Interferon gamma release assays for tuberculosis and their potential as efficacy markers for intervention trials
Adetifa, I.M.O.

Citation for published version (APA):
Adetifa, I. M. O. (2012). Interferon gamma release assays for tuberculosis and their potential as efficacy markers for intervention trials

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
4. Sensitivity of commercial interferon gamma release assays for Tuberculosis in high incidence settings—does the infecting Mycobacterial strain matter?

Ifedayo M. O. Adetifa¹, Martin O. C. Ota¹, Martin Antonio¹, Abdulrahman Hammond¹, Moses D. Lugos¹, Simon Donkor¹, Owiafe Patrick¹, Richard A. Adegbola¹, Philip C. Hill¹,²

¹Medical Research Council Unit, The Gambia, Atlantic Boulevard, Fajara, Po Box 273, Banjul, The Gambia;

²Department of Preventive and Social Medicine, Centre for International Health, University of Otago School of Medicine, Dunedin, New Zealand

Submitted Manuscript (2012)
Sensitivity of commercial interferon gamma release assays for Tuberculosis in high incidence settings—does the infecting Mycobacterial strain matter?

Ifedayo MO Adetifa¹, Martin OC Ota¹, Martin Antonio¹, Abdulrahman Hammond¹, Moses D Lugos¹, Simon Donkor¹, Owiafe Patrick¹, Richard A Adegbola¹, Philip C Hill¹,²

¹ Medical Research Council Unit, The Gambia, Atlantic Boulevard, Fajara, The Gambia
² Centre for International Health, Department of Preventive and Social Medicine, University of Otago School of Medicine, Dunedin, New Zealand

*Corresponding author

Dr. Ifedayo Adetifa
Medical Research Council Unit, The Gambia
P.O.Box 273, Banjul, The Gambia
Phone:+220 4495442-6 ext 3011
Fax: +220 4495919
Email- iadetifa@mrc.gm
ABSTRACT

Background:
Interferon gamma release assays (IGRAs) perform differently in high TB incidence compared to low TB incidence countries. As part of an evaluation of widely available commercial IGRAs for latent TB infection, we assessed the sensitivities of two IGRAs for culture positive TB and the impact of the *Mycobacterium tuberculosis* strain isolated.

Methods:
The sensitivities of T-SPOT.TB and Quantiferon-Gold in-tube (QFT-GIT) assay were compared in sputum culture positive cases of TB. The results were stratified according to whether the infecting strain was *Mycobacterium tuberculosis* or *M. africanum*. Factors associated with discordance and concordance were examined

Results:
In 99 TB cases studied, both IGRAs had high sensitivities, 89.7% and 90.1% for T-SPOT and QFT-GIT respectively and these were not significantly different (p=0.91). When each was combined with TST, there were gains in sensitivity (96.3% and 100% respectively for TST combined with QFT-GIT and T-SPOT. The agreement between IGRAs was 85.4% with relatively poor concordance (κ=0.25 [0.14-0.36, p=0.01]). In *M. africanum* compared to *M. Tuberculosis* cases, there was a trend towards lower sensitivity; lower median spots with T-SPOT and IFN-γ concentrations with QFT-GIT but these did not reach statistical significance.

Conclusion:
IGRAs are significantly more sensitive than TST for the diagnosis of TB disease in The Gambia as a proxy for LTBI. The utility of the sensitivity gained by combining IGRA and TST for LTBI if any is unclear. The impact of infecting Mycobacteria strain on IGRA test characteristics requires further investigation.

Keywords: Tuberculin skin test; interferon gamma release assays; latent infection; Tuberculosis; *Mycobacterium tuberculosis*; *Mycobacterium africanum*
INTRODUCTION

Interferon gamma release assays (IGRAs) detect interferon gamma (IFN-γ) released by T-cells stimulated with *Mycobacterium tuberculosis*-specific antigens that are not found in *Mycobacterium bovis* BCG and most atypical/environmental Mycobacteria. They were primarily developed as replacement for the tuberculin skin test (TST) for immunodiagnosis of latent tuberculosis (TB) infection (LTBI).

In low TB incidence settings, the use of IGRAs is backed by official recommendations for their use where TST is indicated, in a 2-step strategy to confirm a positive TST, and as adjunct tests for diagnosis of active TB. [1,2,3] IGRAs are currently not recommended for use in low and middle income countries (LMICs) because the available evidence suggests IGRAs may not perform as well as they do in high income countries (HICs). [4,5] The reasons for these differences are currently unclear.

