Conference

8th National Conference on
Laboratory Aspects of Tuberculosis

APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES

IGRAs: update on serial testing and predictive value

Dr Madhukar Pai, MD, PhD

Associate Professor, Epidemiology, McGill University

Associate Director, McGill International TB Centre, Montreal, Canada

McGill

McGill International TB Centre

madhukar.pai@mcgill.ca

We know a lot about IGRAs, but these are 3 areas where new evidence is rapidly accumulating

- Predictive (prognostic) value of IGRAs
- Serial testing: use of IGRAs for estimating incidence of new TB infection (i.e. conversions)
- Reproducibility (variability) of IGRAs

Systematic review and meta-analysis of predictive value 15 cohort studies

published in 2012

Predictive value of interferon-γ release assays for incident active tuberculosis: a systematic review and meta-analysis

Molebogeng X Rangaka, Katalin A Wilkinson, Judith R Glynn, Daphne Ling, Dick Menzies, Judith Mwansa-Kambafwile, Katherine Fielding, Robert J Wilkinson, Madhukar Pai

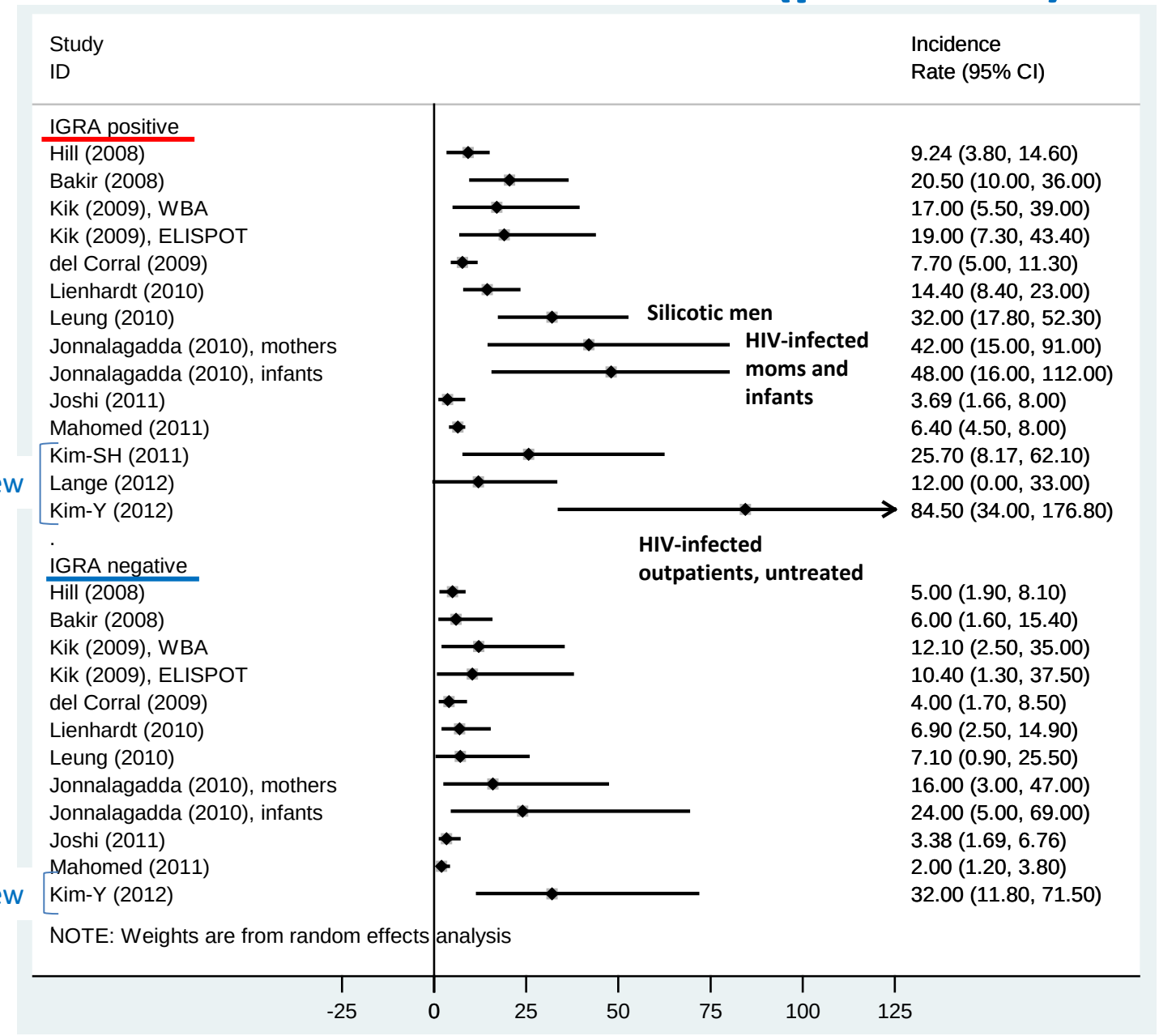
Lancet Infect Dis 2012; 12: 45–55

Interpretation Neither IGRAs nor the TST have high accuracy for the prediction of active tuberculosis, although use of IGRAs in some populations might reduce the number of people considered for preventive treatment. Until more predictive biomarkers are identified, existing tests for latent tuberculosis infection should be chosen on the basis of relative specificity in different populations, logistics, cost, and patients' preferences rather than on predictive ability alone.

We updated the review with 5 new studies since 2012

(15 + 5 new)

TB incidence rates (per 1000)



