Commercial Interferon Gamma Release Assays Compared to the Tuberculin Skin Test for Diagnosis of Latent *Mycobacterium tuberculosis* Infection in Childhood Contacts in the Gambia

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Background: We compared the performance of tuberculin skin test (TST), Quantiferon-TB Gold in-tube (QFT-GIT), and T-SPOT.*TB* in diagnosing latent tuberculosis (LTBI) among childhood TB contacts in a TB endemic setting with high BCG coverage. We evaluated the performance of interferon gamma release assays (IGRAs) and TST when combined in an algorithm.

Methods: Childhood contacts of newly diagnosed TB patients were tested with TST, QFT-GIT, and T-SPOT. The level of exposure in contacts was categorized according to whether they slept in the same room, same house, or a different house as the index case. For the evaluation of combined test performance, prior estimates for prevalence of latent TB were used in Bayesian models that assumed conditional dependence between tests.

Results: A total of 285 children were recruited. Overall, 26.5%, 33.0%, and 33.5% were positive for TST, T-SPOT, or QFT-GIT, respectively. All 3 tests responded to the gradient of sleeping proximity to the index case. Neither TST nor IGRA results were confounded by BCG vaccination. There was moderate agreement ($\kappa = 0.40–0.68$) between all 3 tests. Combination of either IGRA with TST increased sensitivity (by 9.3%–9.6%) especially in contacts in the highest exposure category but was associated with loss of specificity (9.9%–11.3%).

Conclusion: IGRAs and TST are similar in their diagnostic performance for LTBI. An approximate 10% sensitivity benefit for using the TST and an IGRA in combination is associated with a slightly greater specificity loss. Testing strategies combining an IGRA and TST with an "or" statement may be useful only in situations where there is a high pretest probability of latent infection.

Key Words: tuberculin skin test, interferon gamma release assays, children, latent tuberculosis infection, Bayesian modeling

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Worldwide, approximately 2 billion people have latent *Mycobacterium tuberculosis* infection (LTBI). The risk of progression from LTBI to TB disease is highest in children.[1,2] Accurate diagnosis of LTBI in children in TB endemic countries is a priority. The use of tuberculin skin test (TST) for diagnosis of LTBI has well described limitations such as low specificity especially in settings with low TB burden, the need for a repeat visit and boosting effect with repeat testing.[3] Interferon gamma (IFN-γ) release assays (IGRAs; Quantiferon-TB Gold [Cellestis Inc, Carnegie, Australia] and T-SPOT.*TB* [Oxford Immunotec Ltd, Oxford, UK]) are alternative diagnostic tools.[3,4]

Use of IGRAs is recommended in a number of situations such as where the TST is required, for screening for LTBI in immunosuppressed persons, as an adjunct test for diagnosis of active TB where bacteriologic confirmation is not possible, and in a 2-step strategy to confirm positive TST results.[5–7] There are only limited data on their performance in children in tropical TB-endemic settings.[8] Evaluation has been hampered by unavailability of an ideal gold standard for LTBI.[3] TB disease and performance against measures of exposure to a known TB case have been used as surrogate standards.[9,10]

Formal assessment of an in-house ELISPOT test in all age groups in The Gambia suggested lower sensitivity and specificity than TST in diagnosis of LTBI.[10,11] Combination of IGRA and TST for diagnosis of LTBI in the same setting correctly identified more adult contacts that progressed to active TB disease than either test alone.[12] We conducted a formal comparison of T-SPOT.*TB,* QFT-GIT, and the TST for diagnosis of LTBI in Gambian childhood contacts of TB patients and applied Bayesian analytic techniques to evaluate the performance of the IGRAs or TST in combination.

