Serial Testing for TB Infection with IGRAs: Understanding the Sources of Variability

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Overview

• Accuracy and reproducibility of IGRAs
• Sources of IGRA variability
• Future direction
Disclosures

- Banaei:
  - No financial conflicts
  - No industry links or funding
Stanford University Medical Center
Clinical Laboratories

CAP inspected, CLIA certified
Stanford Policy

- Screen new HCW and then annually
- >10,000 screenings per year
Diagnosis of LTBI Until Dec 2006

- Tuberculin Skin Test

- Disadvantages
  - Subjective
  - In vivo
    - Adverse effects
    - Boosting
  - Affected by BCG vaccination
  - Requires two visits

Pulmonary Perspective

Interpretation of Repeated Tuberculin Tests
Boosting, Conversion, and Reversion

DICK MENZIES
Montreal Chest Institute and Respiratory Epidemiology Unit, McGill University, Montreal, Quebec, Canada
IGRAs entered the scene with a lot of promise

More sensitive and specific than TST
More reproducible/objective
More predictive

‘A 21st Century Solution for Latent TB Detection’
Interferon-γ Release Assays

- **Quantiferon (Cellestis Inc.)**
  - Positive $\geq 0.35$ IU/ml
- **T-SPOT.TB (Oxford Immunotec Inc.)**
  - Positive $\geq 6$ spots/well
TST can be positive in all states except the last

IGRAs are likely to be positive in all states as well? Or spontaneous convert/revert?
CDC guidelines in 2005 recommended use of IGRAs for HCW screening with:

- no published data on serial testing
- no independent, peer-reviewed literature on IGRA reproducibility

Simplistic neg to pos change was defined as conversion (since there were no data)
Sensitivity of TST & IGRAs in patients with active TB

TST

Pooled Sensitivity = 0.70 (0.67 to 0.72)
Chi-square = 128.00; d.f. = 24 (p = 0.0000)
Inconsistency (I-square) = 81.3 %

QFT-IT

Pooled Sensitivity = 0.81 (0.78 to 0.83)
Chi-square = 79.90; d.f. = 18 (p = 0.0000)
Inconsistency (I-square) = 77.5 %

T-SPOT. TB

Pooled Sensitivity = 0.875 (0.85 to 0.90)
Chi-square = 65.59; d.f. = 16 (p = 0.0000)
Inconsistency (I-square) = 75.6 %

Diel et al Chest 2010
Sensitivity in patients with latent TB using progression to active TB as gold standard

<table>
<thead>
<tr>
<th>Country [study]</th>
<th>No. of subjects</th>
<th>Follow-up period (yrs)</th>
<th>Active TB cases</th>
<th>Case isolates available for RFLP typing</th>
<th>% sensitivity&lt;sup&gt;(a)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TST&lt;sup&gt;(c)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Netherlands [20]</td>
<td>339</td>
<td>2</td>
<td>9</td>
<td>6(6)</td>
<td>100(100)</td>
</tr>
<tr>
<td>Germany [21]</td>
<td>954</td>
<td>4</td>
<td>19</td>
<td>11(11)</td>
<td>52(36)</td>
</tr>
<tr>
<td>Japan [26]</td>
<td>3012</td>
<td>2</td>
<td>39</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Colombia [27]</td>
<td>2060</td>
<td>3</td>
<td>26</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Gambia [28]</td>
<td>2348</td>
<td>2</td>
<td>26</td>
<td>9(6)</td>
<td>56(67)</td>
</tr>
</tbody>
</table>
CDC guidelines in 2005 recommended use of IGRAs for HCW screening with:

- no published data on serial testing
- no independent, peer-reviewed literature on IGRA reproducibility

**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**

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

Simplistic neg to pos change was defined as conversion (since there were no data)
IGRA Reproducibility in HCWs
Since 2006, >50 studies have assessed IGRAs in HCWs

Interferon-gamma release assays for tuberculosis screening of healthcare workers: a systematic review

Alice Zwerling,¹ Susan van den Hof,²,³ Jerod Scholten,² Frank Cobelens,²,³ Dick Menzies,¹ Madhukar Pai¹

