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First independent evaluation of QuantiFERON-TB Plus performance

To the Editor:

Tuberculosis elimination requires an effective strategy to diagnose and treat people infected with *Mycobacterium tuberculosis* who would otherwise be at high risk of developing and transmitting active disease [1, 2]. The diagnostic tools for latent tuberculosis infection (LTBI) are the tuberculin skin test (TST) and the T-cell interferon-γ release assays (IGRAs). Two IGRAs are commercially available, QuantiFERON-TB Gold In-Tube (QFT-GIT) (Qiagen, Hilden, Germany) and T-SPOT.TB (Oxford Immunotec, Abingdon, UK). Compared to the TST, IGRAs offer operational advantages and higher specificity in the bacille Calmette–Guérin (BCG)-vaccinated population [3], and they are at least as sensitive for LTBI [4]. However, IGRAs have limitations: reduced sensitivity in children and immunocompromised subjects, including HIV-infected individuals [3, 4]; failure to discriminate between active tuberculosis and LTBI; and poor correlation with the risk of progression to active disease [3].

QuantiFERON-TB Plus (QFT-Plus) is a new generation of QTF-GIT [5] that includes an additional antigen tube (TB2). The TB1 tube contains ESAT-6- and CFP-10-derived peptides (TB7.7, present in QFT-GIT, has been removed), designed to elicit cell-mediated immune responses from CD4$^+$ T-helper lymphocytes. TB2 contains newly designed, shorter peptides able to stimulate interferon-γ production by both CD4$^+$ and CD8$^+$ T-cells. Previous studies have reported higher *M. tuberculosis*-specific CD8$^+$ T-cell responses in those with active tuberculosis disease compared to LTBI [6] and in those with recent *M. tuberculosis* exposure [7]. In addition, *M. tuberculosis*-specific CD8$^+$ T-cells producing interferon-γ have been detected in active tuberculosis subjects with HIV co-infection [8] and in young children with TB disease [9].

IGRAs primarily serve as tests for LTBI in clinical practice and, to date, they have no place in active tuberculosis diagnosis [10]. In the absence of a gold-standard test for LTBI against which to compare test accuracy, sensitivity and specificity are estimated using surrogate reference standards. Sensitivity is estimated in confirmed tuberculosis cases, while specificity is estimated in low-risk individuals with no known tuberculosis exposure in low-incidence settings. From November 2014 to September 2015, 119 consecutive individuals with active tuberculosis and 106 low-risk controls were enrolled and tested with QFT-Plus at different sites in Italy (Villa Marelli-Niguarda Hospital and San Raffaele Hospital, Milan; INMI, Rome; Sant'Orsola-Malpighi University Hospital, Bologna; and San Matteo University Hospital, Pavia) and one in the UK (Royal Free Hospital, London). The active tuberculosis group included adult patients (aged ⩾18 years) with microbiologically confirmed tuberculosis (either by nucleic acid amplification or culture), who had received <15 days of anti-tuberculosis drugs. HIV co-infected individuals and immunocompromised patients were not excluded. Additional information was collected regarding: results of QFT-GIT, BCG vaccination status, and demographic, clinical and microbiological data. Healthy controls were recruited among students at the time of enrolment at the University Vita-Salute in Milan (low-risk population, non-BCG vaccinated); all subjects were interviewed and screened for the absence of any risk factors for *M. tuberculosis* exposure. The study was approved by the ethical committees of all the participating centres and informed consent was obtained from study subjects before blood sample collection.

QFT-Plus kits were donated by Qiagen and used according to the manufacturer's instructions [5]. Experiments were performed in three different laboratories (San Raffaele Hospital, Sant'Orsola-Malpighi

University Hospital and the Royal Free Hospital) by trained staff. Levels of interferon-γ quantified by ELISA were converted to international units per millilitre using a standard curve constructed from the QFT-Plus Analysis Software provided with the kit. Test results were interpreted according to manufacturer's criteria [5]. Positivity of a single antigen tube (TB1 or TB2) was sufficient to record the QFT-Plus as positive.

