

Two Interferon Gamma Release Assays and Tuberculin Skin Test in the diagnosis of *Mycobacterium tuberculosis* infection and disease in The Gambia

Ifedayo Adetifa, Martin Ota, Abdulrahman Hammond,
Moses Lugos, Owiafe Patrick, Richard Adegbola, Philip Hill

Bacterial Diseases Programme, MRC (UK) Laboratories, The Gambia

BACKGROUND

- The tuberculin skin test until recently was the only diagnostic test for latent TB infection (LTBI)
- In recent years, the interferon gamma release assays (IGRA) that measure interferon gamma released by sensitized T-cells, have been developed for the diagnosis of LTBI and provides a means of identifying and tracking short lived effector T-cells responding to specific TB antigens.

- IGRAs differ from each other mainly with respect to the technique of IFN- γ detection (enzyme linked immunospot; ELISPOT vs. enzyme linked immunosorbent assay; ELISA) and the samples utilized (peripheral blood mononuclear cells vs. whole blood)
- Two interferon gamma release assays (IGRAs) are now licensed for the diagnosis of LTBI
- The T.SPOT.TB® is ELISPOT-based and uses PBMCs while QuantiFERON-TB Gold® is a whole-blood ELISA test.

- The IGRAs, now available as standardized assays are being evaluated in a variety of settings leading to an increasing body of literature supporting their use
- But there remains insufficient data on test performance in high risk groups such as children
- We had previously compared an in-house IGRA to TST in children across a sleeping gradient of exposure to an index TB case and found it slightly less sensitive than TST in diagnosis of LTBI from recent exposure.

[Hill,et al. Pediatrics 2006;117: 1542-1548]

Objectives

- **Hypothesis-**
 - The diagnostic performance of 2 commercial IGRA assays compared to the TST across a TB exposure gradient is equivalent in Gambian adult and childhood TB contacts.
- **Objectives-**
 - To evaluate the response of the TST, T-SPOT.TB and QuantiFERON TB Gold In Tube (QFT-GIT) tests in childhood TB contacts across a gradient of sleeping proximity to an index case
 - To estimate the sensitivity of all tests in smear positive TB cases

Methods (1)

- Sputum smear positive TB cases aged ≥ 15 years were consecutively recruited.
- Contacts aged 0.5-14 years who have lived for ≥ 3 months in the same compound as the case were also recruited
- They were excluded if they had been treated for TB in the past year or diagnosed with TB within a month of recruitment
- Written informed consent was obtained from all subjects

Methods (2)

- Blood samples taken for both IGRAs, HIV testing and TST given.
- Ascertainment of exposure
 - Tuberculosis contacts were categorized according to where they slept:
 - in the same bedroom as the case,
 - A different bedroom in the same house, or
 - in a different house in the same compound.
- Procedures
 - TST was done with 2 TU PPD RT-23A TST, A 10mm cut off was used. Fieldworkers who gave this test were blinded to lab results
 - All commercial assays were performed and results interpreted according to the manufacturers instructions. Lab personnel were blinded to subjects status and TST results.

