also look at IGRA values

Specificity Prioritized

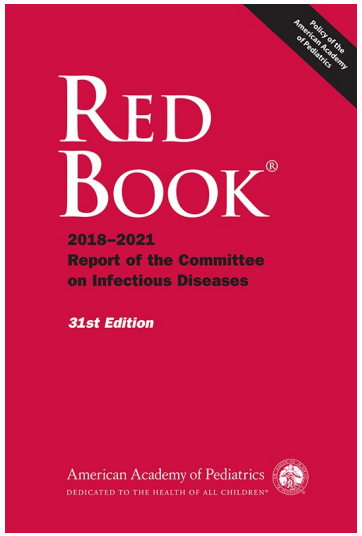
Case Study

23 yo college student from China reports has received BCG x 2.TST is 11mm on college entrance. After discussion with their provider they get a QFT-Plus test which is negative.

Which is true:

A. **Given discrepancy, repeat the TST**
B. **QFT likely false negative**
C. **No treatment, no additional testing at this time**

Screening Guidelines age ≤5 yrs



Preferred test: TST

- Limited evidence suggests TST more sensitive in children
- Prioritize sensitivity over specificity
- Allows for serial testing with TST during "window prophylaxis"

Can use IGRAs in immunocompetent children > 2 years; some experts down to 1 year of age - Esp. if prior BCG vaccination

Lewinsohn *CID* 2017; AAP *2021 Red Book*; Amina 2018

Summary

Latent TB infection

- State of persistent immune response to stimulation by *M. tuberculosis* antigens without evidence of clinically active TB
- Screening in Low Risk Individuals → Don't Do
- Dual Testing → Useful for maximizing sensitivity OR specificity

All LTBI tests (TST, IGRA) have limitations

- Indirect measures of infection
  - Require both Mtb infection and an intact immune system to mount a response for a positive test
- False positives: NTM (TST false positive with BCG)
- False negatives: severe immunosuppression, early in window after infection
- Should not be used to diagnose active TB* or LTBI treatment response

AAP Redbook recommends IGRAs down to age 2 (many experts rec age 1 or younger)

* Can be helpful pediatric TB

References/Resources

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