METHODS

Participants

We recruited household contacts of newly diagnosed TB index cases from major government health centers and the Medical Research Council Laboratories' (MRC Labs) outpatients' clinic. TB cases were sputum smear and culture positive for acid-fast bacilli by Ziehl-Neelsen stain and had a typical chest radiograph. Contacts were 6 months to 14 years of age and lived the majority of the time on the same "compound" as a case. A compound was defined as a separate cluster of homes (or buildings) often owned by members of the same family. Exclusion criteria were history of treatment for active TB in the past year and diagnosis of TB disease within 1 month of recruitment. Written informed consent from parents/guardians was obtained, subjects were examined, and tested for T-SPOT, QFT-GIT, TST, and HIV (after counseling). Samples for QFT-GIT were drawn into three 1 mL tubes provided by the manufacturer. For T-SPOT, blood was drawn into 4 mL

pediatric cell preparation tubes (CPT, Becton Dickinson, Oxford Park, Oxford) for children under 10 years and into 8 mL adult CPTs for those 10 to 14 years old.

TST was carried out with 2 TU (PPD RT23, Statens Serum Institut, Copenhagen, Denmark) immediately after blood samples were collected. Indurations were recorded at 48 to 72 hours. Subjects with a positive TST (induration diameter ≥10 mm) were offered a chest radiograph and those with symptoms underwent a clinical assessment. Those with TB disease were referred to the National TB Control Program for free treatment. BCG is given within the first month of life in The Gambia. Preventive treatment for TB contacts less than 5 years old was recently included in the new National TB Guidelines. However, practical issues have precluded its implementation.

The Joint Gambia Government/MRC Joint Ethics Committee approved this study.

Laboratory Procedures

Sputum smears and all mycobacterial cultures were examined, identified and confirmed using standard procedures.[13]

QFT-GIT Assay

This assay was carried out according to the manufacturer's instructions (http://www.cellestis.com/IRM/content/aust/qtfproducts_tbgoldintube_techinfo.html). The IFN-γ levels were measured in international units (IU) with a Dynex ImmunoAssay System ELISA reader version 6.0 (Dynex Technologies, West Sussex, UK). A positive result was defined as IFN-γ concentration in TB-specific antigen stimulated tube minus that in the negative control (nil) tube ≥0.35 IU/mL. Results were indeterminate if there were no detectable IFN-γ responses in the mitogen tube or IFN-γ concentration in the nil tube >8 IU/mL.

T-SPOT Assay

The manufacturer's instructions were followed in performing this test (http://www.oxfordimmunotec.com/UK%20Pack%20Insert). The spot forming units in each well were counted using an automated ELISPOT reader (AID-GmbH, Strassberg, Germany). Where the negative control had 0 to 5 spots, a positive result was defined as ≥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 in 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 if there were <20 spots in the positive control panel and the ESAT-6 and CFP-10 panels were nonreactive.

HIV Testing

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.

Ascertainment of Exposure to M. tuberculosis

Exposure in contacts was classified according to whether they slept in a different house but on the same compound, the same house or the same room relative to the TB case.[10]

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 and discordance by McNemar's test. Agreement between the tests was classified into categories; Kappa coefficient, poor (if κ <0.20), fair (κ 0.21–0.40), moderate (κ 0.41–0.60), good (κ 0.61–0.80), and very good (κ 0.81–1.00).[14] The relationship between sleeping proximity to an index case and test results was assessed using random effects logistic regression accounting for household clustering. Age and sex were included in the analysis at the outset. Other variables assessed for possible inclusion in the model were ethnicity, BCG scar status and duration of cough of the respective index case. The likelihood ratio test was used to test for interaction between variables. Statistical significance was assumed at a P value <0.05. All these statistical analyses were conducted using Stata software (version 9.2; Stata Corp, College Station, TX).

