

Serial IGRA Testing and the Impact of Tuberculin Skin Testing

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Presenter Disclosures

Richard N. van Zyl-Smit

“No industry or financial relationships to disclose”



Overview of presentation

- Background & introduction
- Variability of IGRA assays
- TST mediated boosting of IGRA responses
- Summary and clinical recommendations

Background

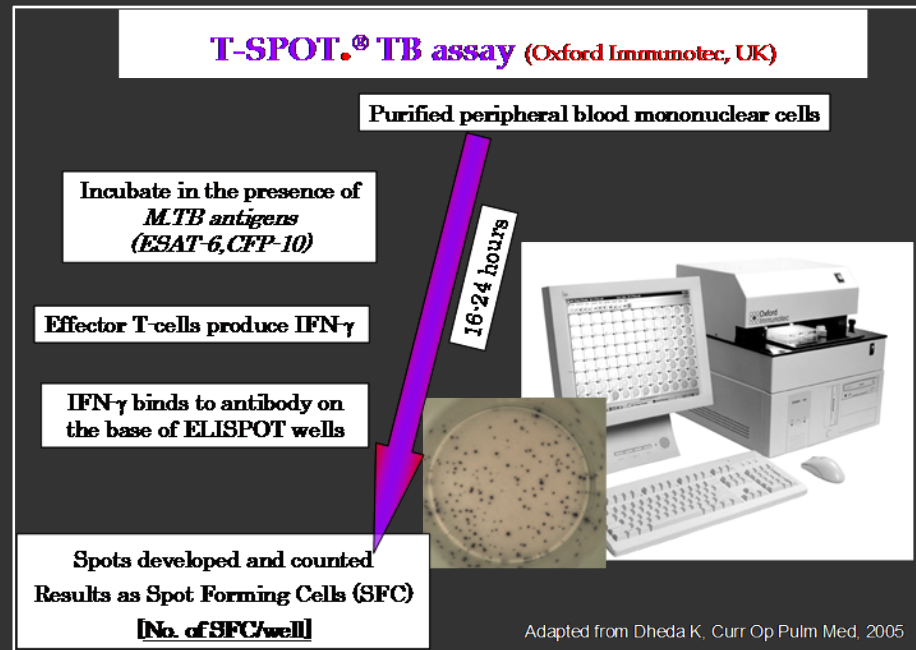
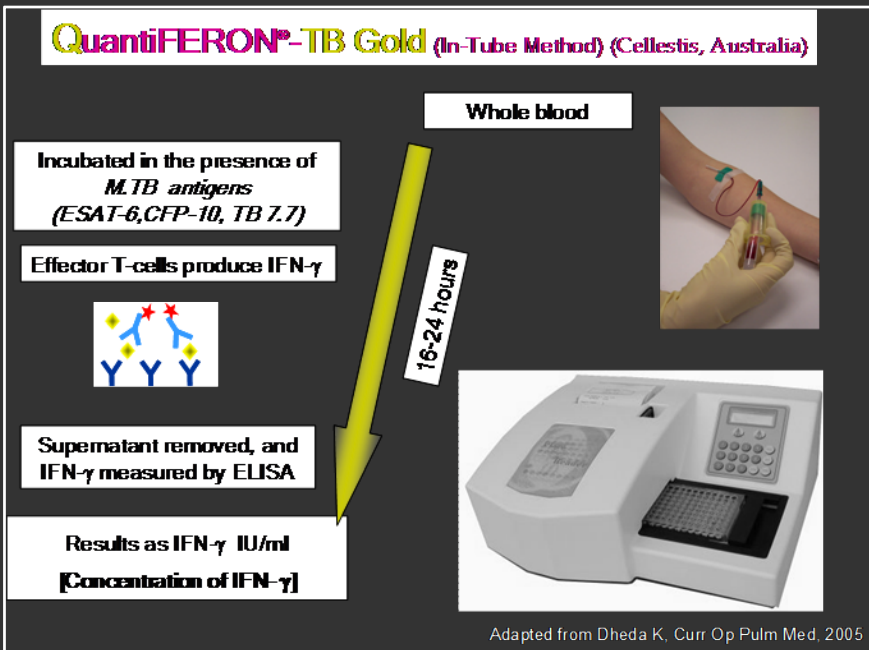
- Latent TB Infection (LTBI) may be defined as the presence of viable *M.TB* organisms in an individual without active disease (asymptomatic).
- The detection of latent infection is important in various clinical contexts:
 1. **Low burden settings**
 - TB control (reducing disease reservoirs)
 2. **High burden settings**
 - High risk individuals (preventing active disease)
 - HIV positive individuals
 - Children
 - Health care workers

Background

- The Tuberculin Skin Test (TST) has traditionally been the basis of the latent TB diagnosis
- The TST has many drawbacks:
 - Cumbersome to perform
 - Poor specificity
 - Poor sensitivity in immunocompromised states
 - Influenced by BCG vaccination
 - Prone to “boosting” if repeated

Background

- Interferon gamma release assays (IGRAs) have been developed to overcome the drawbacks of the TST
- Two commercially available assays
 - QuantiFERON[®]-TB Gold (In-Tube Method) (Cellestis, Australia)
 - T-SPOT.[®] TB (Oxford Immunotec, UK)



How and when to use an IGRA?

- International Guidelines:
 - USA-CDC, Canada, UK, France, Germany...
- CDC 2005 guidelines (MMWR 2005;54(RR-15):49-55): recommends that QFT-G may be used in all circumstances in which the TST is currently used, including contact investigations, evaluation of immigrants
- UK National Institute for Health and Clinical Excellence (NICE) 2006 guidelines: recommends a two-step (hybrid) strategy for LTBI diagnosis: initial screen with TST, and those who are positive (or in whom TST may be unreliable) should then be considered for IGRA testing, if available, to confirm positive TST results.