Thorax 2012
### Reproducibility of QFT-IT in HCW

**Largest study of >2000 HCWs (CDC Task Order 18 study):**

<table>
<thead>
<tr>
<th>Author (reference), year, country</th>
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<tr>
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<td>2 to 30 days</td>
<td>Tuberculin skin test</td>
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<tr>
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<td>1 year</td>
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<td>Torres Costa et al (18), 2011, Portugal</td>
<td>1 year</td>
<td>N/A</td>
</tr>
<tr>
<td>Schablon et al (19), 2010, Germany</td>
<td>High-risk HCWs tested annually, all others evaluated every other year</td>
<td>Reversion rates:</td>
</tr>
<tr>
<td>Ringshausen et al (20), 2010, Germany</td>
<td>18 weeks</td>
<td>N/A</td>
</tr>
<tr>
<td>Park et al (21), 2010, South Korea</td>
<td>1 year</td>
<td>N/A</td>
</tr>
<tr>
<td>Lee et al (22), 2009, South Korea</td>
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**Largest study of 9155 HCWs (Slater et al in revision):**

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**Canadian study in HCWs (Zwerling et al. PLoS ONE 2013):**

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**Canadian study in HCWs (Zwerling et al. PLoS ONE 2013):**

- **TST** = 0%  
- **QFT** = 5.3% conversion rates  
- **T-SPOT** = 8.3% conversion rates

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**TABLE 1**

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

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**Conversions 2% to 15%**  
**Reversions 20% to 40%**  

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Pai and Elwood Can Respir J. 2012
Early adopters of IGRAs for HCW screening in the US are reporting interesting challenges…
(and different hospitals are coming up with their own interpretational criteria and cut-offs!)

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, Thomas P Monson MD, and Gail L Woods MD

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,4 David Marder,2 and Jerome A. Yesavage4,5
Need for a New Cut-off?

Slater et al. in revision
If cut-off is raised to \( \geq 1 \text{IU/ml} \), short-term reproducibility is improved from 55% to 78% BUT 51% of persistent positives are missed.
Need to Identify Sources of IGRA Variability

- Eliminate sources of variability
- Standardize methods
- Account for variability (borderline zone)
Reproducibility studies are now emerging (independent of manufacturers)
Sources of IGRA Variability

- Pre-analytical
- Analytical
- Manufacturing
- Immunological
Quantiferon Gold Intube Assay

1. Collect 1mL of blood into Nil, Antigen and Mitogen tubes. Shake well. Incubate tubes at 37°C for 16-24 hrs.

2. Centrifuge tubes for 15 minutes.

3. Add conjugate, plasma samples and standards to ELISA. Incubate for 120 minutes at room temperature.

4. Wash and add substrate. Read absorbance after 30 minutes.

5. Software calculates results and prints reports.

Assay cut-off ≥0.35 IU/ml

Nil TBAg Mit

Harvested plasma is stable refrigerated for at least 4 weeks.

http://www.cellestis.com
QFT-GIT Assay Standardization

Standardized
- Pre-analytical
  - Skin disinfection
  - Blood volume
  - Shaking of tubes
  - Incubation delay
  - Incubation temp
  - Incubation duration
  - Plasma separation delay
  - Plasma storage

- Analytical
  - ELISA

Not Standardized
- Pre-analytical
  - T cell and APC count
  - Transportation temp

- Diet
- Infection
- Antibiotics
Does Blood Volume Matter?

- Draw 0.8-1.2 ml of blood
Distribution of Blood Volume in QFT Tubes

- No. 30
- Median 0.923
- Range 0.785-1.0

- No. 30
- Median 0.930
- Range 0.81-1.01

Gaur et al submitted
Study Design

Gaur et al submitted
Effect of Blood Volume on TB Response

**Infected Group**
(TST+ QFT+)
n=17

<table>
<thead>
<tr>
<th>Blood Volume (ml)</th>
<th>&lt;0.35</th>
<th>≥0.35</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8 ml</td>
<td>2 (12%)</td>
<td>15</td>
</tr>
<tr>
<td>1.0 ml</td>
<td>5 (29%)</td>
<td>12</td>
</tr>
<tr>
<td>1.2 ml</td>
<td>7 (41%)</td>
<td>10</td>
</tr>
</tbody>
</table>

**Uninfected Group**
(TST- QFT-)
n=33

<table>
<thead>
<tr>
<th>Blood Volume (ml)</th>
<th>&lt;0.35</th>
<th>≥0.35</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8 ml</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>1.0 ml</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>1.2 ml</td>
<td>32</td>
<td>1 (3%)</td>
</tr>
</tbody>
</table>

Gaur et al submitted
Does Shaking Matter?