In West Africa including The Gambia, *Mycobacterium africanum* is responsible for 30-40% of cases.[6] Contacts exposed to *M. africanum* are less likely to progress to tuberculosis disease and *M. africanum* elicits attenuated IFN-γ responses to ESAT-6 in household contacts and TB cases compared to *M. tuberculosis*. [7,8] There are genomic differences between *M. africanum* and *M. tuberculosis* that may influence the performance of IGRAs. [9] Diagnosis of *M. africanum* LTBI is currently not possible because the antigens predicted to be specific to this strain following sequencing of the genome have not been immunogenic in humans.[10]

As part of our evaluation of the 2 widely available commercial IGRAs for diagnosis of LTBI in The Gambia, we compared sensitivity using proven TB disease as a proxy for LTBI and stratified results by infecting Mycobacteria strain.
METHODS

Study Participants
We recruited consecutively diagnosed TB index cases from major government chest clinics and outpatients’ clinics at Medical Research Council (MRC) Unit, The Gambia. They were included if they were aged ≥15 years, had two sputum samples positive for acid-fast bacilli by Ziehl-Neelsen stain and radiological features suggestive of TB on chest x-ray. Each patient had TST with 2 TU (PPD RT23, Statens Serum Institut, Copenhagen, Denmark) immediately after blood samples were collected. Indurations were recorded at 48-72 hours. A positive test was defined as induration diameter ≥ 10mm.

Ethics statement
All study participants gave written informed consent after they received detailed information regarding the study. The Gambia Government/MRC Joint Ethics Committee approved the information sheet, consent checklist and form along with approvals given for conduct of the study.

Laboratory procedures
Sputum was obtained from all recruited cases for diagnosis of TB using conventional methods- Auramine-phenol and Ziehl-Neelson (Z-N) microscopy and cultures in recommended liquid and solid media for isolation of *M. tuberculosis*, as previously described.[11]

Spoligotyping and polymerase chain reaction (PCR) for RD9 and RD702 were performed. Briefly, DNA was extracted from each isolate of *M. tuberculosis complex* using cetyl trimethyl ammonium bromide and chloroform using standard methods[12] following which DNA concentration and purity were assessed by spectrophotometry (Eppendorf BioPhotometer). Spoligotyping was done using membranes (Isogen Biosciences) as recommended.[13] Spoligotyping results were scanned and analyzed with Matlab software (Mathworks), after which manual
editing and confirmation were performed.\[14\] Isolates subjected to spoligotyping were then classified as *M. africanum* or *M. tuberculosis* as described previously. \[7\]

The QFT-GIT assay was performed according to the manufacturer’s instructions in \url{http://www.cellestis.com/IRM/content/aust/qtfproductstbgoldintubetechninfo.html}. The IFN-\(\gamma\) levels were measured in international units (IU) with a Dynex ImmunoAssay System ELISA reader version 6.0 (Dynex Technologies, West Sussex, UK). The raw data were entered into QFT-GIT analysis software. A positive result was defined as IFN-\(\gamma\) concentration in TB-specific antigen stimulated tube minus that in the negative control tube \(\geq 0.35\) IU/mL. Results were indeterminate if there were no detectable IFN-\(\gamma\) responses in the mitogen tube.

T-SPOT (Oxford Immunotec LTD, Oxford, UK) was performed according to the manufacturer’s instructions (\url{http://www.oxfordimmunotec.com/8-UK}) and (\url{http://www.oxfordimmunotec.com/UK%20Technical%20Handbook}). The numbers of spot forming units (SFU) were counted in each well using an automated ELISPOT reader (AID-GmbH, Strassberg, Germany). Where the negative control had 0–5 spots, a positive result was defined as \(\geq 6\) spots in either the ESAT-6 or the CFP-10 panel after subtracting the number of spots found in the negative control panel. In case >6 spots were seen the negative control panel, the ESAT-6 or the CFP-10 panel had to contain at least twice the number of spots found in the negative control panel to obtain a positive result. An indeterminate result was reported if the negative control spot count was >10 spots and as failed if there were <20 spots in the positive control panel and the ESAT-6 and CFP-10 panels were non-reactive.