Unresolved issues relating to the use of IGRAs?

- The applicability to high burden settings: HIV positive individuals, children and health care workers
- Inherent variability of the assays
 - “Within-subject variability”
 - Same subject tested multiple times over a short or long time period
 - “Conversions and reversion”
 - Interpreting serially tested individuals with results close to the assay cut point...

Single previous published paper: Veerapathran 2008

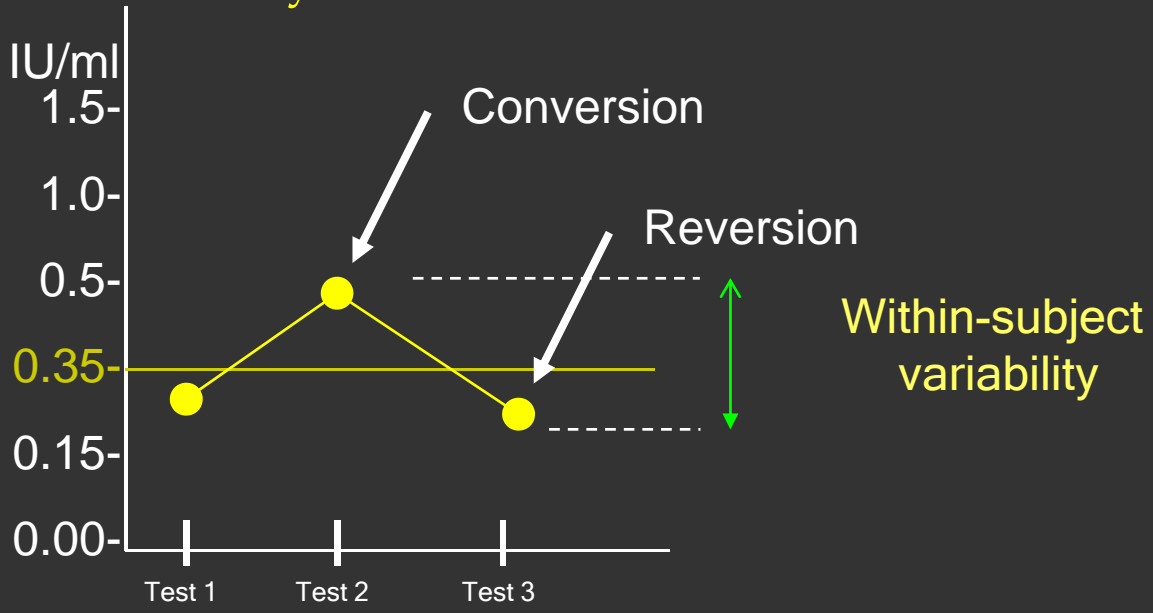
- The effect of TST on IGRA results
 - “TST mediated IGRA boosting”

No large prospective studies using both assays

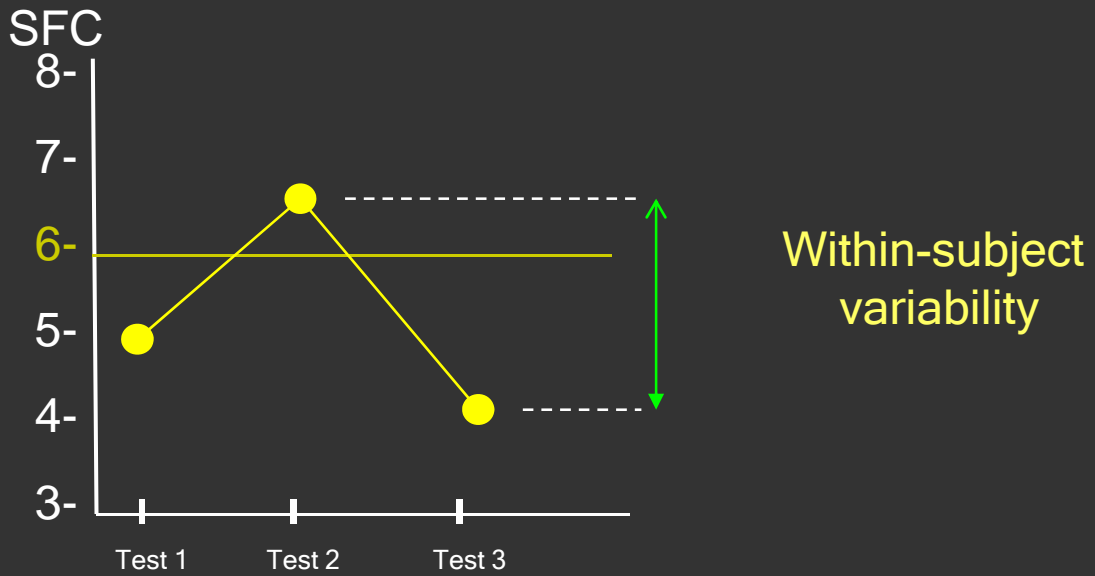
Important definitions:

Within-subject variability and "conversion & reversions"

QuantiFERON®-TB
Gold-IT



T-SPOT.® TB



Boosting

- TST
- IGRA
- TST - IGRA

Published literature

- IGRA within-subject variability studies
 - Veerapathan '08 [Belknap '09 (ATS abstract)]
- IGRA boosting studies
 - “Concluded that boosting occurs”:
 - Baker '09, Vilaplana '08, Choi '08, Perry '08, Leyten '07, Igari '07, Naseer '07, [Belknap '09 (ATS abstract)]
 - “Concluded that boosting does not occur”:
 - Richeldi '08, Leyten '07, Richeldi '06, Nguyen '05, QFT-GIT USA package insert

