Relationship of immunodiagnostic assays for tuberculosis and numbers of circulating CD4+ T-cells in HIV–infection

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Abstract

Infection with the human-immunodeficiency-virus (HIV) is the greatest risk factor for tuberculosis (TB) in Africa. Tuberculin-skin-test (TST), QuantiFERON-TB Gold In-Tube (QFT-G-IT) and T-Spot.TB assays were performed in newly diagnosed HIV-infected individuals with and without active TB and in HIV-uninfected persons at an University outpatient clinic in Kampala, Uganda. A total of 135 individuals were enrolled: 109 with a new diagnosis of HIV-1 infection, but no active TB, 19 with HIV-1 infection and active TB, and 7 HIV-uninfected healthy subjects. In control subjects immune responses were positive in 57.2 % by TST and in 100% by at least one IGRA. In HIV-1 infected patients without active TB, induration in the TST (mm) (rho=0.41, p-value<0.0001) and concentration of IFN-γ in the QFT-G-IT tube with MTB-specific antigens (rho=0.38; p-value:0.0001) were negatively correlated to numbers of circulating CD4+ T-cells (cells/µl) while numbers of IFN-γ producing cells (rho=0.03-0.13; p-value:0.21-0.77) and frequencies of positive test results for T-Spot.TB test among groups of patients with different levels of immunodeficiency remained constant (p=0.46). In HIV-1 infection, TST and QFT-G-IT immune responses are both strongly related to the degree of immunodeficiency, while results of the T-Spot.TB are independent of the level of CD4 T-cell depletion.

Keywords: CFP-10, ESAT-6; HIV; IGRA; LTBI, tuberculosis

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Introduction

According to a previous estimate by the World Health Organization (WHO), approximately one third of the world population is infected with *Mycobacterium tuberculosis* [1]. Latent infection with *M. tuberculosis* (LTBI) is usually defined as infection with *M. tuberculosis complex*, manifested by a pre-defined tuberculin skin test (TST) reaction and/or positive interferon-γ release assay (IGRA) without any sign of active disease [2]. As a result of a successful immune response, only a minority of those individuals who are thought to have become latently infected with *M. tuberculosis* ever develop tuberculosis [3, 4]. Nevertheless, approximately 9 million people are diagnosed with active tuberculosis and nearly 2 million die of the disease annually [5].

Tuberculosis and infection with the Human-immunodeficiency-virus (HIV) are leading public health problems in Africa [6]. A dangerous interaction of these two diseases has been documented that includes increased morbidity and mortality in dually infected subjects [7]. In fact, tuberculosis continues to be the leading cause of death in individuals with HIV-infection [8]. Recent data also indicate that infection with HIV doubles the risk of tuberculosis reactivation early after HIV-seroconversion [9]. Further, in HIV-infected individuals with LTBI the annual risk to develop active tuberculosis is 5 – 10% [10]. Thus, rapid identification and preventive treatment of HIV-infected individuals with LTBI, who are at risk of developing active tuberculosis, is a priority to fight tuberculosis in Africa [11].

However, achieving this goal has been hampered to date by limitations in TST reactivity, particularly in the majority of persons with advanced HIV-1 infection and presumptive LTBI [12-15]. Interferon-γ release assays (IGRA) may prove to be more reliable for the diagnosis of LTBI in individuals with HIV infection [16-21]. Nevertheless, studies comparing TST with the two commercially available IGRA, the QuantiFERON-TB Gold In-Tube
(Cellestis, Australia) and the T-Spot.TB (Oxford Immunotec, UK) in a country of high HIV prevalence and endemic for tuberculosis are limited [21].

To evaluate current methods for the immunodiagnosis of LTBI and understand their relationship to the level of immunodeficiency in patients with HIV-infection, we compared the performance of different IGRA and the TST in an African country with high TB-incidence.