12/20 publications reported TB incidence rates

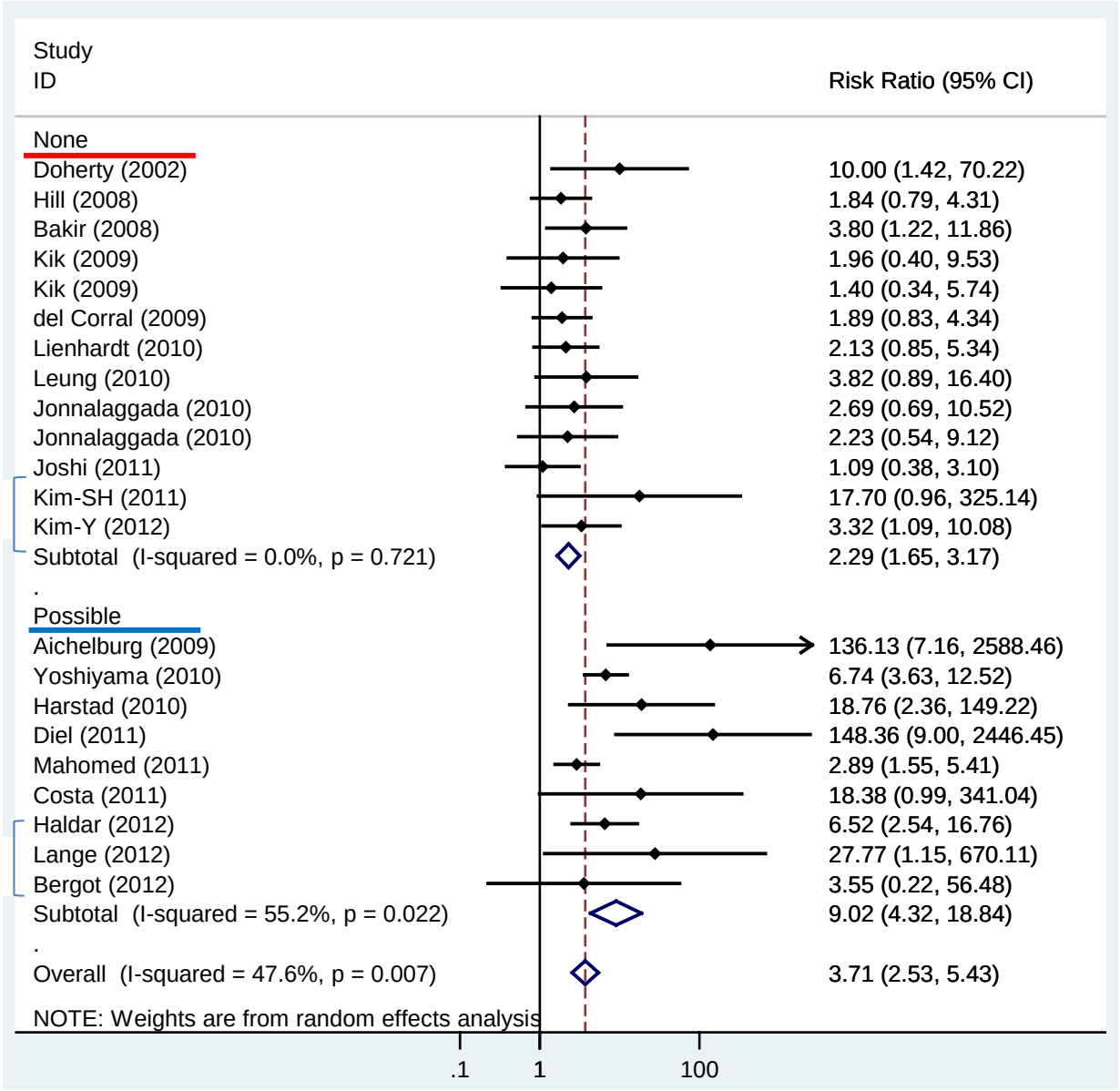
IGRA-positive: Incidence ranges from 0.4 to 8%

IGRA-negative: Incidence ranges from 0.2 to 3%

Sandra Kik, MX Rangaka, Pai M. Unpublished data, confidential

Association between IGRA and incident TB:

RR, stratified by potential incorporation/work-up bias



20/20 publications reported RR

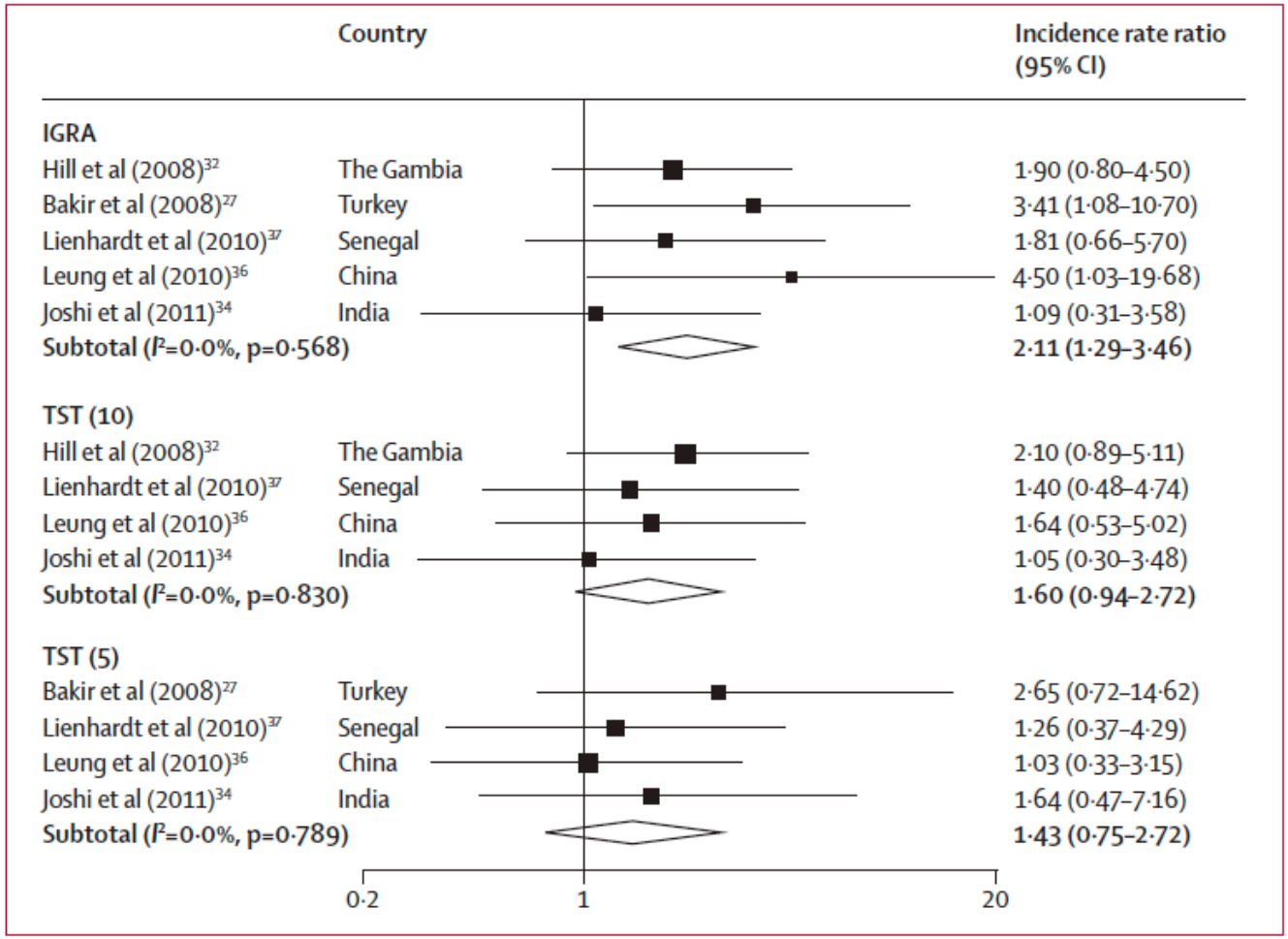
No bias:
Overall RR
2.29 (1.65-3.17)

Possible bias:
Overall RR
9.02 (4.32-18.84)

Sandra Kik, MX Rangaka, Pai M. Unpublished data, confidential

IGRA vs TST comparison:

which has greater predictive value?
(studies that did a head-to-head)



None of the new studies qualified to be included in this analysis;

thus results of Ranganka *et al* still hold.