1. Collect 1mL of blood into Nil, Antigen and Mitogen tubes. Shake well. Incubate tubes at 37°C for 16-24 hrs.

2. Centrifuge tubes for 15 minutes.

3. Add conjugate, plasma samples and standards to ELISA. Incubate for 120 minutes at room temperature.

4. Wash and add substrate. Read absorbance after 30 minutes.

5. Software calculates results and prints reports.

Assay cut-off \( \geq 0.35 \text{ IU/ml} \)

Nil TBAg Mit

Harvested plasma is stable refrigerated for at least 4 weeks.
Effect of Shaking on TB Response

A. Nil (IU/ml) vs. Shaking (Gentle, Vigorous)
   - P < 0.001

B. TB Ag (IU/ml) vs. Shaking (Gentle, Vigorous)
   - P = 0.004

C. TBAg-Nil (IU/ml) vs. Shaking (TBAgGen - NilGen, TBAgVig - NilVig)
   - P = 0.35

D. TBAg-Nil (IU/ml) vs. Shaking (TBAgGen - NilGen, TBAgVig - NilVig)
   - P = 0.004

Gaur et al submitted
Does Incubation Delay Matter?

- QFT
  Transport to incubator: $\leq 16h$

- T-SPOT.TB
  Transport to processing $\leq 8h$
Effect of Incubation Delay on IGRA

Effect of Incubation Delay on the Accuracy of QFT-IT Results

128 study participants

3 QFT-GIT sets collected

Incubation Delay

0 h

6 h

12 h

Low risk & - TST &or QFT
High risk & + TST &or QFT
TB Response Following Immediate and Delayed Incubation

P = 0.007

P = 0.0006

TBAg-Nil IFN-γ (IU/mL)

n=128

0 h 6 h 12 h

Incubation Delay

Doberne et al JCM 2011
TB Response for Subjects with Discordant Results

Reversion rate:
19% (5/26) with 6 h delay
22% (5/23) with 12 h delay
Introduction/Rationale: The QuantiFERON®-TB Gold In-Tube test (QFT-GIT), designed to detect Mycobacterium tuberculosis infection, has become a viable alternative to the tuberculin skin test (TST). QFT-GIT assesses interferon gamma (IFN-γ) response to manufactured peptides representing specific M. tuberculosis peptides using an enzyme-linked immunosorbent assay (ELISA). Excessive unexplained variability may limit test utility. To investigate the cause of within-subject variability, we performed QFT-GIT when certain test parameters were varied.

Methods: Within-subject QFT-GIT variability was assessed when three experimental parameters were varied, including: 1) incubation delay (<1 hr vs. 11-12 hrs), 2) incubation time (23-24 hrs vs. 16-17 hrs), and 3) incubation temperature (35°C vs. 37°C), and when QFT-GIT was repeated without varying these parameters (control comparison with incubation delay < 1 hr, incubation time 23-24 hrs, and incubation temperature 37°C). For each comparison, QFT-GITs were performed on blood collected at the same time from each subject. Qualitative analyses compared test interpretations. Quantitative analyses compared IFN-γ concentrations (IU/mL) in plasma from TB tubes (TB) and Nil tubes (Nil), and TB Responses (TB - Nil). Subjects with incomplete tests were excluded, and subjects with indeterminate results were excluded from the qualitative analyses.

Results: Of the 158 subjects enrolled, QFT-GIT results were available for (1) 149 subjects for the delay to incubation comparison, (2) 152 subjects for the incubation time comparison, (3) 102 subjects for the incubation temperature comparison, and (4) 145 subjects for the control comparison. Qualitative comparisons indicated discordant rates of 5.3%, 6.8%, and 7.8% for incubation time, delay, and temperature comparisons, respectively, compared to a discordance of 4.8% in the control comparison. Significantly lower TB Responses were seen with longer incubation delays (11-12 hrs vs. <1 hr, p = 0.001) and shorter incubation times (16-17 hrs vs. 23-24 hrs, p=0.002). A similar pattern of significance was seen for raw TB values, but not for Nil values. There were no significant differences for any of these measure for the control comparison.

Conclusions: Significantly lower QFT-GIT IFN-γ levels are observed with longer delays to incubation and shorter incubation times despite being within the limits recommended by the manufacturer. Delayed blood incubation and decreased incubation time contributed to quantitative QFT-GIT variability.