**Material and methods**

*Study subjects and design*

The study was conducted in Kampala, Uganda, at the HIV outpatient clinic of the Infectious Diseases Institute of Makerere University. In 2006, the incidence of tuberculosis in Uganda was 355 per 100,000. In recent years, approximately 60 % of tuberculosis patients tested for HIV were HIV-positive in Uganda [22], however, current HIV-prevalence rates in patients with tuberculosis are estimated to be lower at 45% – 50 % (H. Mayanja-Kizza, pers. communication).

The study was approved by the ethical committees of Makerere University, Uganda, Case Western Reserve University in Cleveland, USA, and Lübeck University, Germany. All subjects provided written informed consent, consistent with good clinical practice and ethical guidelines of the US Department of Health and Human Services.

Following voluntary counselling and testing for HIV-1 infection, adult residents of Kampala who were referred to the Infectious Diseases Institute at Makerere University were invited to participate. All study subjects were antiretroviral therapy naïve. Prior tuberculosis,
isoniazid preventive therapy, steroid therapy, pregnancy, and in HIV-infected people, a Karnofsky score \( \leq 60 \) or current opportunistic infection were exclusion criteria. The presence of any one of the symptoms cough, chest pain, recent weight loss, night sweats, fever, loss of appetite, swelling of lymph nodes or generalized tiredness triggered referral to rule out active tuberculosis to the Tuberculosis Research Unit clinic where sputum examinations were performed. All individuals were clinically examined and received a chest X-ray to rule out active pulmonary tuberculosis. Healthy HIV-seronegative controls (staff of the Infectious Diseases Institute at Makerere University) where also enrolled.

HIV-seropositive positive individuals without risk factors for active tuberculosis where recruited at the National Institute for Infectious Diseases L. Spallanzani, Rome, Italy, as additional controls.

_Tuberculin skin testing_

At the first study visit, blood was taken and a 2 TU/0.1 mL TST (RT23, Statens Serum Institute, Copenhagen, Denmark) was placed on the volar aspect of the forearm, and the site was marked with a circle by felt tip pen. At 48 hours, the transverse diameter of TST induration was determined by the ball-point pen and ruler method [23].

_Interferon-γ release assays_

T-Spot.TB (Oxford Immunotec, Abingdon, UK) and QuantiFERON-TB Gold in tube (QFT-G-IT; Cellestis, Melbourne, Australia) were performed according to the manufacturer’s guidelines.

_T-Spot.TB assay_

For the T-Spot.TB assays peripheral blood mononuclear cells (PBMCs) were separated from
heparinized venous blood by centrifugation and the Interferon-\(\gamma\) ELISPOT kit with a 96-well plate was used. 250,000 cells per well were stimulated with phytohaemagglutinin (positive control), were left unstimulated (negative control) or contained peptides of ESAT-6 and CFP-10. After incubation for 18 hours, plates were developed according to the manufacturer's protocol and were read on an Immunospot Analyzer (AID, Strassberg, Germany). A positive result was defined as spot count in the ESAT-6 and/or CFP-10 panel minus spot count in the nil control panel \(\geq\) 5 spots; a negative result was defined as < 5 spots. Results were defined as indeterminate, if more than 10 spot-forming cells (SFC) were present in the nil-control wells (high background) and/or less than 20 SFC in the mitogen positive control wells.

*QuantiFERON-TB-Gold-in-tube assay*

For the QFT-G-IT test three 1mL-blood venous blood samples were collected in 3 evacuated tubes that were precoated with *M. tuberculosis*–specific antigens (ESAT-6, CFP-10, and TB7.7) for the test, phytohaemagglutinin for the positive control, or no antigen for the negative control. Specimens were processed according to the manufacturer’s instructions. Results were considered to be positive, if the level of IFN-\(\gamma\) in the ESAT-6, CFP-10, and TB7.7 antigen–exposed sample minus the level in the negative control was \(\geq 0.35\) IU/mL and \(>25\%\) of the IFN-\(\gamma\) concentration in the negative-control plasma. Indeterminate results were defined as either an unprovoked IFN-\(\gamma\) level of \(\geq 8.0\) IU/mL in the negative-control plasma or an IFN-\(\gamma\) response of \(\leq 0.5\) IU/mL on phytohaemagglutinin stimulation with a level of IFN-\(\gamma\) in the tuberculosis antigen-exposed sample minus the level in the negative control of either <0.35 IU/mL or <25\% of the IFN-\(\gamma\) concentration in the negative-control plasma.
Laboratory evaluation

Plasma HIV viral load was examined by Amplicor (Roche™) and blood CD4+ cell counts were detected by flow cytometry (Becton Dickinson Inc., San Jose, CA, USA).