HIV status was determined by a testing algorithm consisting of enzyme linked immunosorbent assays (Murex 1.2.0, Abbott-Murex Biotec, Dartford, Kent, UK), Hexagon HIV (Human Diagnostics GmbH, Wiesbaden, Germany) and type specific immunoblotting kit (Pepti-LAV I/II, BIORAD, Marnes-la-Coquette, France) for confirmation. HIV infected subjects were referred to the MRC Genitourinary Medicine Clinic for review and follow-up.
Data management and analysis
All data were entered into an ACCESS database and validated using adjudicated double data entry. Concordance was assessed by the calculation of a kappa statistic. McNemar’s test of symmetry was used to assess discordance when comparing two different methods of assessing TB positivity. Tests for significance were done using the medians and Wilcoxon (Mann Whitney) tests for non parametric data. Factors associated with discordance were identified using logistic regression analysis. Statistical significance was assumed at a p-value <0.05. All the statistical analyses described above were conducted using Stata software (version 12.1; Stata Corp, College Station, TX).
RESULTS

Characteristics of the study population

A total of 99 TB cases were recruited from August 2006-March 2007. Cultures for the *Mycobacterium tuberculosis complex*, were positive for 94 of 95 cases for whom results were available; 33 (33%) and 61(61.6%) were *M. africanum* and *M. tuberculosis* respectively. One case had a mixed infection. Most cases were male (71, 71.7%) and 41% were of Mandinka ethnic origin. A BCG scar was visible in 31(31.3%) and 85% of those tested were HIV negative. (See table 1) Most cases (79%) had the maximum sputum smear grade. On chest radiographs, a large minority (41%) had 3-4 lung zones affected by disease. Smear grade was not related to severity of radiological findings (p=0.21) or infecting strain (p=0.47).

Comparison of tests for detecting Mycobacterial infection

After excluding those cases with indeterminate results, the sensitivities of TST, T-SPOT and QFT-GIT using culture positive cases as the gold standard were 63.6% (95% Confidence Interval ([95%CI],40.7-82.8%), 89.7% (95%CI [81.3-95.2%]) and 90.1% (95%CI [81.5-95.6%]) respectively. There was no difference in the sensitivity of the IGRAs (p=0.91) and the overall agreement between IGRAs was 85.4%, although concordance was relatively poor (κ=0.25 [0.14-0.36, p=0.01]). As most cases were positive by both IGRAs as shown in figure 1, discordance was not significant (McNemar test, p=1.00). With test combinations of IGRAs and TST, 96.8% (92 of 95) cases were positive by either T-SPOT or QFT, 96.3% (80 of 83) by either QFT or TST and 100% (85) by T-SPOT or TST.

The numbers of indeterminate results were the same for both tests- T-SPOT (6, 6.1%) and QFT (6, 6.5%). Indeterminate results were not associated with age, HIV status, strain, or smear grade or severity of disease on chest X-ray. All of the indeterminate QFT results showed very low interferon gamma levels In addition, the antigen tube had interferon gamma less than 0.35IU/ml. For T-SPOT, the SFUs in the negative control wells were all above 10 SFUs in those with indeterminate results. However, at least one of the antigen wells and all mitogen wells had SFUs more than twice that in the negative control (See Figure 1 for results profile).
For the T-SPOT, there was a difference in sensitivity -83.3% [95%CI: 70.0-96.7] for *M. africanum* versus 92.7% [95%CI: 85.9-99.6] but this difference was not significant. QFT-GIT sensitivities were very similar for both pathogens (92.6% and 90.4% respectively for *M. africanum* and *M. tuberculosis*).

Overall, the median number of spots for ESAT-6 and CFP-10 were 41 (Interquartile range, IQR 11-86) spots and 29(IQR 7-85) spots respectively. Median numbers of spots for ESAT-6 in the T-SPOT assay were lower for *M. africanum* (33 [IQR 12-78] spots) compared to median in *M. tuberculosis* cases (43 [18-89] spots) but not significantly so (p=0.18). The findings for CFP-10 followed the same pattern as for ESAT-6 (15 [4-70] spots vs. 43 [19-96] spots for *M. africanum* and *M. tuberculosis* respectively, p=0.39)

For QFT-GIT, the median IFN-γ concentration was 1.85 (IQR-0.59-50) IU/mL. The median IFN-γ concentration was lower for *M. africanum* cases (1.47[IQR-0.55-3.62] IU/mL) compared to *M. tuberculosis* cases (2.04[0.69-5.87] IU/mL) but this was also not statistically significant (p=0.18).