Am J Respir Crit Care Med 185;2012:A4735
Internet address: www.atsjournals.org Online Abstracts Issue
Mitogen Results Following Incubation Delay

\[ P < 0.0001 \]
\[ P < 0.0001 \quad P = 0.012 \]

- Doberne et al JCM 2011
Mitogen Results Following Incubation Delay

Herrera et al JCM 2010
T-Cell Xtend® for Extended Storage of Blood at Room Temperature up to 32 hours
Immediate: Within 3-4 h of blood collection

Delay: 24-26 h with or without T-Cell 

Xtend

The addition of TCell Xtend did not significantly reduce the number of conversions/reversions with T-SPOT.TB: 4.55% vs. 4.19%
Does Incubation Duration Matter?

- QFT
  Incubate @37°C 16-24 h

- T-SPOT.TB
  Incubate @37°C 16-24 h
Study Design

Gaur et al submitted
Effect of Incubation Duration on TB Response

**Infected Group**
- n=17

- <0.35: 5, 5, 5
- ≥0.35: 12, 12, 12

**Uninfected Group**
- n=33

- <0.35: 33, 33, 33
- ≥0.35: 0, 0, 0

Gaur et al submitted
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Analytical Precision of QFT-GIT Assay
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Detjen et al Clin Vaccine Immunol 2009
Metcalfe et al AJRCCM 2012
Whitworth et al PLoS ONE 2012
Analytical Precision of QFT-GIT Assay

Table 2. Repeatability of QFT-GIT Test Interpretation

<table>
<thead>
<tr>
<th>Test Result Sequence</th>
<th>Total Subjects (n=543)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant</td>
<td></td>
</tr>
<tr>
<td>Positive / Positive</td>
<td>201 (93%)</td>
</tr>
<tr>
<td>Negative / Negative</td>
<td>135</td>
</tr>
<tr>
<td>Indeterminate / Indeterminate</td>
<td>166 (31%)</td>
</tr>
<tr>
<td>Discordant</td>
<td></td>
</tr>
<tr>
<td>Positive / Negative</td>
<td>15</td>
</tr>
<tr>
<td>Negative / Positive</td>
<td>13</td>
</tr>
<tr>
<td>Indeterminate / Negative</td>
<td>11</td>
</tr>
<tr>
<td>Positive / Indeterminate</td>
<td>2</td>
</tr>
</tbody>
</table>

- Qualitative results: High agreement (kappa 0.84)
- Quantitative results: Considerable between-run variability (CV 14% for all and CV 27% for borderline (0.25-0.80 IU/ml))
- 86% (24/28) of rev/conv had borderline result (0.25-0.8 IU/ml)
Analytical Imprecision of QFT-GIT Assay: Between-Run Variability (n=20 ELISA runs)

CV 14%
Conversion 10% (2/20)

CV 11%
Reversion 20% (4/20)

Gaur et al unpublished
Sources of IGRA Variability

- Pre-analytical
- Analytical
- Manufacturing
- Immunological
The QFT-GIT Surveillance Graph: Daily Positive Rate Mar-Oct 2010
The QFT-GIT Surveillance Graph Showing Daily Positive Rate at Stanford

Elevated rate noted

TBAg lot discontinued
Within-subject Comparison of QFT-GIT Results

31% vs 5%

n=463

p<0.001

Slater et al JCM 2012
Investigation Outcomes

- FDA informed via CDC.

- Cellestis conducted an internal investigation and "could not reproduce" our findings.

- We could not culture viable organisms.
Sources of IGRA Variability

- Pre-analytical
- Analytical
- Manufacturing
- Immunological
Within-Subject Variability and Boosting of T-Cell Interferon-γ Responses after Tuberculin Skin Testing

Richard N. van Zyl-Smit¹, Madhukar Pai², Kwaku Peprah¹, Richard Meldau¹, Jackie Kieck³, June Juritz⁴, Motasim Badri¹, Alimuddin Zumla⁵, Leonardo A. Sechi⁶, Eric D. Bateman¹, and Keertan Dheda¹,⁵,⁷
Amnestic Response to PPD

IGRA Boosting by PPD
- PPD contains RD1 antigens
- In TST+ subjects
- Observed >3 days post TST

van Zyl-Smit et al PLoS ONE 2009
Ritz et al Ritz Int J Tuberc Lung Dis 2011
Sauzullo et al Tuberculosis 2011
Role of PAMPs in Modulating IGRA