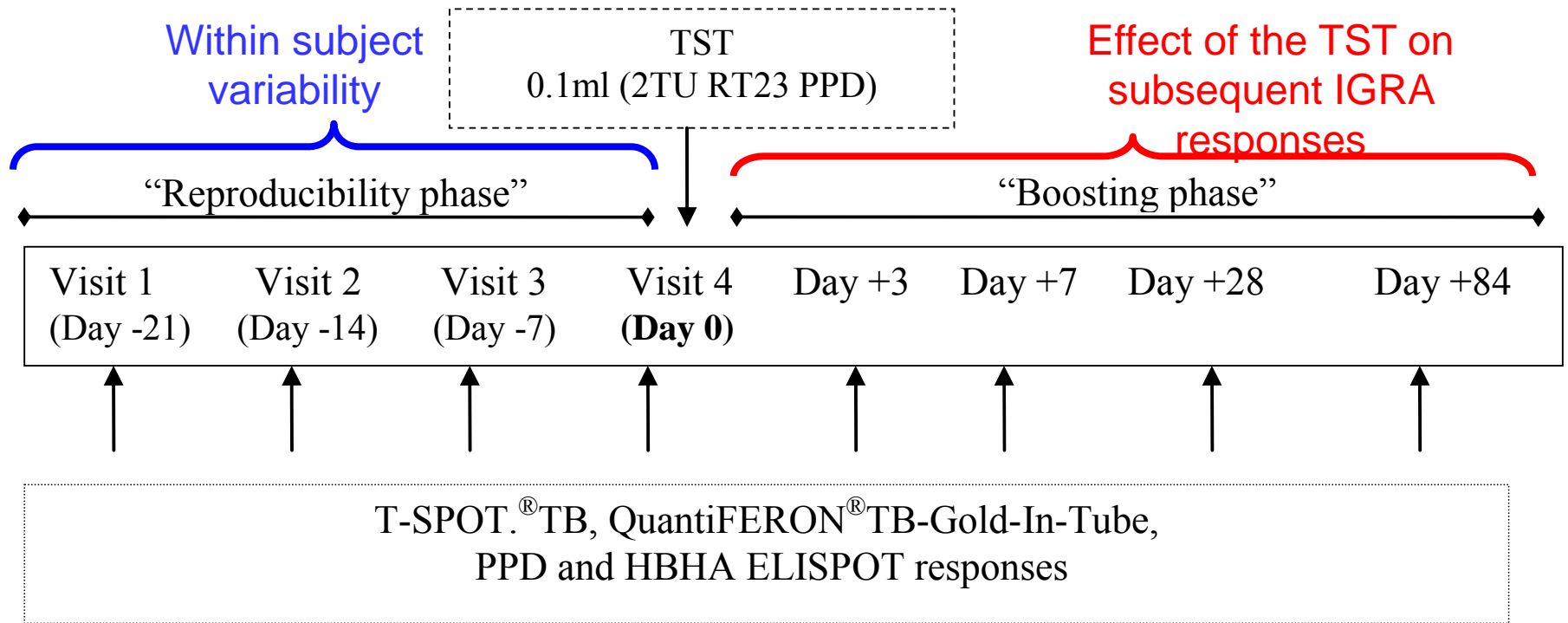
Study Aims

1. To document 'within-subject' variability
(Short term reproducibility over a 3week period)
2. To examine the effect of the TST on IGRA responses
(Does TST 'boost" subsequent IGRA responses)

Methods

- 24 healthy HIV negative recruits, stratified by exposure risk, were recruited to undergo serial IGRA testing over three weeks
- Following a tuberculin skin test, IGRA responses were tested at several interval to 3 months.
- Standardized T-SPOT.[®]TB and QuantiFERON[®]-TB GOLD (In-Tube) assays were used.
 - PPD and HBHA ELISPOT responses were also measured

Study Design



Study participants

	Total	High risk	Medium risk	Low risk
Number of subject	26	11	10	5
Age mean (SD)	39 (10)	43 (7) ⁺	41 (10)*	29 (11) ⁺ *
Racial group				
Black African	4 (15%)	3 (27%)	1 (10%)	0
Mixed ancestry	14 (54%)	7 (63%)	7 (70%)	0
European ancestry	8 (31%)	1 (10%)	2 (20%)	5 (100%)
Current smoker	6 (23%)	2 (18%)	3 (30%)	1 (20%)
HIV status	All HIV negative			
BCG vaccinated	All vaccinated			
Previous TB (n)	1	1	0	0