Statistical analysis

Data were analysed using Stata 9.0 (StataCorp, Stata Statistical Software Release 9, College Station, TX, USA, 2005). Assay responses were evaluated as continuous variables and as categorical variables (positive or negative responses). Comparisons between proportions were performed using Fisher's exact test; the Student's t-test was used for continuous measurements to test relationships in unpaired analysis, while the Wilcoxon-Mann-Whitney test was used when assumed that the dependent variable is a normally distributed interval variable. Group means (> 2) were compared using analysis of variance; its non-parametric version (Kruskal-Wallis test) was used when appropriate. Test concordance was assessed by κ-statistics with agreement considered ‘slight’ for k ≤ 0.20, ‘fair’ for 0.20 < k ≤ 0.40, ‘moderate’ for 0.40 < k ≤ 0.60, ‘substantial’ for 0.60 < k ≤ 0.80 and ‘optimal’ for 0.80 < k ≤ 1.00 All analyses were two-sided, with a p-value of 0.05 considered significant [24, 25].

Results

Of 190 individuals initially evaluated, 135 individuals met the inclusion/exclusion criteria and were included in the analysis. Of those, 109 were newly diagnosed HIV-1 positive patients with no signs or symptoms of active TB, 19 were newly diagnosed HIV-1 positive patients with active TB, and 7 were HIV-uninfected healthy controls. The study design, the demographic and clinical characteristics of the study participants and results of the immunodiagnostic assays are shown in figure 1 and table 1, respectively.
Median numbers of circulating CD4+ T-cells were 182/µl (interquartile range-IQR 118) and 283/µl (IQR 226) in HIV-infected patients with and without active tuberculosis. In HIV-infected patients without active tuberculosis (n = 109), median numbers of circulating CD4+ T-cells were 64/µl (IQR 43), 179/µl (IQR 68) and 368/µl (IQR 206), in those with CD4+ T-cell counts of < 100 (n=10), 100-250 (n=33) and > 250 (n=66) cells/µl. HIV-viral load was inversely related to CD4+ T-cell counts as expected (p< 0.01) (table 1). Gender and age were equally distributed among patients from the different CD4+ T-cell count strata.

HIV-uninfected controls were younger (mean=25.7 years) than HIV-positive individuals (mean=33.4 years and 34.1 years in those with and without active tuberculosis). TST results were not available for 20/109 (18.3%) individuals from the group of HIV-infected persons without active tuberculosis, as individuals did not return for test reading. In patients with active tuberculosis, no TST was performed. All HIV-uninfected controls had a TST-reaction of ≥ 5 mm induration and in 6/7 (85.7 %) and 4/7 (57.2 %) HIV-uninfected individuals, the TST induration was ≥ 10 mm and ≥ 15 mm, respectively. Whereas in the control group TST results corresponded to the Gaussian distribution, HIV-infected patients were mostly TST negative or only had small TST-diameters (range 5 – 10mm) (table 1). In HIV-infected patients induration in the TST (mm) was directly correlated to numbers of circulating CD4+ T-cells (cells/µl) (spearmans rho=0.41; p-value<0.0001) (table 2). Thirty of 89 (33.7 %) HIV-infected patients without active tuberculosis had a TST induration of 0 mm.

QFT-G-IT test and T-Spot.TB results were positive in all (7/7) and 5/7 (70 %) of healthy controls, respectively. The HIV-infected individuals without tuberculosis were stratified according to CD4+ T-cells counts of > 250, 100-250 and < 100 cells/µl.