A Bayesian approach was used to estimate the sensitivity and specificity changes caused by combining tests. Beta distributions.[15] were used to model the prevalence of latent TB, single test sensitivity and specificity, with model parameters estimated from the literature.[3,4,16] The Bayesian models were programmed in Matlab v7.6 (The Mathworks, Nantwick, MA). Within a Gambian setting the Mantoux test has high specificity[11] and the same sensitivity and specificity parameters were chosen for both the IGRA tests and the Mantoux test. The sensitivity priors had a mean of 85% and a 95% CI of ±5% and the specificity priors had a mean of 90% with a 95% CI of ±5%. The prior prevalence of latent TB infection in the farthest to the nearest proximity, had means of 30% (95% CI: ±5%), 40% (95% CI: ±10%), and 50% (95% CI: ±10%), respectively. Conditional dependence between tests was included using the methods described by Dendukuri.[17] A Gibbs sampling scheme,[17] was used to generate the posterior distributions for the parameters given the cross tabulations of each pair of tests were estimated. After a burn in of 500 samples, the chains from the Gibbs sampler were run for 20,000 estimates with a thinning parameter of 10. Convergence was tested from a number of different starting values and different chain lengths and none of the autocorrelations up to lag 20 were significant at the 5% level. The converged values were used to estimate sensitivity gains and specificity losses.[18]

RESULTS

We recruited 285 childhood household contacts (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A342); 140 (49.1%) contacts were male and 3 (1.1%) were HIV-positive. No child had active TB disease. There were 215 valid TST results and 245 for both T-SPOT and QFT-GIT. There were no failed or indeterminate results with T-SPOT while there were 2 (0.8%) indeterminate QFT-GIT results. Overall, there were 215 subjects with valid results for all 3 tests (Fig., Supplemental Digital Content 2, http://links.lww.com/INF/A343). The proportion of contacts with positive results were 26.5% (95% CI: 20.7%–32.9%), 33.0% (26.8%–39.7%), and 33.5% (27.2%–40.2%) for TST, T-SPOT, or QFT-GIT, respectively.

A BCG scar was seen in 127 (59.1%) of 199 children when those with uncertain scars were excluded. The univariate odds ratio of a positive test in those with a visible scar compared with those without, was 0.89 (0.50–1.70, P = 0.72), 1.1 (0.61–2.09, P = 0.70), and 1.1 (0.60–2.0, P = 0.85) for TST, T-SPOT, and QFT-GIT, respectively. This implies none of the tests was confounded by the presence of a BCG scar.

Twenty-eight of 71 (39.4%) T-SPOT positive contacts were TST negative while 14 of 57 (24.5%) TST positive contacts were T-SPOT negative. Twenty-nine of 72 (40.3%) QFT-GIT positives were TST negative while 14 (24.6%) TST positive contacts were QFT-GIT negative. For the IGRAs, 20 (28.2%) T-SPOT positive contacts were QFT-GIT negative, while 22 (30.6%) QFT-GIT positive contacts were T-SPOT negative. There was moderate to

good agreement between TST and T-SPOT (concordance 80.5%, $\kappa = 0.54$ [95% CI: 0.41–0.68], $P < 0.0001$), TST and QFT-GIT (79.8% $\kappa = 0.52$ [0.40–0.66] $P < 0.0001$) and between T-SPOT and QFT-GIT (79.5%, $\kappa = 0.55$ [0.41–0.69] $P < 0001$). There was discordance between each IGRA and TST (T-SPOT vs. TST [$\chi^2 = 4.67$, $P = 0.03$] and QFT-GIT vs. TST [$\chi^2 = 7.23$, $P = 0.03$]) but not between the IGRAs ($\chi^2 = 2.10$, $P = 0.55$).

The proportions of contacts positive by all 3 tests increased with increasing exposure to the index case, most dramatically for TST. Table 1 shows the univariate and multivariate odds ratios for a positive result for all 3 tests across the gradient of exposure. When LTBI was defined as a positive TST or IGRA result, all test combinations responded significantly to the TB exposure gradient and the most significant change was for the T-SPOT/TST combination (Table 2).

Combining 2 tests with an "OR" statement implies the number of positive results can only stay the same or increase and consequently the sensitivity can only remain the same or increase. In exactly the same manner as the number of negative results remains the same or decreases, the specificity must remain the same or decrease. The estimated sensitivity gains and specificity losses across the different categories of exposure to a TB case are shown in Table 3. The mean estimated gains in sensitivity were similar for all test combinations and ranged from 9.3% to 9.6%. The mean estimated loss of specificity ranged from 9.9% to 11.3% and was highest for the T-SPOT or QFT combination. Changes in sensitivity and specificity of combined tests were robust to reductions in the mean prior estimates for TST specificity.