Conclusions of this updated review

- Incidence rates of TB, even in IGRA positive individuals, are low, suggesting that a vast majority (>95%) of IGRA+ individuals do not progress to TB disease during follow-up. This is similar to the TST.

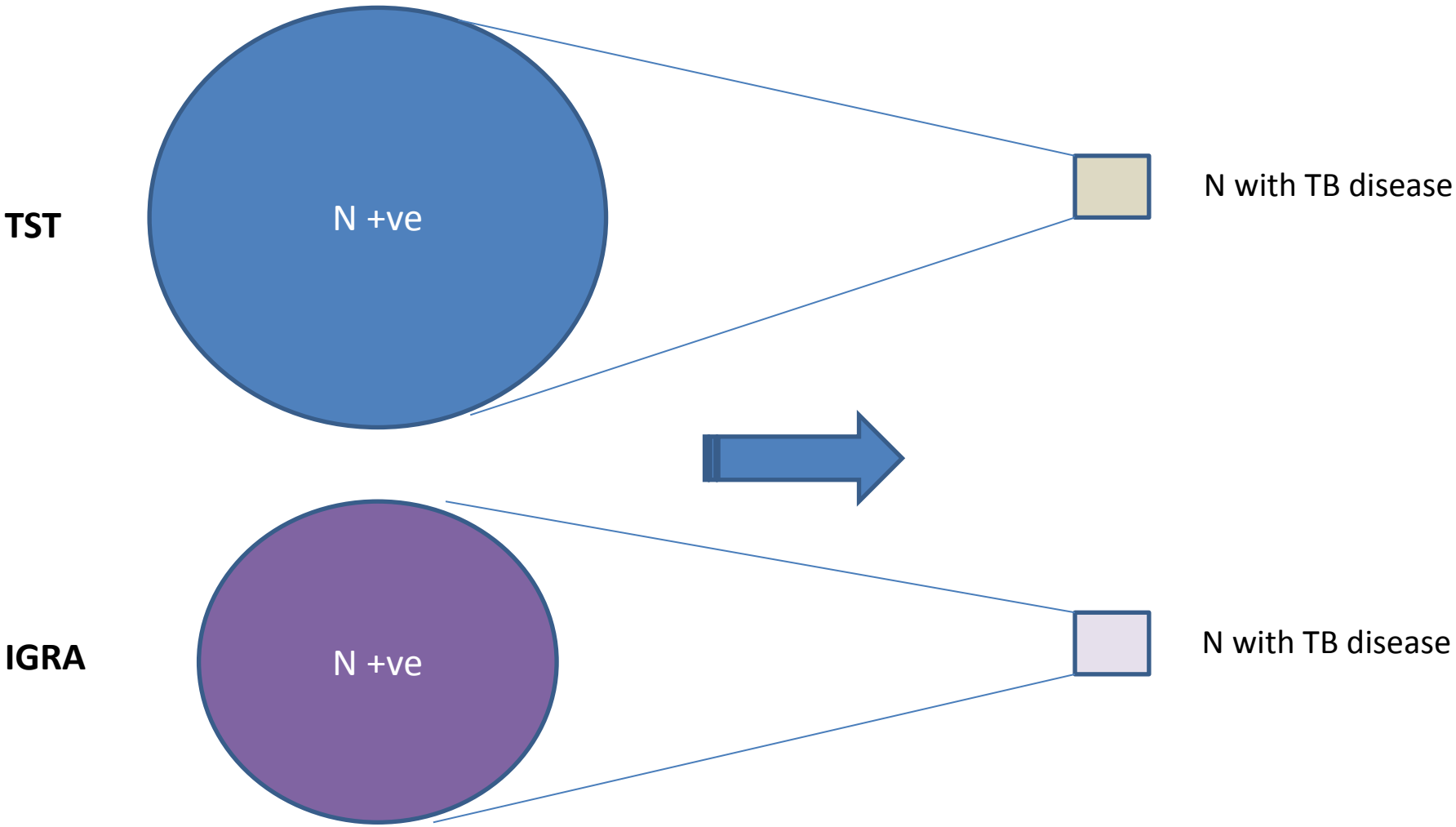
- In some settings (mostly low TB incidence), the % IGRA+ will be less than % TST+, reducing the number needed for preventive therapy.

- Based on the evidence thus far, IGRAs appear to have similar predictive value as the TST (perhaps slightly higher, but statistically not significant).

- All existing LTBI tests (TST and IGRAs) have only modest predictive value and may not help identify those who are at highest risk of progression to disease.

Sandra Kik, MX Rangaka, Pai M. Unpublished data, confidential

Reduction in number needed for IPT



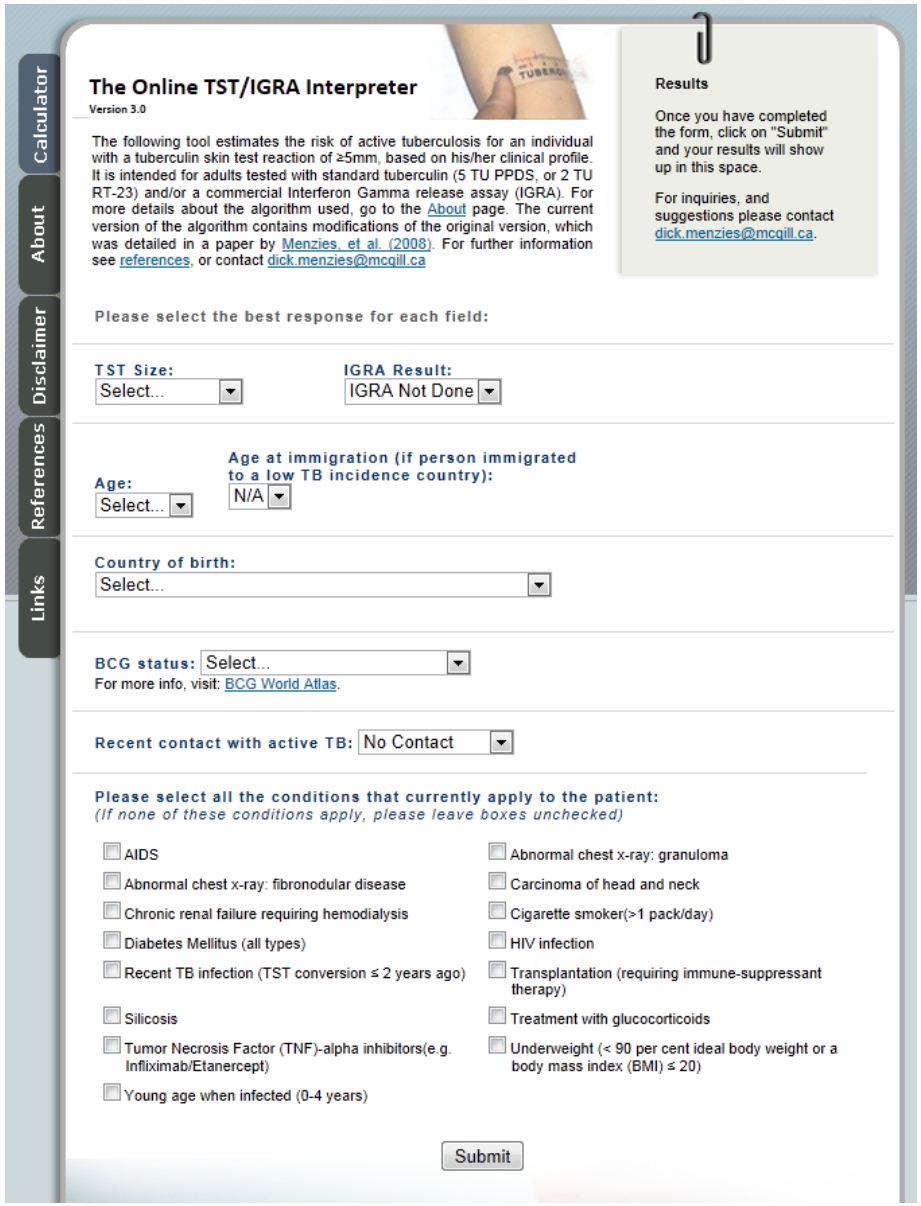
How can we squeeze predictive value out of IGRAs?