Frequencies of positive results for QFT-G-IT test declined significantly from 77.3 % to 66.7 % and 10 % (p<0.0001) respectively, while frequencies of positive test results
for T-Spot.TB test were 50 %, 57.6 % and 70 %, respectively, and were not significantly different from the frequency of positive T-Spot.TB test result in the group of HIV-uninfected controls (p=0.76) (table 1, figure 2).

Indeterminate test results in QFT-G-IT test and T-Spot.TB test were observed in a total of 3/109 (2.8 %) and 4/109 (3.7 %) of HIV-infected patients without active tuberculosis. Indeterminate test results were not related to levels of circulating CD4+T-cells (p>0.45). In HIV-infected patients, the concentration of IFN-γ in the tube with the specific antigens QFT-G-IT was directly correlated to numbers of circulating CD4+ T-cells (cells/µl) (Spearman’s rho= 0.38; p-value: 0.0001). In contrast, numbers of spots in the ESAT-6 or CFP-10 antigen wells in the T-Spot.TB test were not correlated to numbers of circulating CD4+ T-cells (cells/µl) (Spearman’s rho= 0.03; p-value: 0.77 and Spearman’s rho= 0.13; p-value: 0.21, respectively) (table 2).

Concordance between immunodiagnostic tests is shown in table 3. In the group of HIV-infected persons without active tuberculosis, overall concordant test results between the T-Spot.TB and the QFT-G-IT, between the T-Spot.TB and the TST and between the QFT-G-IT and the TST were observed in 60.8% (κ =0.17; SE = 0.09), 40.4% (κ =0.37; SE = 0.11) and 66.3% (κ =0.34; SE = 0.1), respectively. In HIV-infected individuals, overall test result concordance was best in the group of patients with >250 circulating CD4+ T-cells. In this group, highest concordance among test results was observed between the TST and the T-Spot.TB test 73.1% (κ =0.46; SE = 0.14) (table 3).

In patients with active tuberculosis, 17/19 (89 %) and 2/19 (11%) had a positive and indeterminate T-Spot.TB test result, respectively (figure 1). Results of the QFT-G-IT were positive in 13/19 (68%) and negative in 6/19 (32%) of those with active tuberculosis.
In 10 HIV seropositive individuals (10 male) from Italy, who were included as additional negative controls to document test specificity, the median patient age was 41.4 (IQR: 34-51) years, median number of circulating CD4+ T-cell counts was 480 (IQR 303.5-778) cells/microL. Results of the TST (> 5mm), the QTF-IT and the T-Spot.TB were positive in 0/10, 0/10 and 1/10 patients respectively. Indeterminate results in the QTF-IT and T-Spot.TB assays were observed in 0/10 in both tests (figure 1).

**Discussion**

Combating co-infection of HIV with *M. tuberculosis* is a major goal of health care interventions in Africa [26]. Tuberculosis is still the leading cause of HIV-related deaths in the developing world. Early identification and treatment of LTBI in individuals with HIV-infection is efficient [27] and important to reduce the spread of tuberculosis in Africa [28]. Detection of LTBI relies on immunodiagnostic tests, the TST and, more recently, IGRA, but comparative data on their performance in individuals from high incidence countries of tuberculosis are still limited [20, 21].

In this study, HIV-infected individuals from a country of high incidence of tuberculosis were enrolled to directly compare the diagnostic accuracy of the TST, the T-Spot.TB test and the QFT-G-IT test for the diagnosis of LTBI. Results from this comparison add substantially to the knowledge on the performance of these tests in individuals with different levels of immunodeficiency in HIV-infection. In a cohort of African individuals from a community in Uganda, where tuberculosis is highly endemic, we found in patients without active tuberculosis that 57.2 % of the HIV-uninfected individuals had a positive TST reaction of an induration of > 15mm, the recommended cut-off according to the ATS criteria [29] (the TST induration in all HIV-uninfected controls was > 5mm, which is the
recommended cut-off for HIV-infected individuals [29]). Only 12.5% of HIV-infected persons with < 100 circulating CD4+ T-cells exhibited a positive TST reaction, and the diameter of TST induration was significantly correlated to numbers of circulating CD4+ T-cells in the HIV-infected individuals, as previously demonstrated [30].