DISCUSSION

The results from this comparison of commercially available IGRAs with the TST in children are largely in agreement with our previous assessment of our in-house interferon gamma (IFN-γ) ELISPOT test in adults and children and shed new light on the use of these tests in combination.[10,19] TST was most responsive to TB exposure among the 3 tests and none was affected by prior BCG vaccination. The performance of both IGRAs in relation to the gradient of TB exposure was improved especially for T-SPOT when combined with TST. Modeling suggests that sensitivity gains of approximately 10% with TST and IGRA in combination were associated with slightly greater specificity losses.

That the TST correlates better with TB exposure compared with the IGRAs is consistent with our previous findings across all age groups in The Gambia when we used our in-house ELISPOT test.[10,19,20] Others have also reported comparable performance for IGRAs and TST,[21] while studies, mainly from settings with low TB burden found improved specificity and sensitivity of IGRAs in diagnosis of LTBI.[3,4,9] Although the actual number of children with LTBI is unknown, there are a few reasons for the appearance of better sensitivity of TST over IGRAs in detecting LTBI in Gambian TB contacts. Delayed presentation by TB cases (mean 9 weeks) in the presence of early reversion of IGRAs may contribute.[22–24] Also, a third of TB cases in The Gambia are infected with *M. africanum* which exhibits an attenuated response to ESAT-6 in both cases and their contacts.[25]

Surprisingly higher specificity of TST compared with IGRAs to the exposure gradient is difficult to explain, but the higher specificity of TST when compared with the IFN-γ ELISPOT response to the same PPD antigens,[11] suggests that the type of immune response elicited is at least partially responsible.

We had previously shown that when LTBI was defined as a positive test for TST or IGRA (using our in-house ELISPOT), 71% of household contacts that progressed to disease were correctly identified compared with only about 50% of those positive by both

TABLE 1. Univariable and Multivariable Odds Ratios Determined by Logistic Regression (With Household Clustering as a Random Effect), for Sleeping Proximity as a Surrogate Marker of Exposure to *M. tuberculosis* in Household TB Contacts

	T-SPOT.TB (n = 215)				Quantiferon (n = 215)				TST* (n = 215)			
	Positive Results No. (%) Contacts	Unadjusted OR (95% CI)	Adjusted† OR (95% CI)	*P*	Positive Results No. (%) Contacts	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	*P*	Positive Results No. (%) Contacts	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	*P*
Sleep proximity												
Different house	18 (25.0)	1	1		19 (26.4)	1	1		10 (14.0)	1	1	
Different room	39 (33.1)	2.0 (0.8–5.1)	2.6 (0.9–7.1)		39 (33.1)	1.2 (0.6–2.6)	1.5 (0.7–3.1)		32 (27.1)	2.4 (1.0–5.8)	2.9 (1.3–6.7)	
Same room	14 (56.0)	5.3 (1.5–18.5)	6.6 (1.7–25.2)	0.02‡	14 (56.0)	3.2 (1.2–9.1)	4.0 (1.4–11.4)	0.03‡	15 (60.0)	10.1 (3.2–32.1)	15.0 (4.7–47.2)	<0.0001‡

*Positive result defined as a mean induration diameter of ≥10 mm.
†Adjusted for age, sex and ethnic group.
‡*P* values for change in adjusted results across exposure categories (test for trend).

TABLE 2. Univariable and Multivariable Odds Ratios for Combined IGRA and TST Results Determined by Logistic Regression (With Household Clustering as a Random Effect), for Sleeping Proximity as a Surrogate Marker of Exposure to *M. tuberculosis* in Household TB Contacts