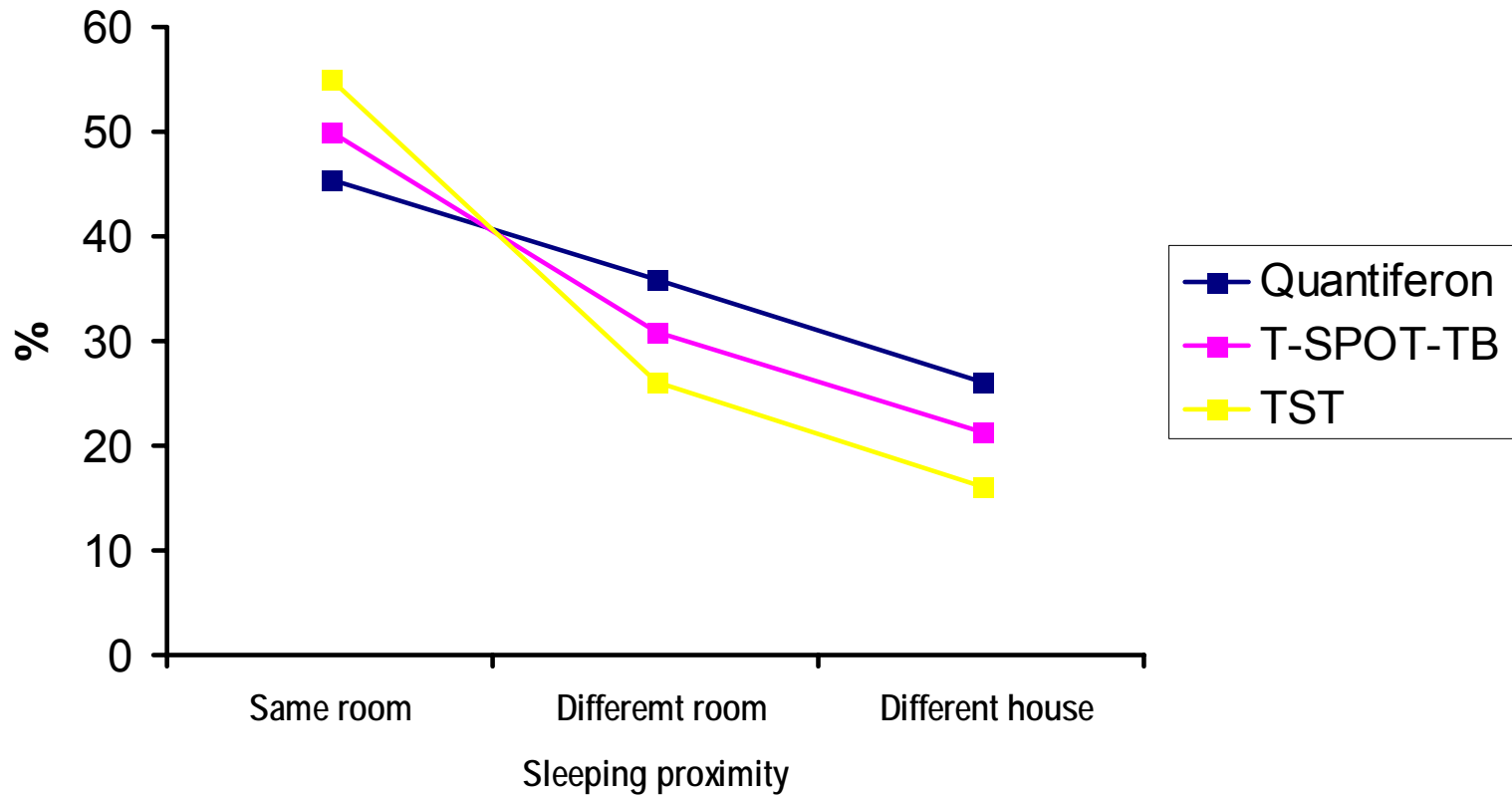


Preliminary Results (1)



- 385 subjects recruited, 100 cases and 285 contacts
- The sensitivities all tests in TB cases were
 - 82.8% [95%CI 81.5-94.9%] for T-SPOT.TB,
 - 85.4% [95%CI 81.4-95.8%] for QFT-GIT
 - 66.7% [95%CI 46.3-87.0%] for TST
- The prevalence of LTBI by
 - TST, 26.5% [95%CI 21.0-32.0%]
 - T-SPOT-TB 27.3% [95%CI 24.2-36.1%]
 - QFT-GIT 34.1% [95%CI 27.0-41.5%]

Percentage of contacts positive for TST, T-SPOT.TB and QFT-GIT by *M. tuberculosis* exposure



Univariable and multivariable odds ratios determined by logistic regression for sleeping proximity as a surrogate marker of exposure to *M. tuberculosis*