All HIV-uninfected individuals without active tuberculosis had a positive reaction to one of the ex vivo IGRA to test for memory against M. tuberculosis specific antigens, the QFT-G-IT or the T-Spot.TB test. Detection of high frequencies of LTBI in healthy individuals provide a basis to study M. tuberculosis specific immune responses in relation to the degree of immunosuppression in HIV-infected individuals from the same community [31].

We found a striking difference in the frequencies of positive immune responses assayed by the different tests in HIV-infected individuals without active tuberculosis at different levels of circulating CD4+ T-cell counts. When we compared the size of TST induration and the concentration of IFN-γ (in IU/ml) from the tubes containing the M. tuberculosis specific antigens ESAT-6, CFP-10 and TB7.7 in the QTF-G-IT, they correlated negatively to numbers of circulating CD4+ T-cells in individuals with HIV-infection. This correlation was not found for numbers of antigen specific spots in the T-Spot.TB test. When we evaluated frequencies of positive immune responses in groups of patients with different levels of immunodeficiency, again the TST and QFT-G-IT were dependent upon the level of circulating CD4+ T-cell counts, while this relationship was not observed for the T-Spot.TB test. The highest frequency of positive TST and QFT-G-T test results was observed in HIV-infected individuals with > 250 CD4+ cells/µl, indicating a dependency of these tests on the absolute number of circulating CD4+ T-cells. In contrast, frequencies of positive immune responses in the T-Spot.TB test were independent of the level of immunodeficiency. Rate of positive T-Spot.TB
results were comparable in HIV-infected patients with < 100, 100-250 and > 250 CD4+ T-cells/µl and HIV-uninfected controls.

Consequently, there is a high level of discordant results among immunodiagnostic tests for the detection of LTBI in HIV-infected individuals [21, 32-35]. In our study, overall concordance among the results of all three tests was not more than fair in HIV-infected individuals without active tuberculosis. Similar to the results of the above cited study from Germany, the lowest agreement among test results was observed between the two IGRA (κ = 0.17). Moderate test agreement was only observed among the TST and T-Spot.TB in patients with more than 250 circulating CD4+ T-cells (κ = 0.46).

Our results strongly support previous observations that show robustness of antigen specific immune responses in the Elispot assays, irrespective of T-cell stratification in patients with HIV-infection [20, 34, 36, 37]. In the largest comparable trial by Rangaka et al. who enrolled 74 HIV-infected individuals and 86 HIV-uninfected individuals in a township in the Cape region of South Africa, frequencies of T-Spot.TB results in HIV-infected individuals with > 250 CD4+ T-cells/µl were comparable to those of individuals with < 250 CD4+ T-cells/µl, while frequencies of QTF-G-IT test results were lower in patients with more advanced HIV-infection. However, results did not reach statistical significance in that study [21].

Recently, immunodiagnostic tests for LTBI were compared among HIV-infected individuals in Germany, a country of low tuberculosis incidence [34]. In agreement with the results obtained in this study, positive results of the T-Spot.TB were independent of the numbers of circulating CD4+ T-cells.
In contrast to these observations, the proportion of patients with a positive response to an in-house *M. tuberculosis* specific Elispot decreased with the numbers of circulating CD4+ cells in HIV-infected individuals from Dakar, Senegal [38]. This effect was particularly observed in individuals with circulating CD4+ T-cell counts of less than 50/µl, indicating that indeterminate or negative Elispot test results for the immunodiagnosis of LTBI in severely immunosuppressed individuals need to be interpreted with caution.