1. Only test those who are at high risk
2. Incorporate biomarkers with other known risk factors (age, recent conversion, HIV etc.) into a composite scoring system to generate multivariable risk prediction models
3. Identify new biomarkers that are more predictive
4. Use a higher cut-off for prediction (as compared to diagnosis)
5. Use serial testing to resolve underlying phenotypes (e.g. stable conversions)

Use composite risk prediction models: test + risk factors

http://www.tstin3d.com

Composite risk prediction models that incorporate biomarker and risk factors

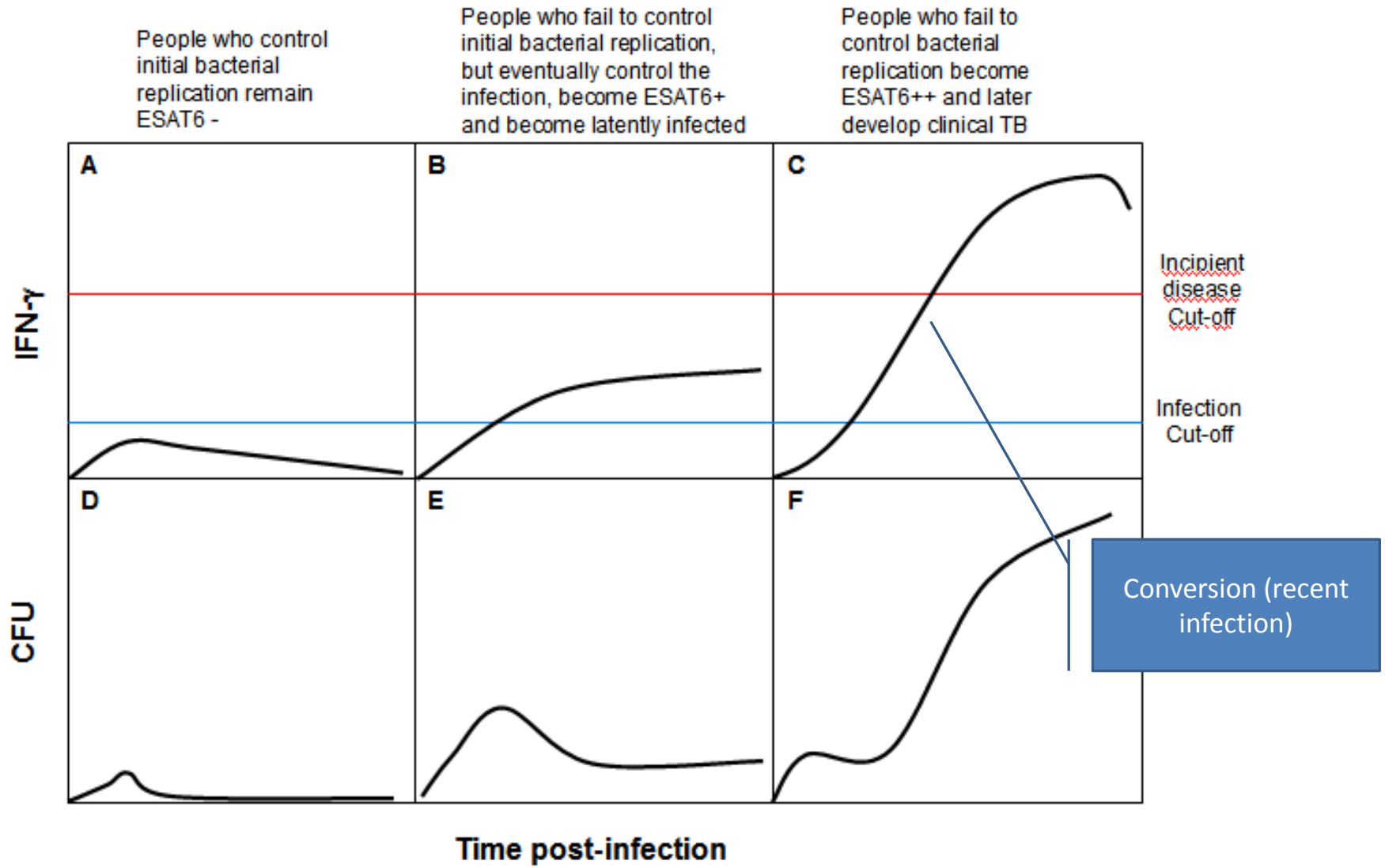


Age

Recent infection

HIV

Use a higher cut-off for disease prediction



Andersen P, Doherty TM, Pai M, Weldingh K; *Trends Mol Med 2007*

A single IGRA or TST = limited predictive value

Can we use serial testing to resolve the phenotypes and estimate incidence of new TB infections?