+ p=0.05 High vs. Low * p< 0.01 Medium vs. Low

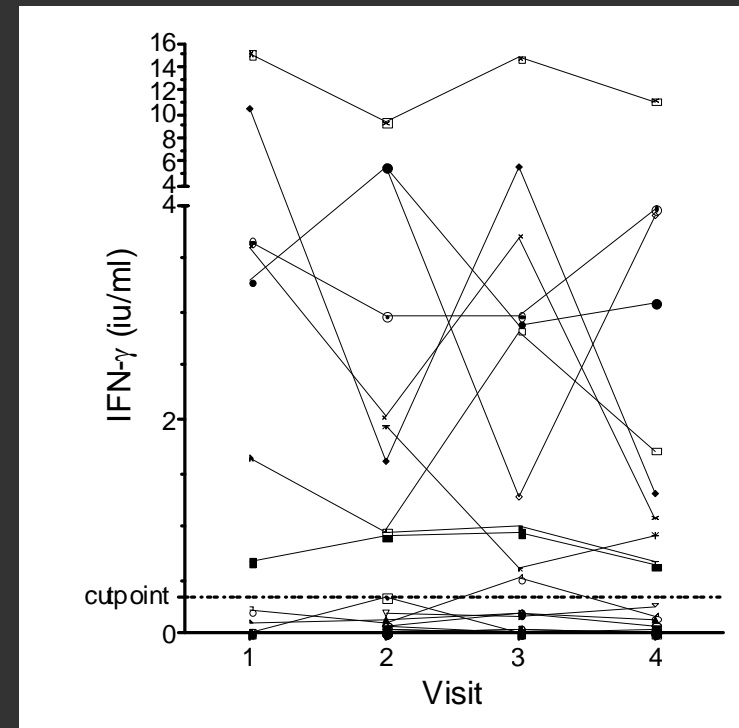
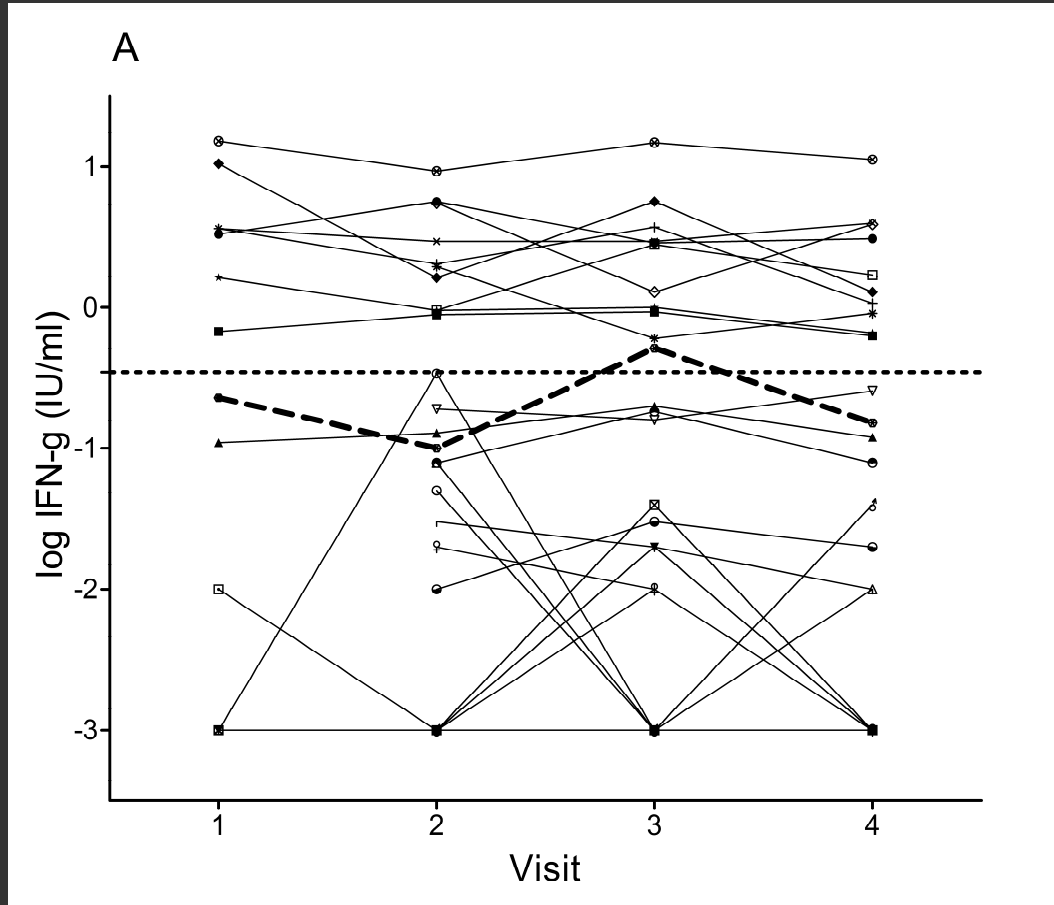
van Zyl-Smit RN et al. AJRCCM 2009