The low frequency of positive results in the QTF-G-IT test in persons with advanced CD4+ T-cell depletion in our cohort was not explained by a high number of indeterminate results in this group. Frequencies of indeterminate results were only observed in 3.6 % of HIV-infected individuals by either IGRA, independently of the circulating CD4+ T-cell count. These data differ from other studies where high numbers of indeterminate results were found by the QTF-G-IT assay [39, 40] or by “in house” tests of Elispot-assays [33, 41]. This is probably due to the fact that in the present study, the group of patients with CD4+ T-cells < 100/µl was relatively small to have a profound effect on the number of indeterminate results, compared to other studies, and the majority of the HIV+ individuals with circulating CD4+ T-cells above 100/µl did not have active disease. These individuals were, therefore, less prone to the immune deficiency induced by tuberculosis disease per se [42-44].

In the present study, we demonstrated that T-Spot.TB positive results were independent from CD4+ T-cell counts; differently from QTF-G-IT results. These data can be explained by the fact that the detrimental effects of lymphopenia associated with the late stages of HIV disease in reducing the efficiency of IGRA tests can be overcome or reduced by the Elispot procedure. In fact, Elispot involves a normalization of the input of PBMC used in the assays (always 250,000 PBMC are plated in each well). Therefore, although QTF-G-IT produces
easier logistic calculation in which the cell input is not normalized, it could suffer from a high rate of negative results among those HIV-infected patients with advanced immunodeficiency [19, 40, 45, 46] as also shown in this study.

The limitations of our study need to be addressed. While we performed the largest comparison of the three currently available immunodiagnostic tests for latent tuberculosis infection in HIV-infected individuals in a country of high tuberculosis incidence to date, numbers of HIV-infected individuals with less than 100 circulating CD4+ T-cells and numbers of HIV-uninfected controls were still relatively small in our study. However, the results are robust and are strengthened by different statistical methods, including a very high correlation between the magnitude of immune-responses in the TST and QFT-G-IT and the numbers of CD4+ T-cells in the blood. HIV-uninfected controls were younger than HIV-infected individuals, leading to a possible bias in frequencies of positive immune responses in this group. However, HIV-infected individuals were on average younger than 35 years and it is not expected that age influenced the test results substantially.

There are now growing numbers of studies reporting cross sectional data on frequencies of immune responses by IGRA in immunocompromised individuals without active tuberculosis. While these data provide important information, longitudinal studies on the predictive values of IGRA for the development of active tuberculosis are now urgently needed [47].

In conclusion, in HIV-infection, immune responses in the TST and QFT-G-IT are both strongly related to the degree of immunodeficiency, while the T-Spot.TB seems to function independently of the level of CD4+ T-cell depletion.
REFERENCES


Figures and tables

Figure 1: Study design and main outcomes

Figure 2: Frequencies of positive immune-responses in HIV-infected persons from Kampala, Uganda, with < 100, 100-250 and > 250 CD4 T cells and HIV-uninfected controls in the T-Spot.TB test (white bars), QuantiFERON-TB-Gold In-Tube test (black bars), and the tuberculin-skin-test (induration cutoffs: light grey bars ≥5mm, medium grey bars ≥10 mm, dark grey bars ≥15 mm)
Table 1: Characteristics and antimycobacterial immune responses in 109 HIV+ persons, 19 HIV+/TB coinfected patients and 7 healthy individuals in Kampala, Uganda.

Table 2: Correlation (*significant values) of numbers of circulating CD4+ T-cells with the skin induration in the TST (mm), IFN-γ concentration in the ESAT-6, CFP-10 and TB 7.7 containing tube in the QuantiFERON-Gold In-Ttube test and with numbers of spot forming cells (sfc) in response to incubation with ESAT-6 and CFP-10 in the T-Spot.TB test