Lymphoid Tissue

Th0

IL-12

PAMPs

Th1

IFN-γ

Th2

Site of Infection

Th1

IFN-γ

Mφ

TNF-α

APC

+
## Recognition of Different PAMPs by PRRs

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
<th>Fungi</th>
<th>Protozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP, DNA, RNA</td>
<td>DNA, LP, PG, LTA</td>
<td>DNA, Porin, PG, LPS</td>
<td>Zymosan, Mannan, β-glycan</td>
<td>GPI anchors, DNA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TLRs:</th>
<th>TLR2, 4, TLR9, TLR3, 7/8</th>
<th>TLR9, TLR2, TLR2</th>
<th>TLR9, TLR4, TLR5</th>
<th>TLR2, TLR2, TLR2,4</th>
<th>TLR9, TLR2,4</th>
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</thead>
<tbody>
<tr>
<td>RLRs:</td>
<td>RIG-I/MDA5 (PKR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NLRs:</td>
<td>NALP3, NALP3</td>
<td>NALP3, NOD2, NALP1/3</td>
<td>NALP3, NOD2, NALP1/3</td>
<td>IPAF</td>
<td></td>
</tr>
<tr>
<td>DNA sensors:</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mogensen Clin Mic Rev 2009
TLR Agonists Activate Adaptive Immune Responses

- Cell recruitment
- DC maturation: ↑Costimulatory molecules, ↑MHC
- Antigen presentation
  Cross-presentation
- Naïve T-cell activation

Mogensen Clin Mic Rev 2009
Role of PAMPs in Modulating IGRA
Effect of Microbiota on IGRA Response

- CD4
- IFN-γ
- APC
- ESAT6
- CFP-10
- TB7.7
PAMPs Increase TB Response in QFT-IT Assay

Healthy Controls

Subjects with LTBI

N=10 per grup

Gaur et al PLoS ONE 2012
Poly(I:C) & LPS Induce Inflammatory Cytokines & IFN-α in Whole Blood in QFT Nil Tube

N=8
LPS Induce Maturation of Monocytes in Whole Blood in QFT Nil Tube
PAMPS Trigger Earlier and Greater IFN-γ Release

![Graph showing IFN-γ response over time with different treatments.](image)
Nasal
Oral
Skin
Gastro-intestinal
Urogenital

Clarke et al Nat Med 2010
Ichinohe et al PNAS 2011
Microbiota regulates immune defense against respiratory tract influenza A virus infection

Takeshi Ichinohe\textsuperscript{a,b,1}, Iris K. Pang\textsuperscript{a,1}, Yosuke Kumamoto\textsuperscript{a}, David R. Peaper\textsuperscript{c}, John H. Ho\textsuperscript{a}, Thomas S. Murray\textsuperscript{c,d}, and Akiko Iwasaki\textsuperscript{a,2}

\textsuperscript{a}Department of Immunobiology, \textsuperscript{d}Department of Pediatrics, and \textsuperscript{c}Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06520; and \textsuperscript{b}Department of Virology, Faculty of Medicine, Kyushu University, Fukuoka 812-8582, Japan

A

\begin{itemize}
  \item Anti-PR8 titer (total IgG)
  \item Anti-PR8 titer (IgG2a)
  \item Anti-PR8 titer (IgA)
\end{itemize}

\begin{axis} [ybar, ymode=log, title={Anti-PR8 titer}, ylabel={IgG concentration}, symbolic x coords={Water, Antibiotics}, xtick=data, enlarge y limits={0.2,upper}, bar width=0.3, error bars/.cd, y dir=both, y explicit, error bars/.cd, y dir=both, y explicit]
  \addplot coordinates {(Water, 10^6) (Antibiotics, 10^5)}
  \addplot coordinates {(Water, 10^4) (Antibiotics, 10^3)}
  \addplot coordinates {(Water, 10^2) (Antibiotics, 10^1)}
  \addplot+[error bars/.cd, y dir=both, y explicit] coordinates {(Water, 10^0) (Antibiotics, 10^-1)}
\end{axis}