	T-SPOT or TST (n = 208)				T-SPOT or QFT-GIT (n = 213)				QFT-GIT or TST* (n = 213)			
	Positive Results No. (%) Contacts	Unadjusted OR (95% CI)	Adjusted† OR (95% CI)	*P*	Positive Results No. (%) Contacts	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	*P*	Positive Results No. (%) Contacts	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	*P*
Sleep proximity												
Different house	21 (30.0)	1	1		22 (31.4)	1	1		22 (31.4)	1	1	
Different room	51 (38.1)	1.8 (0.8–3.8)	2.0 (0.9–4.5)		53 (44.9)	2.0 (0.9–4.2)	2.1 (1.0–4.5)		47 (39.8)	1.5 (0.8–2.9)	1.7 (0.8–3.6)	
Same room	21 (72.0)	11.1 (2.4–27.7)	9.6 (2.7–33.8)	0.002‡	17 (68.0)	5.0 (1.7–15.2)	5.3 (1.8–16.1)	0.01‡	17 (68.0)	4.9 (1.7–13.8)	6.4 (2.1–193)	0.005‡

*Positive result defined as a mean induration diameter of ≥10 mm.
†Adjusted for age, sex and ethnic group.
‡*P* values for change in adjusted results across exposure categories (test for trend).

TABLE 3. Table Showing Estimates of Sensitivity Gain and Specificity Loss With Pair-Wise Combinations of the Three Tests for Diagnosis of Latent TB Infection Across Gradient of TB Exposure

Test Combination	Sleeping Proximity to Index TB Case		
	Different House	Same House	Same Room
Sensitivity gain			
TST or T-SPOT	9.4%	8.7%	10.1%
TST or QFT	9.1%	9.8%	9.0%
T-SPOT or QFT	7.4%	11.4%	9.9%
Specificity loss			
TST or T-SPOT	8.6%	10.0%	11.3%
TST or QFT	8.6%	9.3%	11.9%
T-SPOT or QFT	11.7%	10.4%	11.7%

IGRA and TST.[12] This approach also identifies more contacts as latently infected in this study. The best strategy for diagnosis of LTBI with these 2 tests remains unclear. With reports of boosting of subsequent IGRA tests by earlier placed TST, the 2-step test approach may require re-examination.[24,26,27] In addition, there is very limited information on the relative predictive value of the results from different test algorithms.

Statistical tools such as Bayesian mixture modeling techniques have been explored to obtain robust estimates of LTBI in TB endemic settings.[28,29] However, in this study we have been able to estimate the specificity cost of using IGRA and TST in combination for the first time by using the "Gambian TB exposure gradient." This reproducible surrogate for TB exposure, in the absence of a gold standard for the diagnosis of LTBI, is a valuable tool for assessing the performance of new tests.

Discordance between both IGRAs and between IGRAs and the TST has been well described.[9,30,31] The agreement between the 2 IGRAs and their discordance with TST in part support the idea that different mechanisms are involved in these tests. Among them, the production of and response to chemokines involved in the TST response may have a limited influence on the priming of antigen-specific T cells.[32] Although these distinct immunologic processes are thought to play a role, there is still no consensus as to the biologic basis.[33–35]

There is increasing information, from settings such as ours, reporting non confounding of TST and IGRA results by BCG vaccination as we have found.[10,20,36–38] This is probably explained by the reported rapid reversion of positive TST post BCG vaccination in tropical settings compared with its persistence in temperate locations, where BCG is often given at an older age.[39]

This study has some limitations. Our cross sectional design does not allow for any assessment of changes in IGRA over time. In addition, we did not use the quantitative outputs from each test which could have varied with increasing exposure but restricted our analysis to results in categorical format. HIV prevalence is relatively low in The Gambia compared with some other TB endemic/high burden settings.[40] Thus our results may not apply to settings with higher HIV prevalence. Our models for estimating sensitivity and specificity are not perfect, especially as they depend on assumptions of priors for sensitivity and specificity; and of conditional dependence. The absence of a gold standard to assess IGRAs is the same reason why models like this are required.