Table 3: Concordance between three tests for the immunodiagnosis of latent tuberculosis infection in HIV-seropositive persons and healthy controls from Kampala, Uganda.
Table 1. Characteristics and antmycobacterial immune responses in 109 HIV+ persons, 19 HIV+/TB coinfected patients and 7 healthy individuals from Kampala, Uganda.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HIV+ patients</th>
<th>HIV+/TB patients</th>
<th>Healthy controls</th>
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<tr>
<td></td>
<td>CD4 cell counts &lt; 100 / µl n=10</td>
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<tr>
<td>Age (years, mean ± SD)</td>
<td>34.3 ± 6.6</td>
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<td>CD4 cell count / µl (median; IQR)</td>
<td>64; 43</td>
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<tr>
<td>HIV viral load (copies/ml, median; IQR)</td>
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<tr>
<td>HIV viral load (log copies/ml, mean ± SD)</td>
<td>5 ± 0.6</td>
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<td>TST (%)</td>
<td>8 (80.1)</td>
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<td>TST induration (median; IQR)</td>
<td>0; 2.5</td>
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<td>T-SPOT TB positive result (%)</td>
<td>7 (70.7)</td>
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<td>T-SPOT TB indeterminate result (%)</td>
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<td>QFT-G positive result (%)</td>
<td>1 (10)</td>
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<td>QFT-G indeterminate result (%)</td>
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<td>ESAT-6/CFP-10/TB7.7 (median; IQR)</td>
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<td>T-Spot positive result (%)</td>
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<tr>
<td>T-Spot indeterminate result (%)</td>
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<tr>
<td>ESAT-6 (median; IQR)</td>
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<tr>
<td>CFP-10 (median; IQR)</td>
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*Fisher’s exact test  
**Two independent samples t-test  
***Analysis of Variance  
****Wilcoxon-Mann-Whitney test  
*****Kruskal-Wallis test  
IQR: Inter-Quartile Range  
TST: tuberculin Skin Testing  

^ Comparison of the demographic and clinical characteristics among HIV+ patients (n= 109) with CD4 cell counts < 100 / µl, those with 100-250 / µl and those with > 250 / µl
Comparison of the demographic and clinical characteristics among HIV+ patients (n= 109) and HIV+/TB coinfected patients (n= 19)

Comparison of the demographic and clinical characteristics among HIV+ patients (n= 109), HIV+/TB coinfected patients (n= 19) and healthy controls (n= 7)
Table 2. Correlation (*significant values) of numbers of circulating CD4+ T-cells with the skin induration in the TST (mm), IFN-γ concentration in the ESAT-6, CFP-10 and TB 7.7 containing tube in the QuantiFERON-Gold-in tube test and with numbers of spot forming cells (sfc) in response to incubation with ESAT-6 and CFP-10 in the T.-Spot-TB test

<table>
<thead>
<tr>
<th>Correlation of CD4+ T-cells (cells/µl) with</th>
<th>Spearman’s rho</th>
<th>p-value</th>
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<tr>
<td>TST (mm)</td>
<td>0.41</td>
<td>&lt;0.0001*</td>
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<tr>
<td>QuantiFERON-Gold-in tube (IFN-γ)</td>
<td>0.38</td>
<td>0.0001*</td>
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<td>T-SPOT.TB ESAT-6 (sfc)</td>
<td>0.03</td>
<td>0.77</td>
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<td>0.13</td>
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<td>0.01</td>
<td>0.31</td>
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Table 3. Concordance between three tests for the immunodiagnosis of latent tuberculosis infection in HIV-infected persons and healthy controls from Kampala, Uganda.

<table>
<thead>
<tr>
<th>Concordance (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CD4 cell counts</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 100 / µl</td>
<td>100-250 / µl</td>
</tr>
<tr>
<td>T-SPOT.TB/QuantiFERON (k; SE)</td>
<td>44.4 (0.12; 0.16)</td>
<td>61.3 (0.16; 0.18)</td>
</tr>
<tr>
<td>TST/T-SPOT.TB (k; SE)</td>
<td>37.5 (0.09; 0.15)</td>
<td>68 (0.39; 0.18)</td>
</tr>
<tr>
<td>TST/QuantiFERON (k; SE)</td>
<td>71.4 (-0.17; 0.38)</td>
<td>55.6 (0.22; 0.15)</td>
</tr>
</tbody>
</table>

* Cohen’s kappa index, in order to evaluate agreement inter assay, could not be calculated because of the small number of persons.