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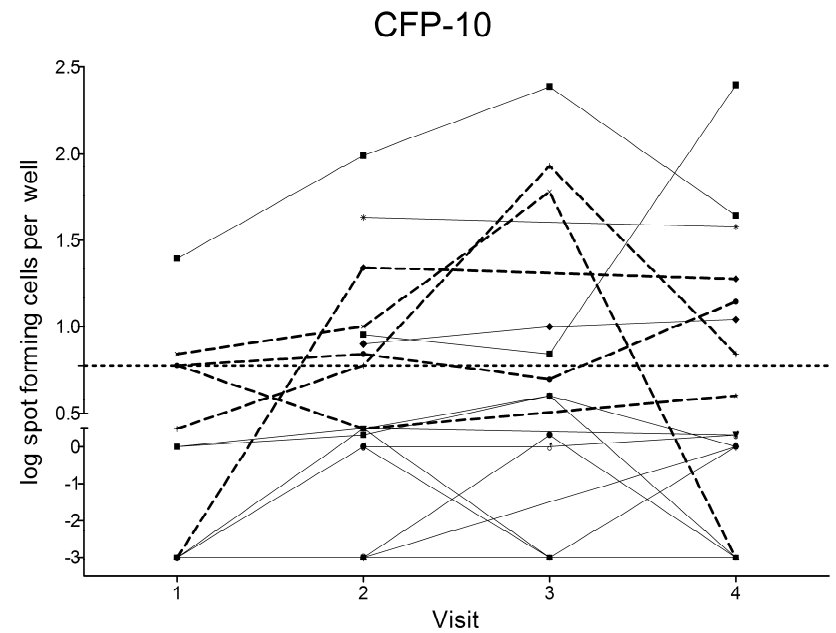
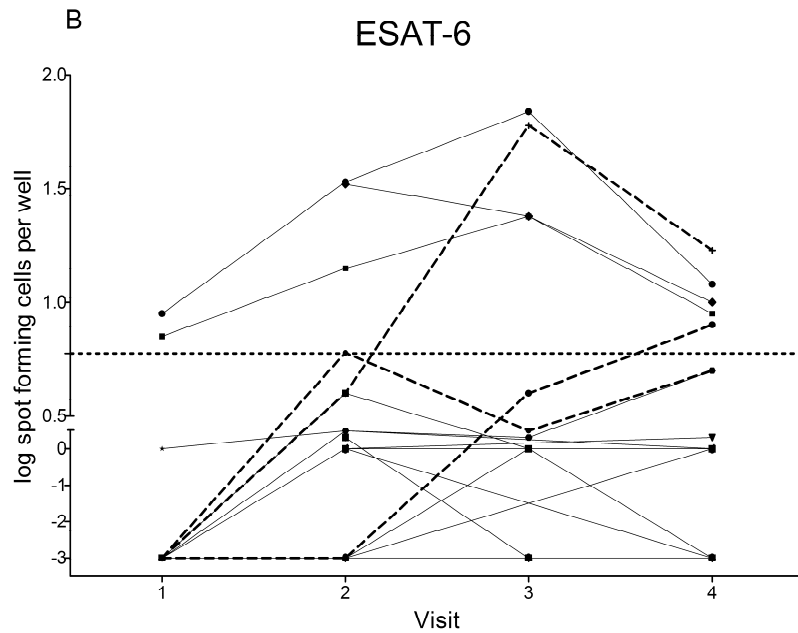
Within-subject variability

QuantiFERON[®] TB GIT



Within-subject variability

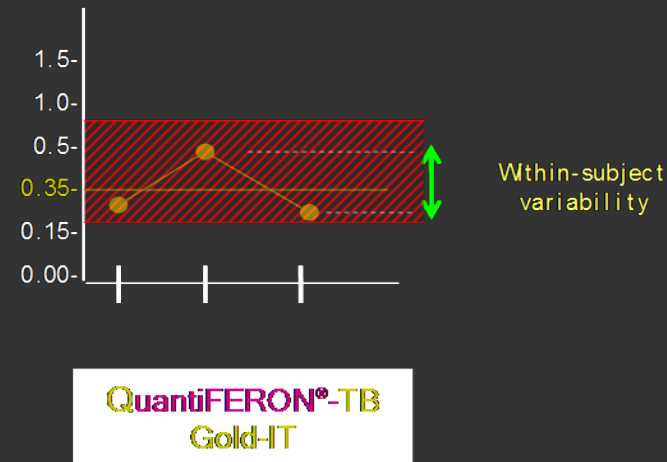
T-SPOT.[®]TB



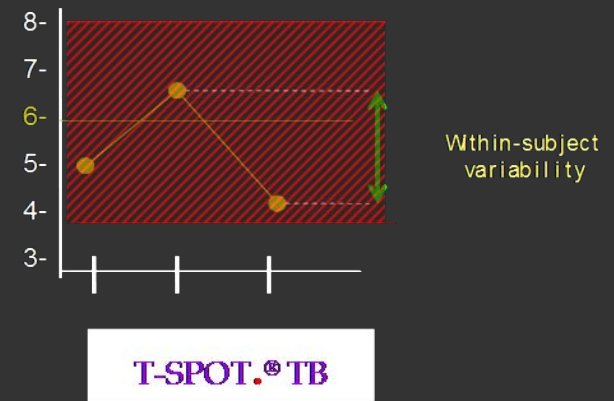
Within-subject variability

What does it mean to the clinician?

- QuantiFERON[®]TB -GIT
 - Serially tested IFN- γ responses may vary in an individual by 80% from any given initial value week to week...