B

\begin{axis} [ybar, ymode=log, title={IFN-\gamma concentration}, ylabel={IFN-\gamma (ng/ml)}, xtick=data, enlarge y limits={0.2,upper}, bar width=0.3, error bars/.cd, y dir=both, y explicit, error bars/.cd, y dir=both, y explicit]
  \addplot coordinates {(Water, 10^0) (Antibiotics, 10^1)}
  \addplot+[error bars/.cd, y dir=both, y explicit] coordinates {(Water, 10^1) (Antibiotics, 10^2)}
  \addplot+[error bars/.cd, y dir=both, y explicit] coordinates {(Water, 10^2) (Antibiotics, 10^3)}
\end{axis}

C

\begin{axis} [ybar, ymode=log, title={IFN-\gamma concentration}, ylabel={IFN-\gamma (ng/ml)}, xtick=data, enlarge y limits={0.2,upper}, bar width=0.3, error bars/.cd, y dir=both, y explicit, error bars/.cd, y dir=both, y explicit]
  \addplot coordinates {(Water, 10^0) (Antibiotics, 10^1)}
  \addplot+[error bars/.cd, y dir=both, y explicit] coordinates {(Water, 10^1) (Antibiotics, 10^2)}
  \addplot+[error bars/.cd, y dir=both, y explicit] coordinates {(Water, 10^2) (Antibiotics, 10^3)}
\end{axis}

D

\begin{axis} [ybar, ymode=log, title={CD8+ T cell count}, ylabel={CD8+ T cell count (x10^6/lung)}, xtick=data, enlarge y limits={0.2,upper}, bar width=0.3, error bars/.cd, y dir=both, y explicit, error bars/.cd, y dir=both, y explicit]
  \addplot coordinates {(Mock, 10^0) (Water, 10^1) (Antibiotics, 10^2)}
  \addplot+[error bars/.cd, y dir=both, y explicit] coordinates {(Mock, 10^0) (Water, 10^0) (Antibiotics, 10^0)}
\end{axis}

E

\begin{axis} [ybar, ymode=log, title={Pulmonary Viral Titer}, ylabel={Pulmonary Viral Titer (PFU/ml)}, xtick=data, enlarge y limits={0.2,upper}, bar width=0.3, error bars/.cd, y dir=both, y explicit, error bars/.cd, y dir=both, y explicit]
  \addplot coordinates {(Water, 10^0) (Antibiotics, 10^1)}
  \addplot+[error bars/.cd, y dir=both, y explicit] coordinates {(Water, 10^0) (Antibiotics, 10^0)}
\end{axis}
Nasal
Oral
Skin
Gastro-intestinal
Urogenital
Skin Flora and Antisepsis
Effect of Microbiota and Environmental Bacteria on IGRA Response
Effect of *Staph aureus* on IGRA Response in Negative Subjects

<table>
<thead>
<tr>
<th>CFU</th>
<th>&lt;0.35</th>
<th>≥0.35</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 CFU</td>
<td>47</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>1000 CFU</td>
<td>37</td>
<td>14 (28%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CFU</th>
<th>&lt;0.35</th>
<th>≥0.35</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 CFU</td>
<td>48</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>1000 CFU</td>
<td>34</td>
<td>17 (33%)</td>
</tr>
</tbody>
</table>

Gaur et al unpublished

N=51
Effect of *E. coli* on IGRA Response in Negative Subjects

\[\begin{array}{c|c|c}
\text{TB Ag}_{ECo} - \text{Nil} (\text{U}/\text{ml}) & 10 \text{ CFU} & 1000\text{CFU} \\
<0.35 & 50 & 49 \\
\geq0.35 & 1 (2\%) & 2 (3\%) \\
\end{array}\]

\[\begin{array}{c|c|c}
\text{TB Ag}_{ECo} - \text{Nil} (\text{U}/\text{ml}) & 10 \text{ CFU} & 1000\text{CFU} \\
<0.35 & 49 & 47 \\
\geq0.35 & 2 (4\%) & 4 (8\%) \\
\end{array}\]

\[\begin{array}{c|c|c}
\text{TB Ag} - \text{Nil} (\text{U}/\text{ml}) & 10 \text{ CFU} & 1000\text{CFU} \\
<0.35 & 51 & 51 \\
\geq0.35 & 0 & 0 \\
\end{array}\]

\[\begin{array}{c|c|c}
\text{TB Ag} - \text{Nil} (\text{U}/\text{ml}) & 10 \text{ CFU} & 1000\text{CFU} \\
<0.35 & 49 & 47 \\
\geq0.35 & 2 (4\%) & 4 (8\%) \\
\end{array}\]

\[\begin{array}{c|c|c}
\text{TB Ag} - \text{Nil} (\text{U}/\text{ml}) & 10 \text{ CFU} & 1000\text{CFU} \\
<0.35 & 51 & 51 \\
\geq0.35 & 0 & 0 \\
\end{array}\]