In conclusion, the TST and commercial IGRAs perform similarly in diagnosis of LTBI among childhood TB contacts in

The Gambia. The sensitivity benefit for using the TST and an IGRA in combination is associated with a slightly larger estimated specificity loss. Therefore, a testing strategy combining an IGRA and TST with an or statement may be of use only in certain situations, including those where there is a high pretest probability or risk of TB. This is particularly relevant as there are data supporting a boosting effect on subsequent IGRA responses when TST is done first.[24,41]

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REFERENCES

1. Marais BJ, Gie RP, Schaaf HS, et al. Childhood pulmonary tuberculosis: old wisdom and new challenges. *Am J Respir Crit Care Med*. 2006;173: 1078–1090.
2. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol*. 1974; 99:131–138.
3. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med*. 2008;149:177–184.
4. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. *Ann Intern Med*. 2007;146:340–354.
5. National Collaborating Centre for Chronic Conditions. Tuberculosis: clinical diagnosis and management of tuberculosis, and measures for its prevention and control. *Clin Guidel.* 2006;33:1–215.
6. Mazurek GH, Jereb J, Lobue P, et al. Guidelines for using the QuantiFERON-TB gold test for detecting *Mycobacterium tuberculosis* infection, United States. *MMWR Recomm Rep*. 2005;54:49–55.
7. Canadian Tuberculosis Committee. Updated recommendations on interferon gamma release assays for latent tuberculosis infection. *Can Commun Dis Rep*. 2008;34:1–13.
8. Pai M, Dheda K, Cunningham J, et al. T-cell assays for the diagnosis of latent tuberculosis infection: moving the research agenda forward. *Lancet Infect Dis*. 2007;7:428–438.
9. Arend SM, Thijsen SF, Leyten EM, et al. Comparison of two interferon-gamma assays and tuberculin skin test for tracing tuberculosis contacts. *Am J Respir Crit Care Med*. 2007;175:618–627.
10. Hill PC, Brookes RH, Adetifa IM, et al. Comparison of enzyme-linked immunospot assay and tuberculin skin test in healthy children exposed to *Mycobacterium tuberculosis*. *Pediatrics*. 2006;117:1542–1548.
11. Hill PC, Brookes RH, Fox A, et al. Surprisingly high specificity of the PPD skin test for *M. tuberculosis* infection from recent exposure in The Gambia. *PLoS One*. 2006;1:e68.
12. Hill PC, Jackson-Sillah DJ, Fox A, et al. Incidence of tuberculosis and the predictive value of ELISPOT and Mantoux tests in Gambian case contacts. *PLoS One*. 2008;3:e1379.
13. Adegbola RA, Hill P, Baldeh I, et al. Surveillance of drug-resistant *Mycobacterium tuberculosis* in The Gambia. *Int J Tuberc Lung Dis*. 2003;7:390–393.
14. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33:159–174.
15. Joseph L, Gyorkos TW, Coupal L. Bayesian estimation of disease prevalence and the parameters of diagnostic tests in the absence of a gold standard. *Am J Epidemiol*. 1995;141:263–272.
16. Pai M, Riley LW, Colford JM Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis*. 2004; 4:761–776.
17. Dendukuri N, Joseph L. Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests. *Biometrics*. 2001;57:158–167.
18. Vacek PM. The effect of conditional dependence on the evaluation of diagnostic tests. *Biometrics*. 1985;41:959–968.
19. Hill PC, Brookes RH, Fox A, et al. Large-scale evaluation of enzyme-linked immunospot assay and skin test for diagnosis of *Mycobacterium tuberculosis* infection against a gradient of exposure in The Gambia. *Clin Infect Dis*. 2004;38:966–973.
20. Adetifa IM, Lugos MD, Hammond A, et al. Comparison of two interferon gamma release assays in the diagnosis of *Mycobacterium tuberculosis* infection and disease in The Gambia. *BMC Infect Dis*. 2007;7:122.