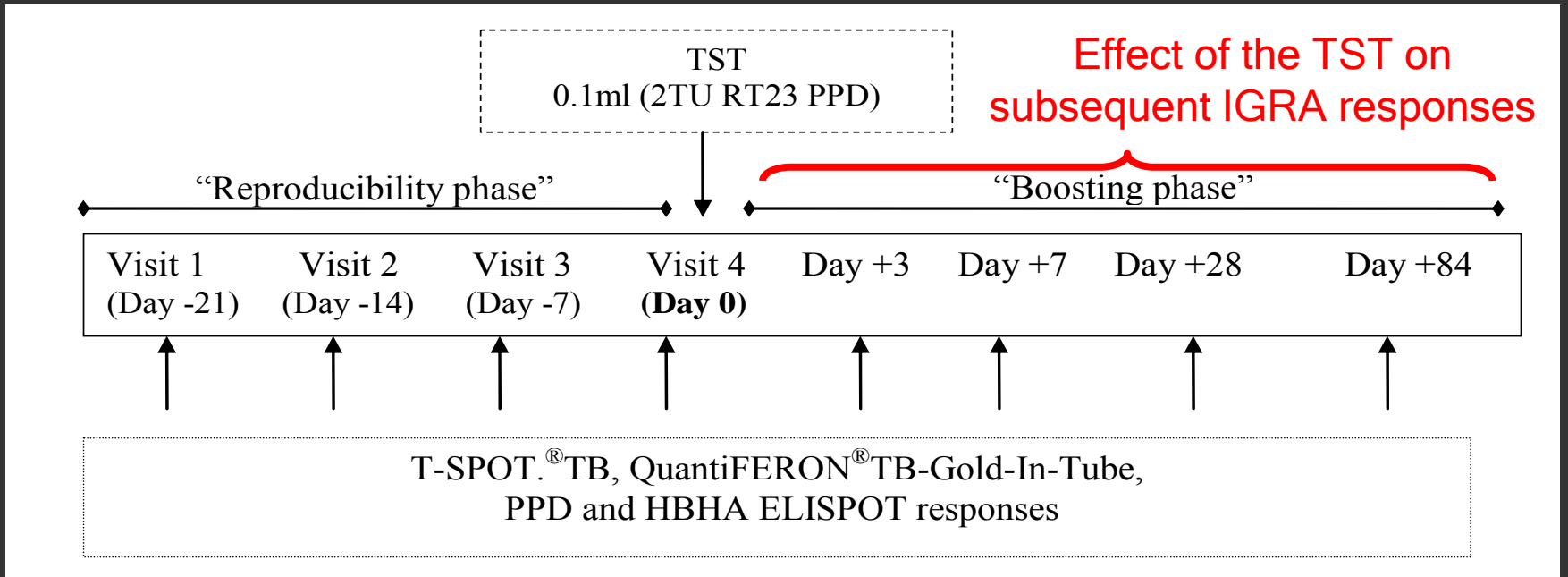
- T-SPOT.[®]TB
 - Serially tested IFN- γ spot forming counts may vary in an individual by 3 spots from any given initial value week to week...



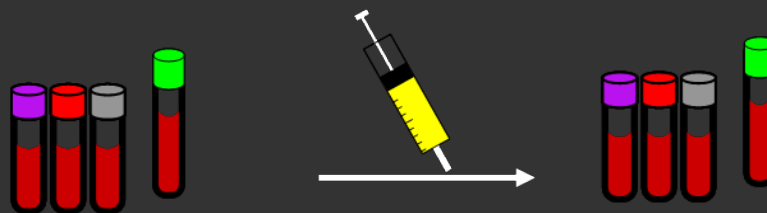
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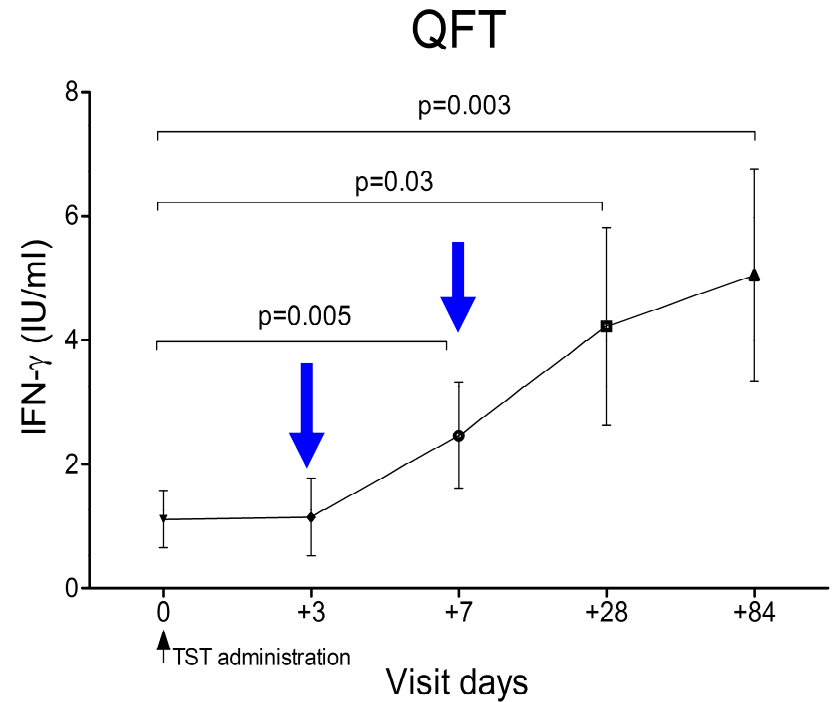
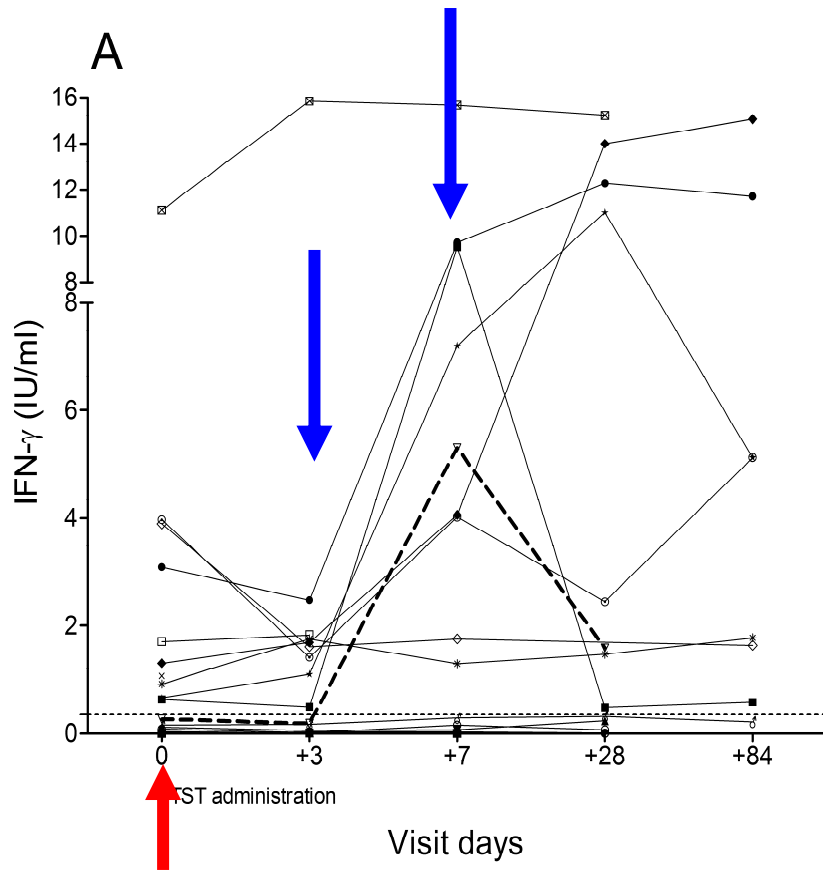
The effect of TST on subsequent IGRA responses