N=51

Gaur et al unpublished
Effect of *P. aeruginosa* on IGRA Response in Negative Subjects

<table>
<thead>
<tr>
<th>CFU</th>
<th>10 CFU</th>
<th>1000 CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.35</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>≥0.35</td>
<td>1 (2%)</td>
<td>2 (3%)</td>
</tr>
</tbody>
</table>

N=51

Gaur et al unpublished
Bacterial Survival in Whole Blood

N=60

- **Staphylococcus aureus**: P<0.001
- **Escherichia coli**: P<0.001
- **Pseudomonas aeruginosa**: P<0.001
Can we eliminate predictable sources of variability and predict the net effect of random sources of variability on TB response?

<table>
<thead>
<tr>
<th>Source</th>
<th>Error Type</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-analytical</td>
<td>Random &amp; Systematic</td>
<td>↑↓</td>
</tr>
<tr>
<td>Analytical</td>
<td>Random</td>
<td>↑↓</td>
</tr>
<tr>
<td>Manufacturing</td>
<td>Random</td>
<td>↑↓</td>
</tr>
<tr>
<td>Immunological</td>
<td>Random &amp; Systematic</td>
<td>↑↓</td>
</tr>
</tbody>
</table>
Can we eliminate predictable sources of variability and predict the net effect of random sources of variability on TB response?
Can we eliminate predictable sources of variability and predict the net effect of random sources of variability on TB response?
Can we eliminate predictable sources of variability and predict the net effect of random sources of variability on TB response?
Construct a sequential series of models to evaluate the different components of variability

- 1\textsuperscript{st} model that will include all sources of variability
- 2\textsuperscript{nd} model will remove sources of variability that could be reduced through quality control measures
- 3\textsuperscript{rd} model will further remove sources of variability that could only be reduced with highly-standardized techniques
Goal of Modeling Study

- Determine true and false conversions
- Develop an evidence-based conversion rule for the interpretation of QFT results in serial testing

![Diagram](image)

**Figure 11:** Schematic of hypothetical cut-off for conversion and uncertainty zone analysis.
Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial

Michele D Tameris*, Mark Hatherill*, Bernard S Landry, Thomas J Scriba, Margaret Ann Snowden, Stephen Lockhart, Jacqueline E Shea, J Bruce McClain, Gregory D Hussey, Willem A Hanekom, Hassan Mahomed†, Helen McShane†, and the MVA85A 020 Trial Study Team

Summary

Background BCG vaccination provides incomplete protection against tuberculosis in infants. A new vaccine, modified Vaccinia Ankara virus expressing antigen 85A (MVA85A), was designed to enhance the protective efficacy of BCG. We aimed to assess safety, immunogenicity, and efficacy of MVA85A against tuberculosis and Mycobacterium tuberculosis infection in infants.

Methods In our double-blind, randomised, placebo-controlled phase 2b trial, we enrolled healthy infants (aged 4–6 months) without HIV infection who had previously received BCG vaccination. We randomly allocated infants (1:1), according to an independently generated sequence with block sizes of four, to receive one intradermal dose of MVA85A or an equal volume of Candida skin test antigen as placebo at a clinical facility in a rural region near Cape Town, South Africa. We actively followed up infants every 3 months for up to 37 months. The primary study outcome was safety (incidence of adverse and serious adverse events) in all vaccinated participants, but we also assessed efficacy in a protocol-defined group of participants who received at least one dose of allocated vaccine. The primary efficacy endpoint was incident tuberculosis incorporating microbiological, radiological, and clinical criteria, and the secondary efficacy endpoint was Mycobacterium tuberculosis infection according to QuantiFERON TB Gold In-tube conversion (Cellestis, Australia). This trial was registered with the South African National Clinical Trials Register (DOH-27-0109-2654) and with ClinicalTrials.gov on July 31, 2009, number NCT00953927.