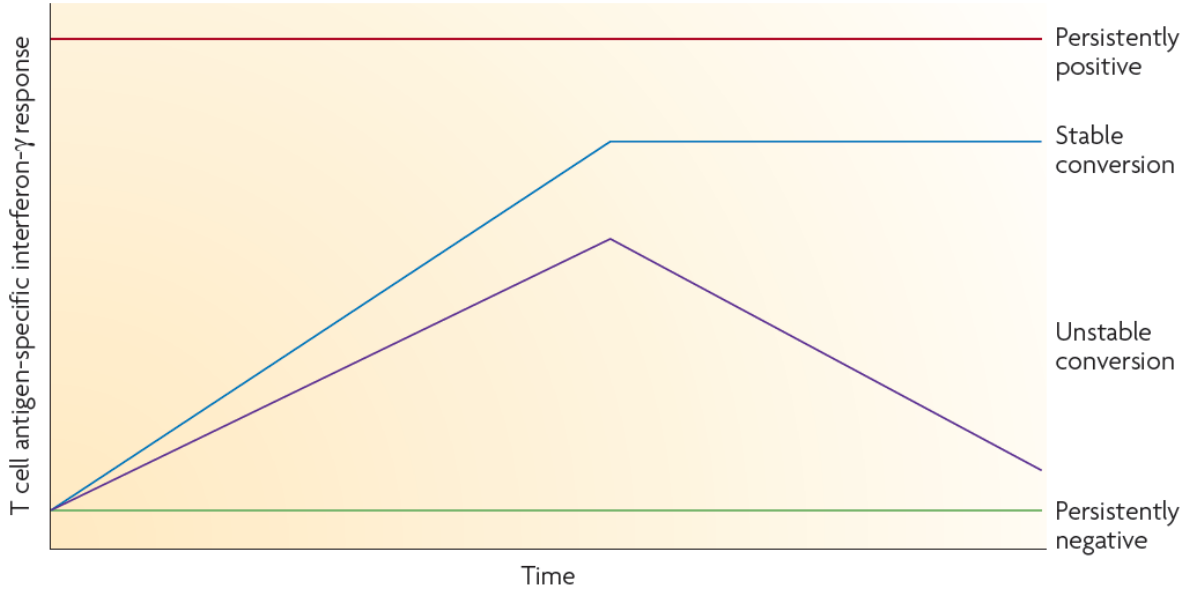


Figure 1 | **Serial testing with antigen-specific T cell interferon-γ release assays reveals underlying phenotypes that are unlikely to have the same prognosis.** The persistently positive pattern is seen in individuals who are repeatedly interferon-γ release assay (IGRA)-positive for a long time. Unstable conversion refers to individuals who convert their IGRA result from negative to positive and then revert again to negativity. Stable conversion refers to individuals who convert their IGRA result and stay converted, at least in the short term. Persistently negative refers to individuals who stay repeatedly IGRA-negative for a long time.

Pai. Nat Rev Microbiol 2010

What is the prognosis of these phenotypes?

Conversions (RR=8) are more predictive than a single test result (RR=2.5)

Predictive Value of Recent QuantiFERON Conversion for Tuberculosis Disease in Adolescents

Shingai Machingaidze[1,2,3], Suzanne Verver[4], Humphrey Mulenga[1,2], Deborah-Ann Abrahams[1,2], Mark Hatherill[1,2], Willem Hanekom[1,2], Gregory D. Hussey[1,2,3], and Hassan Mahomed[1,2]

[1]South African Tuberculosis Vaccine Initiative, Institute of Infectious Diseases and Molecular Medicine; [2]School of Child & Adolescent Health; [3]Vaccines for Africa Initiative, Division of Medical Microbiology, University of Cape Town, Cape Town, South Africa; and [4]KNCV Tuberculosis Foundation, The Hague and CINIMA, Academic Medical Centre, Amsterdam, The Netherlands

QFT conversion indicated an approximately eight-fold higher risk of progression to TB disease within 2 years when compared with non-converters.

TABLE 2. OVERALL TUBERCULOSIS INCIDENCE AND CUMULATIVE INCIDENCE BY QUANTIFERON GROUP

Study Group	n	TB Incident Cases	Observation Time (*person-yr*)	Incidence Rate per 100 person-yr (95% CI)	Cumulative Incidence (%) (95% CI)
All TB cases					
QFT converters	534	15	1,026	1.46 (0.82–2.39)	2.8 (1.58–4.59)
QFT nonconverters	629	2	1,169	0.17 (0.02–0.62)	0.32 (0.03–1.14)
Protocol-defined TB cases					
QFT converters	534	8	1,026	0.78 (0.34–1.53)	1.4 (0.65–2.93)
QFT nonconverters	629	1	1,169	0.08 (0.002–0.48)	0.16 (0.004–0.88)

Definition of abbreviations: CI = confidence interval; QFT = QuantiFERON; TB = tuberculosis.

But, even among QFT converters, the incidence rate was only 1.5 per 100 py!

AJRCCM 2012

To interpret serial IGRA testing results, we need clearly understand the test reproducibility and define cut-offs for conversions and reversions

Guidelines for Using the QuantiFERON®-TB Gold Test for Detecting *Mycobacterium tuberculosis* Infection, United States

Prepared by
Gerald H. Mazurek, MD, John Jereb, MD, Phillip LoBue, MD, Michael F. Iademarco, MD, Beverly Metchock, PhD, Andrew Vernon, MD
Division of Tuberculosis Elimination, National Center for HIV, STD, and TB Prevention

Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-Care Settings, 2005

BOX 2. Interpretations of tuberculin skin test (TST) and QuantiFERON®-TB test (QFT) results according to the purpose of testing for *Mycobacterium tuberculosis* infection in a health-care setting

Purpose of testing	TST	QFT
1. Baseline	1. ≥10 mm is considered a positive result (either first- or second-step)	1. Positive (only one-step)
2. Serial testing without known exposure	2. Increase of ≥10 mm is considered a positive result (TST conversion)	2. Change from negative to positive (QFT conversion)
3. Known exposure (close contact)	3. ≥5 mm is considered a positive result in persons who have a baseline TST result of 0 mm; an increase of ≥10 mm is considered a positive result in persons with a negative baseline TST result or previous follow-up screening TST result of ≥0 mm	3. Change to positive

Simplistic neg to pos change was defined as conversion (since there were no data at that time)

First serial testing study was published in 2006

Serial Testing of Health Care Workers for Tuberculosis Using Interferon-γ Assay

Madhukar Pai, Rajnish Joshi, Sandeep Dogra, Deepak K. Mendiratta, Pratibha Narang, Shriprakash Kalantri, Arthur L. Reingold, John M. Colford, Jr., Lee W. Riley, and Dick Menzies

Divisions of Epidemiology and Infectious Diseases, School of Public Health, University of California, Berkeley; Division of Pulmonary and Critical Care Medicine, San Francisco General Hospital, University of California, San Francisco, California; Departments of Medicine and Microbiology, Mahatma Gandhi Institute of Medical Sciences, Sevagram, India; and the Montreal Chest Institute, McGill University, Montreal, Quebec, Canada

"our results suggest that health care facilities that switch to IGRAs for serial testing might observe higher conversion rates than those with TST, especially if the less stringent definition is used for conversion… Therefore, research is needed to understand the biological basis of IGRA conversions and reversions, to optimize test reproducibility and thresholds, and to determine risk factors for conversions and reversions."