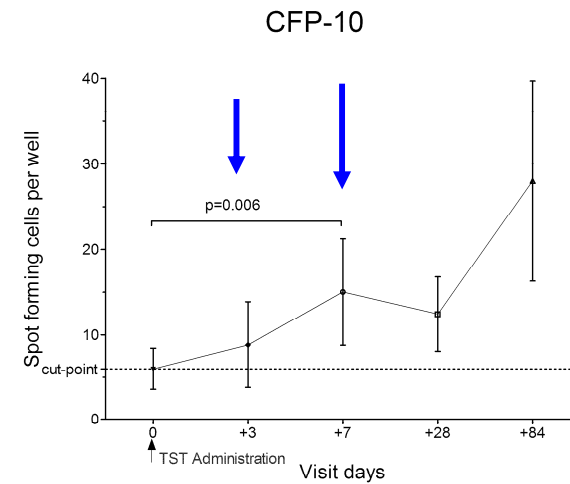
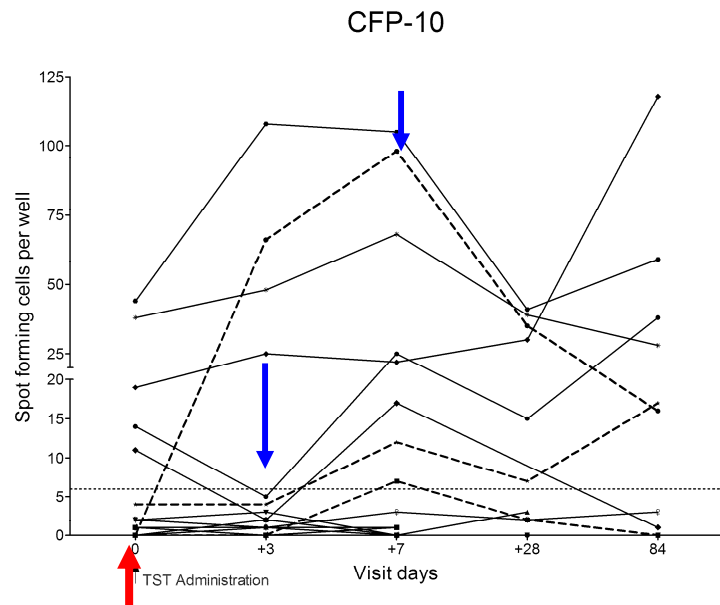
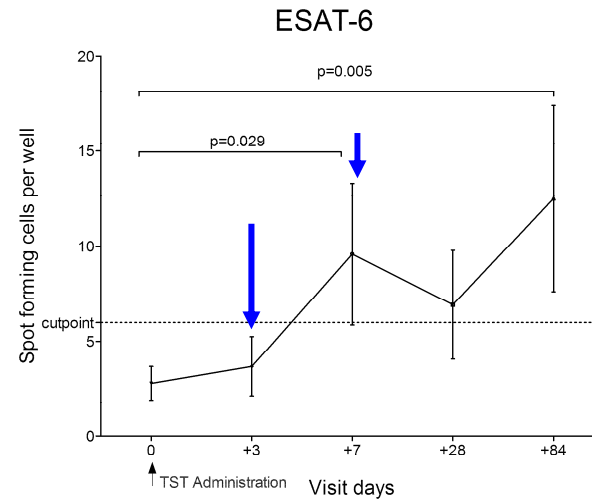
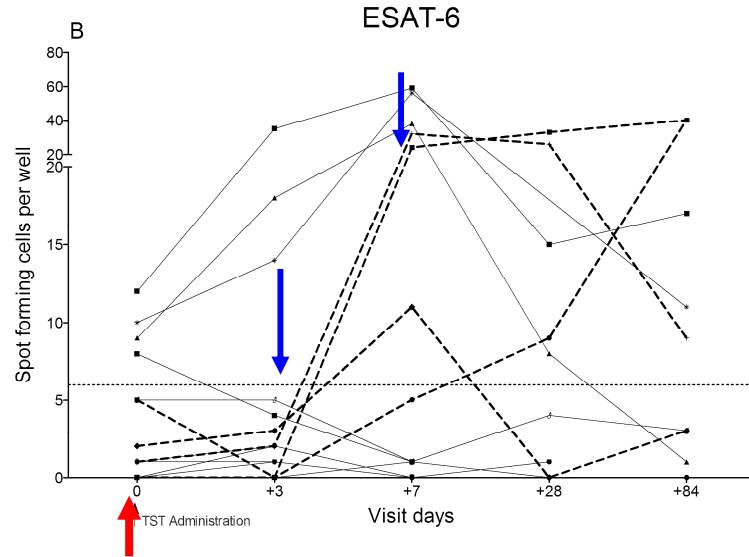
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QFT-GIT boosting...