Findings Between July 15, 2009, and May 4, 2011, we enrolled 2797 infants (1399 allocated MVA85A and 1398 allocated placebo). Median follow-up in the per-protocol population was 24.6 months (IQR 19.2–28.1), and did not differ between groups. More infants who received MVA85A than controls had at least one local adverse event (1251 [89%] of 1399 MVA85A recipients and 628 [45%] of 1396 controls who received the allocated intervention) but the numbers of infants with systemic adverse events (1120 [80%] and 1059 [76%]) or serious adverse events (257 [18%] and 258 [18%]) did not differ between groups. None of the 648 serious adverse events in these 515 infants was related to MVA85A. 32 (2%) of 1399 MVA85A recipients met the primary efficacy endpoint (tuberculosis incidence of 1.15 per 100 person-years [95% CI 0.79 to 1.62]; with conversion in 178 [13%] of 1398 infants [95% CI 11.0 to 14.6]) as did 39 (3%) of 1395 controls (1.39 per 100 person-years [1.00 to 1.91]; with conversion in 171 [12%] of 1394 infants [10.6 to 14.1]). Efficacy against tuberculosis was 17.3% (95% CI –31.9 to 48.2) and against Mycobacterium tuberculosis infection was –3.8% (–28.1 to 15.9).
Standardizing the QFT assay

Niaz Banaei of Stanford University
Need for standardization

Qualification/validation of IGRA as a study endpoint must include the development of Standard Operating Procedures (SOPs) for the following areas:

- Skin disinfection for blood draw
- Specimen volume
- Specimen mixing
- Specimen transfer to central laboratory
- Specimen processing
- Assay interpretation
Skin disinfection for blood draw

• Problem – As few as 10 bacteria in tube can affect assay results
  – It is important to take the cleaning of the skin seriously.

• Proposed solutions
  – Clean skin area well with an alcohol wipe.
  – Don’t touch the skin again once it is wiped.
  – Discard the first portion of blood that may contain the skin plug (with bacteria)
  – Use a different wipe to clean the septum of the throwaway “plug” tube, Nil and TB Ag tubes
Specimen volume

• Problem – standardizing blood volume
  – Current practice is to draw 0.8 to 1.2 mL of blood directly into each of three assay tubes
  – It is difficult to ensure that precisely same volume is drawn into each tube
  – Niaz has shown that using less blood for IGRAs (0.8 vs. 1.0 vs. 1.2) gives a better response* in LTBI cases

• Proposed solutions
  – Use a glass 5 mL green top and draw ≥3 mL. Aliquot 0.8 mL of blood into each of the three QFT tubes.

* higher TB Ag-Nil results
Specimen mixing

• Problem – standardizing blood mixing procedures
  – Current practice is to use 10 inversions (no frothing)

• Proposed solutions
  – Mix Nil and TB Ag tube in same hand so as to mix both with the same vigor.
  – Automate with blood roller.
Specimen transfer to central laboratory

• Problem – standardizing initiation of incubation
  – Current package insert instructions allow for postponed incubation
  – Niaz has shown that it is best to incubate immediately

• Proposed solutions
  – Purchase portable incubators for each site.
  – Incubate samples immediately upon draw.
Specimen incubation time

• Problem – instructions on incubation time allow for great variability
  – Current package insert instructions state that the tubes can be incubated from 16 to 24 hours
  – This allows for too much variability.

• Proposed solutions
  – Standardize incubation time across sites.
  – Include time in portable incubator in total incubation time.
Conclusions and suggestions

• Existing guidelines for HCW screening needs to be revised
  – We need to ask if so many low risk HCWs need repeated screening
    • We should avoid testing low risk people
    • If we do test them, we will need to deal with false-positive results

• Companies, health institutions and labs must do everything they can to standardize testing protocols, to minimize variation
  • IGRA manufacturers should consider tightening the ranges in the current product insert (e.g. time to incubation)
Conclusions and suggestions

• Need to measure reproducibility in the absence of predictable sources of variability
  • Extent to which we can improve reproducibility?
  • Can we start to interpret continuous IGRA results (like TST)?
  • Need a borderline zone to account for random sources of variability
  • We need to figure out the sens/spec of the new cut-offs
  • Need a strategy to handle conversions and reversions (e.g. re-testing)
Acknowledgements

Stanford University
  Victor Herrera
  Rajiv Gaur
  David Doberne
  Mady Slater
  Julie Parsonnet

McGill
  Madhukar Pai
  Claudia Denkinger

Financial Support
  Stanford Pathology
  Stanford SPARK/ Global Health