	T-SPOT.TB (n=231)				QuantiFERON (n=171)				TST (n=248)			
	Positive results No.(%) of contacts	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	p-value	Positive results No. (%) of contacts	Unadjusted OR (95% CI)	Adjusted OR(95% CI)	p-value	Positive results No.(%) of contacts	Unadjusted OR(95% CI)	Adjusted OR(95% CI)	p-value
<u>Sleep proximity</u>												
Different house	16 (21.3)	1	1		14(25.9)	1			12(16.2)	1	1	
Different room	40 (30.8)	2.9 (1.0-8.2)	3.4 (1.1-10.3)		34(35.8)	1.8 (0.7-4.9)	1.6 (0.7-3.9)		37(25.9)	1.8 (0.8-4.1)	2.4 (1.1-5.1)	
Same room	13 (50.0)	7.4 (2.0-28.2)	10.0 (2.4-41.4)	0.007**	10(45.5)	2.6 (0.7-9.4)	3.5 (1.0-11.7)	0.13**	17(54.8)	3.2 (1.2-8.9)	9.2 (3.3-25.8)	0.0001**

TST \geq 10mm defined as positive, **Test for trend



Results (2)

Agreement/Discordance Analysis



- The agreement in contacts between
 - T-SPOT.TB&QFT was 83% ($\kappa=0.60$, discordance $p=0.05$),
 - TST&QFT-GIT 75.5% ($\kappa=0.44$, discordance $p=0.006$),
 - TST&T-SPOT-TB 73.3% ($\kappa=0.43$, discordance $p=0.0003$),

- The agreement in index cases between
 - T-SPOT.TB&QFT was 85.3% ($\kappa=0.27$, discordance $p=0.76$),
 - TST&QFT-GIT 88.9% ($\kappa=0.72$, discordance $p=0.37$),
 - TST&T-SPOT-TB 59.1% ($\kappa=0.1$, discordance $p=0.09$)

- Effect/influence of BCG vaccination
 - T-SPOT.TB: OR 1.3 (0.7-2.4), $p=0.43$
 - QFT: OR 1.1 (0.6-2.2), $p=0.77$
 - TST: OR 0.7 (0.4-1.4), $p=0.35$

- Effect/influence of sputum smear grade in TB case
 - T-SPOT.TB: OR 1.0 (0.5-2.2), $p=0.97$
 - QFT: OR 1.6 (0.7-3.9), $p=0.28$
 - TST: OR 0.9 (0.4-1.7), $p=0.67$

- The detection of LTBI was similar with all 3 tests although the QFT tended towards more positive results
- All 3 tests responded to the *M.tuberculosis* exposure gradient but significantly so for TST and TSPOT compared to QFT.
- There was good concordance between T-SPOT.TB and QFT but significant discordance between TST&T-SPOT-TB and between the TST&QFT-GIT

CONCLUSIONS

- The IGRAs have much better sensitivity in TB cases compared to TST but sensitivity in all tests remain suboptimal for the diagnosis of TB
- These results do not support the replacement of TST by IGRAs for diagnosis of LTBI in The Gambia



Future perspectives

- Need to understand the nature and reason for discordance between IGRAs and TST
- Evaluate the utility of IGRAs as biomarkers for treatment or vaccine efficacy
- What is the value of IGRAs in predicting progression from LTBI to TB disease?
- To understand test and biologic variability



Acknowledgements



- All study subjects especially parents and their children,
- Cellestis (Carnegie, Australia),
- EDCTP,
- MRC (Uk) Labs, The Gambia
- MRC Bacterial Diseases Programme- Prof.Adegbola, Philip Hill, Martin Ota, Moses Lugos, Abdulrahman Hammond, Patrick Owiafe, Simon Donkor and data team, Jacob Otu and team, Entire TB Field team and supervisors
- Gambia National TB and Leprosy Control Programme
- Oxford Immunotec (Oxford),