No Mycobacteria strain related differences were seen in TB cases with discordant and concordant results for all combinations of IGRAs and IGRAs with TST (data not shown) after adjusting age, sex, ethnicity, sputum grade, HIV and BCG vaccination status.
DISCUSSION

The results from this comparison of commercially available IGRAs in proven TB cases show high levels of sensitivity for both IGRAs. These findings were unaffected by the strain of infecting mycobacterium. To our knowledge, this is the first study that has assessed the likely impact of pathogen related differences on the performance of the two widely available IGRAs in a high TB burden LMIC setting.

The comparable sensitivity found here for both IGRAs is in contrast to our earlier reports where our in-house ELISPOT-based IGRA performed better than the QFT in The Gambia [15]. In that study, the version of QFT-GIT used did not have a positive control tube (which was used with the version here), so false negative outcomes could have been accepted as valid results. In addition, the sensitivity seen here with T-SPOT is higher than for our pre-commercial in-house version of the ELISPOT assay. This is most likely the result of the more stringent diagnostic cut off criteria used for the in-house assay.

While some studies show T-SPOT (ELISPOT platform) is more sensitive than the QFT-GIT (ELISA platform) [16,17], our results similar to other investigators show they are comparable.[18,19,20] Most published reports show data in support of the superior performance of IGRAs compared to TST in active TB disease.[16,17] Although this study does not aim to compare IGRAs with TST, we find the former are significantly more sensitive than TST for active TB disease similar to earlier findings with our pre-commercial ELISPOT assay [21,22]. Analyses of pooled published data suggest a trend towards higher T-SPOT sensitivity but this is not significantly different from that seen with QFT-GIT.[5,23]

Considering the different clinical phenotypes and epidemiological differences seen between TB cases infected with *M. africanum* compared to *M. tuberculosis*,[6,7,8] it is reasonable to expect pathogen related differences in the performance of IGRAs in active TB disease between high TB burden vs. low burden countries. *M. africanum* was found in more than a third of TB cases here as reported before[6] and there was a trend towards high sensitivity for the T.SPOT assay in *M. Tuberculosis* cases but this
did not reach statistical significance. Although lower median ESAT-6 and CFP-10 spots were seen in the T-SPOT test for TB cases with M. africanum compared to M. tuberculosis, these were not significantly different. The same pattern was seen for IFN-γ concentrations with the QFT-GIT. The trends towards differential impact of Mycobacterial strain on the performance of both IGRAs suggest that larger sample size may be required to examine the role of the infecting Mycobacteria strain on the sensitivity of commercial IGRAs.

Improved sensitivity for active TB when an IGRA and TST are combined is expected in the presence of poor concordance and we have also shown this is the case for LTBI as well. [24,25,26] Discordance between both IGRAs and between IGRAs and the TST has been well described [15,27,28,29] Their distinct immunologic processes are thought to play a role in this, but there is still no consensus as to the pathophysiologic mechanisms responsible for this finding. [24,25,30] Although there was some sensitivity gain when each IGRA was combined with TST, this will be accompanied by specificity losses as we have reported before with LTBI.[31]

The utility of this combined testing strategy if any, for excluding or confirming LTBI and in which situations are currently unknown in high burden settings. In the LMIC settings with poor health systems capacity, scarce human capacity and laboratory infrastructure, introduction of any IGRAs will introduce additional costs. Therefore, better quality evidence of their predictive value for active TB before their inclusion in diagnostic work up of TB contacts and suspects is required.[32]

This study has some limitations. Our cross sectional design does not allow for any assessment of changes in IGRA over time since rapid reversions and conversions of IGRAs occur.[33] HIV reduces the performance of and increases the number of failed tests and indeterminate results of IGRAs. [34] We did not examine HIV-related impact on test characteristics of IGRAs in this study. Any generalization of results reported here to LTBI can only be done with the understanding that IGRAs cannot distinguish between LTBI and active TB disease. Moreover, we have studied active TB disease, a condition that has the Mycobacterial antigens contained in the IGRAs in
more abundance than LTBI as a proxy for LTBI, and this is likely to diminish the sensitivities of the two tests on small sample size as ours.