AJRCCM 2006

Several new studies from low-incidence countries: all show high rates of conversions and reversions

TABLE 1
Serial testing studies of interferon-gamma release assays in health care workers (HCWs) in low and intermediate incidence countries

Author (reference), year, country	Duration between testing	Conversion, n/N (%)		IGRA reversions*, n/N (%)
		Tuberculin skin test	IGRA*	
Joshi et al (15), 2012, USA	2 to 30 days	N/A	N/A	18/45 (40)
Rafiza et al (16), 2012, Malaysia	1 year	N/A	69/703 (9.8)	14/59 (23.7)
Fong et al (17), 2012, USA	1 year or 1 to 6 months for repeat of positive IGRA	N/A	52/1857 (2.8)	8/10 (80)†
Torres Costa et al (18), 2011, Portugal	1 year	61/199 (30.7) Reversion rates: 4/188 (2.1)	51 /462 (11)	46/208 (22.1)
Schablon et al (19), 2010, Germany	High-risk HCWs tested annually, all others evaluated every other year	N/A	15/245 (6.1)	13/42 (32.6)
Ringshausen et al (20), 2010, Germany	18 weeks	N/A	3/162 (1.9)	6/18 (33.3)
Park et al (21), 2010, South Korea	1 year	N/A	14/244 (5.7)	N/A
Lee et al (22), 2009, South Korea	1 year	16/75 (21.3)	21/146 (14.4)	N/A
Chee et al (23), 2009, Singapore	1 year	0/18 (Note: denominator includes only baseline concordant positives)	9/182 (4.9)	N/A
Yoshiyama et al (24), 2009, Japan	2 and 4 years	N/A	5/277 (1.8)	13/32 (41)
Pollock et al (25), 2008, USA	1 to 7 months	N/A	2/43 (4.6). Selected HCWs at 'increased risk' and negative at baseline	N/A

**All conversions/reversions using simple negative/positive; †Testing was performed among individuals with positive QuantiFERON-TB (Cellestis Ltd, Australia) results close to the cut-off point. IGRA Interferon-gamma release assay; N/A Not available*

Pai & Elwood. Can Resp J 2012

>2000 HCWs in 4 US hospitals (CDC TO18 study):
- TST = 0.9%
- QFT = 6.1%
- T-SPOT = 8.3% conversion rates

Canadian study in HCWs (Zwerling et al. PLoS ONE 2013):
- TST = 0%
- QFT = 5.3% conversion rates

Arkansas study of >2000 HCWs (Joshi M. CHEST 2012):
- TST = 0.1% (historical)
- QFT = 3.2% conversion rates

Stanford study of >9000 HCWs (Slater et al. AJRCCM 2013):
- TST = 0.4% (historical)
- QFT = 4.4% conversion rates

Early adopters of IGRAs for HCW screening in North America are reporting challenges…

(and different hospitals are coming up with their own interpretational criteria, cut-offs and re-testing strategies!)

Challenges of Interferon-γ Release Assay Conversions in Serial Testing of Health-care Workers in a TB Control Program

Kimberlee S. Fong, DO; J. Walton Tomford, MD; Lucileia Teixeira, MD; Thomas G. Fraser, MD; David van Duin, MD, PhD; Belinda Yen-Lieberman, PhD; Steve M. Gordon, MD; and Cyndee Miranda, MD

Questionable Effectiveness of the QuantiFERON-TB Gold Test (Cellestis) as a Screening Tool in Healthcare Workers

Sumanth Gandra, MD, MPH; William S. Scott, MD, MPH; Vijaya Somaraju, MD, MPH; Huaping Wang, PhD; Suzanne Wilton, APN, CNP; Michelle Feigenbaum, RN

Use of interferon-gamma release assays in a health care worker screening program: Experience from a tertiary care centre in the United States

Manish Joshi MD FCCP[1,2], Thomas P Monson MD[2], Gail L Woods MD[2]

Joshi M, Monson T, Woods G. 2012. Performance and Practicality of IGRA in Serial Testing for Latent TB Infection in US Healthcare Workers- A Real World Experience. [Abstract]. Chest **142**:142A.

Delineating a Retesting Zone Using Receiver Operating Characteristic Analysis on Serial QuantiFERON Tuberculosis Test Results in US Healthcare Workers

Wendy Thanassi,[1,2,3,4] Art Noda,[4,5] Beatriz Hernandez,[4,5] Jeffery Newell,[4] Paul Terpeluk,[6] David Marder,[7] and Jerome A. Yesavage[4,5]

Repeat IGRA Testing in Canadian Health Workers: Conversions or Unexplained Variability?

Alice Zwerling[1,2], Andrea Benedetti[1,2,3], Mihaela Cojocariu[2,5], Fiona McIntosh[5], Filomena Pietrangelo[4], Marcel A. Behr[1,2,3], Kevin Schwartzman[2,3], Dick Menzies[1,2,3], Madhukar Pai[1,2,*]

Challenges with QuantiFERON-TB assay for large-scale, routine screening of US healthcare workers

Madeline L. Slater[1], Gary Welland[2], Madhukar Pai[3], Julie Parsonnet[1], Niaz Banaei[1,4,5]

Serial testing challenges have put the spotlight on reproducibility

OPEN ACCESS Freely available online PLoS one

T-Cell Assays for Tuberculosis Infection: Deriving Cut-Offs for Conversions Using Reproducibility Data

Anandharaman Veerapathran[1,2], Rajnish Joshi[1,2,3], Kalyan Goswami[1,2], Sandeep Dogra[4], Erica E. M. Moodie[5], M. V. R. Reddy[1,2], Shriprakash Kalantri[1,2], Kevin Schwartzman[5,6], Marcel A. Behr[5,7], Dick Menzies[5,6], Madhukar Pai[5,6]*

Within-Subject Variability of *Mycobacterium tuberculosis*-Specific Gamma Interferon Responses in German Health Care Workers

Felix C. Ringshausen,[1]* Albert Nienhaus,[1] José Torres Costa,[2] Heiko Knoop,[3] Stephan Schlösser,[4] Gerhard Schultze-Werninghaus,[3] and Gernot Rohde[5]

Reproducibility of QuantiFERON-TB Gold In-Tube Assay

Sharon Perry,[1]* Luz Sanchez,[1] Shufang Yang,[1] Zubin Agarwal,[1] Philip Hurst,[2] and Julie Parsonnet[1]

Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, California,[1] and Santa Clara Valley Health and Hospitals System, San Jose, California[2]

Preanalytical Delay Reduces Sensitivity of QuantiFERON-TB Gold In-Tube Assay for Detection of Latent Tuberculosis Infection