T-SPOT.[®]TB boosting



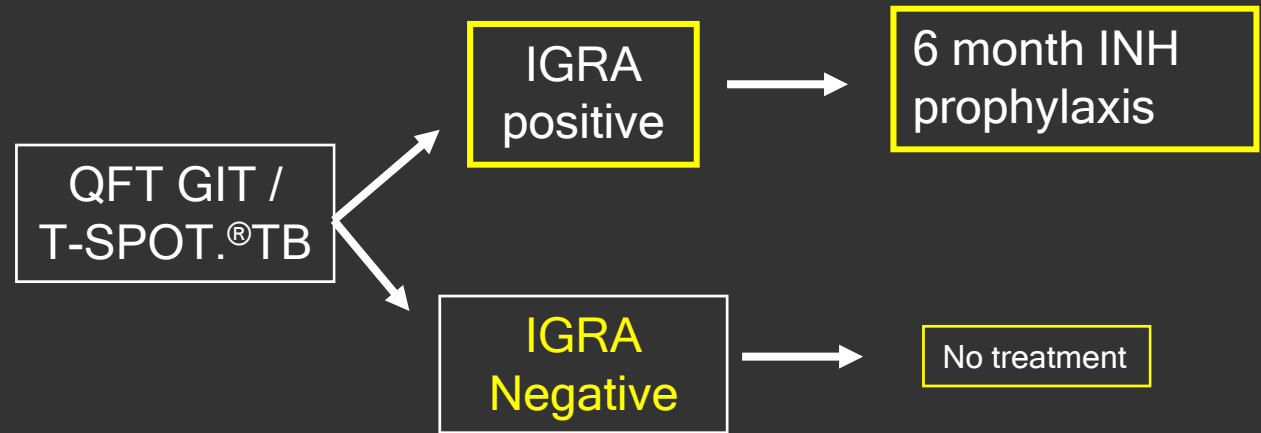
TST induced IGRA response boosting

What does it mean to the clinician?

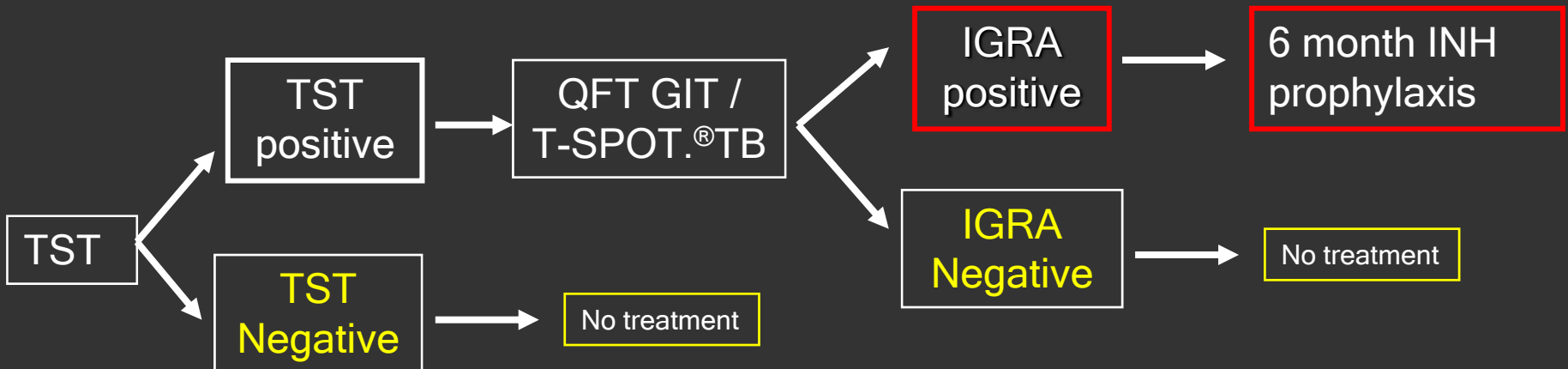
- Boosting occurs:
 - After day 3 post TST
 - In both T-SPOT.[®]TB and QFT-GIT assays
 - Predominantly in IGRA +ve subjects
 - Also occurs in IGRA negative subjects
 - Smaller but significant percentage (12.5%)

Screening for LTBI...

One step strategy



Two step strategy



Summary

i. Within-subject variability is significant in high burden settings.

- Proposed ‘uncertainty zone’
- Proposed threshold for conversions and reversions

	QuantiFERON [®] TB GIT	T-SPOT. [®] TB
Manufacturer defined assay cut-point	> 0.35 IU/ml	≥ 6 Spots *
Within- subject short term variability	± 80% of IFN-γ response	± 3 Spots
Borderline or uncertainty zone	0.2-0.7 IU/ml	4 – 8 spots (inclusive)
Proposed conversion threshold	Increase from below 0.35 to above 0.7 IU/ml	Increase from below 6 to above 9 spots (inclusive)

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Within-subject variability must be taken into consideration when interpreting serial test results particularly those close to the assay cut-point.

Summary

- ii. The Tuberculin Skin Test boosts IGRA responses.
 - Occurs after day 3
 - Predominantly in IGRA positive subjects
 - Significant number of IGRA negative subjects.

If the 'two step strategy' is to be used, IGRAs should ideally be performed within three days after the TST to avoid confounding by the TST.

Further large scale prospective studies in high and low burden settings are required to validate our findings.

Acknowledgements

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