In conclusion, commercial IGRA and both IGRA are significantly more sensitive than TST for the diagnosis of TB disease in The Gambia as a proxy for LTBI. There is a suggestion, consistent with results from our in-house assay, for Mycobacterial strain to influence the sensitivity of commercial IGRA, but a larger study is required to investigate this further. Although combination of an IGRA and TST provides some sensitivity gain here, the utility of this as testing strategy for TB for this setting is questionable.
Acknowledgements: We thank all study participants (TB patients, and parents/guardians), the MRC TB research field team, staff of the MRC TB diagnostic laboratory especially Abigail Ayorinde, the Gambian National Leprosy and TB Programme for ongoing collaborations, data room staff and laboratory assistants especially Joseph Mendy for their hard work.

Conflict of interest statement: All authors do not have financial relationships with any commercial entity that has an interest in the subject of this manuscript.

Financial support: The Medical Research Council (United Kingdom) funded this study. Cellestis Limited, Carnegie, Australia provided all of the QFT-GIT and Oxford Immunotec Ltd., Abingdon, Oxford, gave about a third of T-SPOT.TB kits utilized for this study free. IMOA received funding from the European and Developing Countries’ Clinical Trials Partnership (EDCTP) through a career development fellowship. No funder/donor had an input in the preparation of this manuscript.

Author Contributions
Conceived and designed study: IA, PH, MO, RA
Performed experiments; AH, ML, PO, MA
Analyzed the data; IA, PH
Contributed reagents/materials/analysis tools: MO, MA, RA, SD
Approved paper for submission; IA, PH, MO, SD, RA, MA, PO, AH, ML
### Table 1. Demographic and clinical characteristics of study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>30.7</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>28(23-35)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>71(71.7)</td>
</tr>
<tr>
<td><strong>Ethnic group</strong></td>
<td>28(28.3)</td>
</tr>
<tr>
<td>Madinka</td>
<td>41(41.4)</td>
</tr>
<tr>
<td>Jola</td>
<td>22(22.2)</td>
</tr>
<tr>
<td>Wollof</td>
<td>12(12.1)</td>
</tr>
<tr>
<td>Fula</td>
<td>6(6.1)</td>
</tr>
<tr>
<td>Others</td>
<td>18(18.2)</td>
</tr>
<tr>
<td><strong>Clinical Findings</strong></td>
<td></td>
</tr>
<tr>
<td>BCG scar</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>54(54.5)</td>
</tr>
<tr>
<td>Present</td>
<td>31(31.3)</td>
</tr>
<tr>
<td>Uncertain</td>
<td>14(14.2)</td>
</tr>
<tr>
<td><strong>Mycobacterium strain</strong></td>
<td></td>
</tr>
<tr>
<td><em>M. africanum</em></td>
<td>33 (33.3)</td>
</tr>
<tr>
<td><em>M. tuberculosis</em></td>
<td>61 (61.6)</td>
</tr>
<tr>
<td><strong>HIV results</strong></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>11(11.1)</td>
</tr>
</tbody>
</table>

Data are no. (%) of persons unless stated otherwise.

BCG, Bacille Calmette-Guérin

*Data are for 99 cases

**Data are for 95 cases
Figure 1

Distribution of T-SPOT and QFT-GIT results in TB cases
99 smear and culture positive TB cases results

T-SPOT results unavailable for 1 case, QFT-GIT results

92 cases with T-SPOT and QFT-GIT results

T-SPOT Results for 92

76 (82.6%) positive
6 (6.5%) indeterminate
10 (10.9%) negative

67 (88.2%) T-SPOT and QFT-GIT positive
6 (7.9%) T-SPOT positive but QFT-GIT negative
3 (30%) T-SPOT negative and QFT-GIT negative
3 (3.9%) T-SPOT positive but QFT-GIT indeterminate

1 (10%) T-SPOT negative but QFT-GIT indeterminate

67 (87%) QFT-GIT and T-SPOT positive
6 (7.8%) QFT-GIT positive and T-SPOT negative
4 (5.4%) QFT-GIT positive but T-SPOT negative
6 (6.6%) QFT negative and T-SPOT positive

77 (83.7%) positive
6 (6.5%) indeterminate
9 (9.8%) negative

6 (6.5%) QFT-GIT negative but T-SPOT indeterminate
3 (3.3%) QFT-GIT negative and T-SPOT negative
0 (0%) QFT-GIT negative but T-SPOT positive

6 (7.8%) QFT-GIT and T-SPOT negative
6 (7.8%) QFT-GIT negative and T-SPOT negative
6 (6.6%) QFT negative and T-SPOT positive
References