David Doberne,[1] Rajiv L. Gaur,[2] and Niaz Banaei[1,2,3]*

Intra-assay reliability and robustness of QuantiFERON®-TB Gold In-Tube test in Zambia

K. Shanaube,* P. De Haas,*† A. Schaap,*† M. Moyo,* B. Kosloff,*† A. Devendra,† E. Raby,† P. Godfrey-Faussett,† H. Ayles*†

Test Variability of the QuantiFERON-TB Gold In-Tube Assay in Clinical Practice

John Z. Metcalfe[1,2], Adithya Cattamanchi[1,2], Charles E. McCulloch[3], Justin D. Lew[4], Ngan P. Ha[4], and Edward A. Graviss[4]

Assay Parameters Affecting Variability Of Quantiferon®-Tb Gold In-Tube Assay Results

W. C. Whitworth[1], G. H. Mazurek[2], D. J. Goodwin[3],

[1]CDC, Atlanta, GA, [2]Centers for Disease Control and Prevention, Atlanta, GA, [3]USAF School of Aerospace Medicine, Wright Patterson AFB

Within-Subject Variability and Boosting of T-Cell Interferon-γ Responses after Tuberculin Skin Testing

Richard N. van Zyl-Smit[1], Madhukar Pai[2], Kwaku Peprah[1], Richard Meldau[1], Jackie Kieck[3], June Juritz[4], Motasim Badri[1], Alimuddin Zumla[5], Leonardo A. Sechi[6], Eric D. Bateman[1], and Keertan Dheda[1,5,7]

Short-Term Reproducibility of a Commercial Interferon Gamma Release Assay

A. K. Detjen,[1]* L. Loebenberg,[2] H. M. S. Grewal,[3] K. Stanley,[2] A. Gutschmidt,[2] C. Kruger,[2] N. Du Plessis,[2] M. Kidd,[4] N. Beyers,[1] G. Walzl,[2] and A. C. Hesseling[1]

Within-Subject Interlaboratory Variability of QuantiFERON-TB Gold In-Tube Tests

William C. Whitworth[1]*, Lanette R. Hamilton[2], Donald J. Goodwin[3¤a], Carlos Barrera[2], Kevin B. West[4], Laura Racster[3¤b], Laura J. Daniels[1,5], Stella O. Chuke[1,6], Brandon H. Campbell[1], Jamaria Bohanon[3,5], Atheer T. Jaffar[3,5¤c], Wanzer Drane[7], David Maserang[8¤d], Gerald H. Mazurek[1]

Investigation of False-Positive Results Given by the QuantiFERON-TB Gold In-Tube Assay

Madeline Slater,[a] Julie Parsonnet,[a] and Niaz Banaei[a,b,c]

Division of Infectious Diseases and Geographic Medicine, Departments of Medicine[a] and Pathology,[b] Stanford University School of Medicine, Palo Alto, California, USA, and Clinical Microbiology Laboratory, Stanford University Medical Center, Palo Alto, California, USA[c]

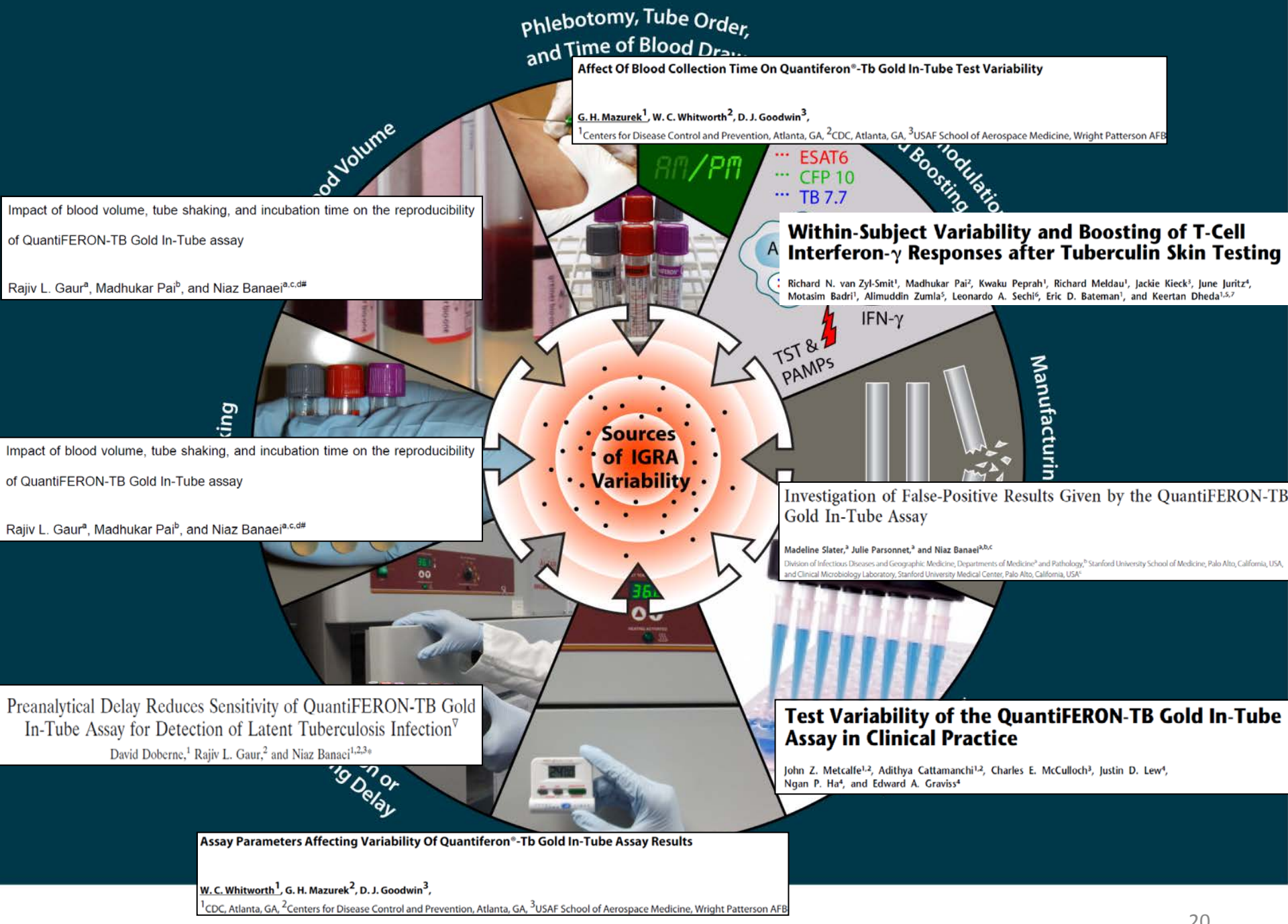
Impact of blood volume, tube shaking, and incubation time on the reproducibility of QuantiFERON-TB Gold In-Tube assay