21. Tavast E, Salo E, Seppala I, et al. IGRA tests perform similarly to TST but cause no adverse reactions: pediatric experience in Finland. *BMC Res Notes*. 2009;2:9.
22. Rathman G, Sillah J, Hill PC, et al. Clinical and radiological presentation of 340 adults with smear-positive tuberculosis in The Gambia. *Int J Tuberc Lung Dis*. 2003;7:942–947.
23. Hill PC, Brookes RH, Fox A, et al. Longitudinal assessment of an ELISPOT test for *Mycobacterium tuberculosis* infection. *PLoS Med*. 2007;4:e192.
24. van Zyl-Smit RN, Pai M, Peprah K, et al. Within-subject variability and boosting of T Cell IFN-{gamma} responses following tuberculin skin testing. *Am J Respir Crit Care Med.* 2009;180:49–58.
25. de Jong BC, Hill PC, Brookes RH, et al. *Mycobacterium africanum* elicits an attenuated T cell response to early secreted antigenic target, 6 kDa, in patients with tuberculosis and their household contacts. *J Infect Dis*. 2006;193:1279–1286.
26. Vilaplana C, Ruiz-Manzano J, Gil O, et al. The tuberculin skin test increases the responses measured by T cell interferon-gamma release assays. *Scand J Immunol*. 2008;67:610–617.
27. Igari H, Watanabe A, Sato T. Booster phenomenon of QuantiFERON-TB gold after prior intradermal PPD injection. *Int J Tuberc Lung Dis*. 2007; 11:788–791.
28. Pai M, Dendukuri N, Wang L, et al. Improving the estimation of tuberculosis infection prevalence using T-cell-based assay and mixture models. *Int J Tuberc Lung Dis*. 2008;12:895–902.
29. Trebucq A, Guerin N, Ali Ismael H, et al. Prevalence and trends of infection with *Mycobacterium tuberculosis* in Djibouti, testing an alternative method. *Int J Tuberc Lung Dis*. 2005;9:1097–1104.
30. Lee JY, Choi HJ, Park IN, et al. Comparison of two commercial interferon-gamma assays for diagnosing *Mycobacterium tuberculosis* infection. *Eur Respir J*. 2006;28:24–30.
31. Ferrara G, Losi M, D'Amico R, et al. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with *Mycobacterium tuberculosis*: a prospective study. *Lancet*. 2006;367:1328–1334.
32. Orme IM, Lee BY, Appelberg R, et al. T cell response in acquired protective immunity to *Mycobacterium tuberculosis* infection. *Bull Int Union Tuberc Lung Dis*. 1991;66:7–13.
33. Menzies D. Using tests for latent tuberculous infection to diagnoses active tuberculosis: can we eat our cake and have it too. *Ann Intern Med*. 2008;148:398–399.
34. Dosanjh DP, Hinks TS, Innes JA, et al. Improved diagnostic evaluation of suspected tuberculosis. *Ann Intern Med*. 2008;148:325–336.
35. Goletti D, Stefania C, Butera O, et al. Accuracy of immunodiagnostic tests for active tuberculosis using single and combined results: a multicenter TBNET-study. *PLoS One*. 2008;3:e3417.
36. Mudido PM, Guwatudde D, Nakakeeto MK, et al. The effect of bacille Calmette-Guerin vaccination at birth on tuberculin skin test reactivity in Ugandan children. *Int J Tuberc Lung Dis*. 1999;3:891–895.
37. Dogra S, Narang P, Mendiratta DK, et al. Comparison of a whole blood interferon-gamma assay with tuberculin skin testing for the detection of tuberculosis infection in hospitalized children in rural India. *J Infect*. 2007;54:267–276.
38. Dominguez J, Ruiz-Manzano J, De Souza-Galvao M, et al. Comparison of two commercially available gamma interferon blood tests for immunodiagnosis of tuberculosis. *Clin Vaccine Immunol.* 2008;15:168–171.
39. Fine PE, Bruce J, Ponnighaus JM, et al. Tuberculin sensitivity: conversions and reversions in a rural African population. *Int J Tuberc Lung Dis*. 1999;3:962–975.
40. Schim van der Loeff MF, Jaffar S, Aveika AA, et al. Mortality of HIV-1, HIV-2 and HIV-1/HIV-2 dually infected patients in a clinic-based cohort in The Gambia. *AIDS*. 2002;16:1775–1783.
41. Naseer A, Naqvi S, Kampmann B. Evidence for boosting Mycobacterium tuberculosis-specific IFN-gamma responses at 6 weeks following tuberculin skin testing. *Eur Respir J*. 2007;29:1282–1283.