Rajiv L. Gaur[a], Madhukar Pai[b], and Niaz Banaei[a,c,d#]

Affect Of Blood Collection Time On Quantiferon®-Tb Gold In-Tube Test Variability

G. H. Mazurek[1], W. C. Whitworth[2], D. J. Goodwin[3],

[1]Centers for Disease Control and Prevention, Atlanta, GA, [2]CDC, Atlanta, GA, [3]USAF School of Aerospace Medicine, Wright Patterson AFB

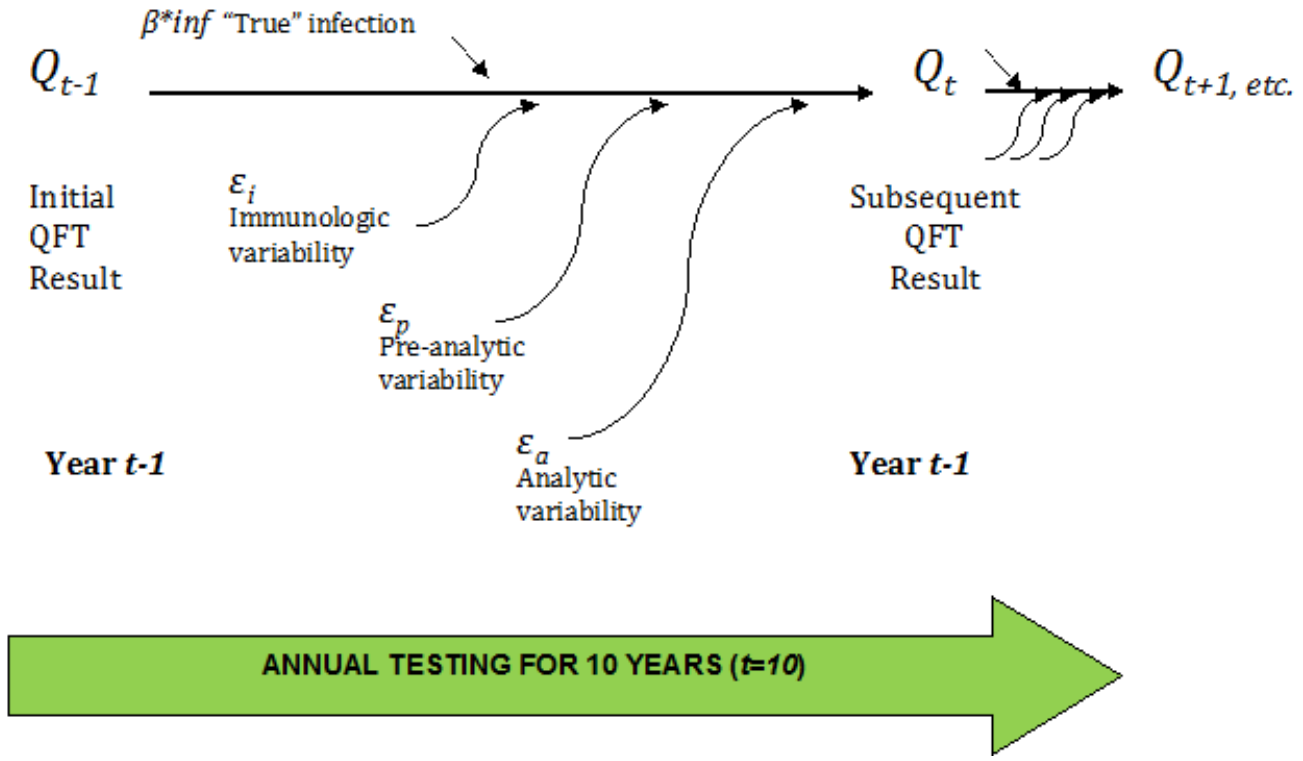


Pai M… Banaei N et al. Clin Micro Rev 2014 (in press)

Sources of variability	QFT	T-SPOT.TB
Manufacturing		
Between-lot variability	↑↓	↑↓
Pre-analytical		
Time of blood draw (AM versus PM)	↑ PM	?
Skin disinfection	?	?
Traumatic blood draw	?	?
Blood volume (0.8-1.2 ml)	↓	NA
Shaking of tubes (gentle-vigorous)	↑	NA
T-cell and APC count	?	?*
Transportation temperature	?	↓ spots
Delay in incubation (0-16 hr)	↓ response	↓ spots
Incubation time (16-24 hr)	Possible effect	?
Plasma separation delays (sec-hr)	?#	NA
Plasma storage (+4-–80°C)	No effect	NA
Analytical		
Within-run imprecision	↑↓	↑↓
Between-run imprecision	↑↓	↑↓
Between-operator imprecision	↑↓	↑↓
Between-laboratory imprecision	↑↓	↑↓
Immunological		
Boosting by PPD	↑ response	↑ spots
Modulation by PAMP	↑↓	?

Pai M… Banaei N et al. *Clin Micro Rev* 2014 (in press)

Work in progress: modeling the total variation



$$Q_t = Q_0 + \beta^*inf + \varepsilon_{i1} + \varepsilon_{i2} + \ldots + \varepsilon_{ir} + \varepsilon_{p1} + \varepsilon_{p2} + \ldots + \varepsilon_{pn} + \varepsilon_{a1} + \varepsilon_{a2} + \ldots + \varepsilon_{am},$$

Hypothesis: different components that contribute to the random and systematic variability of the test will be able to explain at least 50% of the observed conversions and reversions in the different studies, and pre-analytical sources will be the most important source of variability.

Denkinger CM, Dowdy D, Banaei N, Metcalfe JZ, Cattamanchi A, Pai M [CIHR grant funded work]

Conclusions

- IGRAs are an incremental advance; not transformational*
- We are still looking for a highly predictive LTBI test that can help target preventive therapy
- We need new biomarkers and composite risk prediction models that can help resolve the various phases of LTBI spectrum
- If used in serial testing, high rates of IGRA conversions will occur and not be compatible with local TB epidemiology
  - Hospitals and labs must do everything they can to standardize testing protocols, to minimize variation
  - Simple negative to positive cut-off for conversions is not acceptable
  - We need a borderline zone or some other strategy (e.g. re-testing) to handle conversions and reversions
  - To derive better cut-offs, we need to estimate all the sources of variation, and compute the overall expected random variation

*LoBue P & Castro K. JAMA 2012

Thank you!

Stanford
- Niaz Banaei
- Rajiv Gaur
- Mady Slater

McGill University
- Sandra Kik
- Claudia Denkinger
- Alice Zwerling
- Dick Menzies




LSHTM / UCT
- Lele Rangaka

UCSF
- John Metcalfe
- Adithya Cattamanchi


Hopkins
- David Dowdy