Results are reported in table 1. In the active tuberculosis group, three (2.52%) out of 119 patients had indeterminate results. Among the 116 TB patients who had valid test results, 102 were positive, leading to a sensitivity in patients with active tuberculosis disease of 87.93% (95% CI 80.76–92.67%). 95 out of 116 were positive for TB1 and TB2, one patient was positive for TB1 only, and six patients were positive for TB2 only.

Of the 106 low-risk control subjects, three had a positive QFT-Plus test, giving a specificity of 97.17% (95% CI 92.01–99.03%) in this non-BCG-vaccinated population. Two of these three positive results were positive in one antigen tube only (one in the TB1 and one in the TB2), with interferon-γ values close to the cut-off.

Considering the quantitative data, the overall TB2 antigen interferon-γ response was higher than in TB1 (median (interquartile range): TB1 2.09 (0.83–6.52), TB2 2.88 (1–7.89) IU·mL^{-1}; Wilcoxon test p=0.0002). TB2 interferon-γ values were greater than TB1 for 63.16% (72 out of 114; for five patients, the exact value was not available) of the tuberculosis patients and 44.34% (47 out of 106) of the low-risk control group.

As the TB1 tube elicits a CD4$^+$ T-cell response while the TB2 tube elicits both a CD4$^+$ and a CD8$^+$ T-cell response, the difference between these tubes might provide a surrogate marker of the magnitude of CD8$^+$ T-cell responses. In active tuberculosis patients, we found that this difference was higher in smear-positive than smear-negative patients (Mann–Whitney test p=0.0135).

Results of the commercially available QFT-GIT were available for 73 of the active tuberculosis group subjects. The head-to-head comparison of the QFT-GIT and the QFT-Plus in this group demonstrated agreement in 68 results. Four patients scored positive only with QFT-Plus (three of them scored positive for the TB2 antigens only) and one patient was QFT-GIT positive but QFT-Plus indeterminate.

This cross-sectional, multicentre study is the first independent assessment of the performance characteristics of QFT-Plus. Estimated diagnostic sensitivity (88% including all patients) was higher than the upper confidence limit reported among the culture-confirmed, active tuberculosis patients in the most recent meta-analysis for QFT-GIT [3], suggesting that the QFT-Plus does indeed offer improved sensitivity. However, the performance characteristics of this test remain insufficient for use as a rule-out test for active tuberculosis.

Immunocompromised patients were not excluded from the study population. Four HIV/tuberculosis co-infected subjects all had a positive QFT-Plus test. Among the five patients with cancer undergoing chemotherapy, we found one negative result and one indeterminate. A negative result was also observed in

TABLE 1 QuantiFERON-TB Plus (QFT-Plus) performance characteristics in different study groups

| | Frequency | QFT-Plus result | | | Positive results in each tube | | IFN-γ concentration¶ IU·mL^{-1} | | TB2−TB1 IU·mL^{-1} |
		Indeterminate	Negative#	Positive#	TB1	TB2	TB1	TB2	
Low-risk controls	106	0	103 (97.17)	3 (2.83)	2	1	0.1 (0.09–0.13)	0.11 (0.09–0.13)	0 (−0.01–0.01)
Active TB	119	3	14 (12.07)	102 (87.93)	96	101	2.09 (0.83–6.52)	2.88 (1–7.89)	0.14 (−0.13–0.79)
Sex									
Male	72	1	7 (9.86)	64 (90.14)	59	64	2.08 (0.86–6.38)	2.91 (1.17–7.62)	0.23 (−0.14–0.88)
Female	47	2	7 (15.56)	38 (84.44)	37	37	2.09 (0.71–7.03)	2.85 (0.88–7.80)	0.11 (−0.11–0.50)
Smear									
Negative	65	1	12 (18.75)	52 (81.25)	51	52	2.12 (0.89–9.51)	2.69 (1.01–9.66)	0.05 (−0.17–0.55)
Positive	54	2	2 (3.85)	50 (96.15)	45	49	2 (0.67–6.04)	3.26 (0.92–6.31)	0.29 (0–1.16)
Localisation									
PTB	79	3	9 (11.84)	67 (88.16)	62	66	1.93 (0.57–6.04)	2.82 (0.75–6.22)	0.26 (−0.12–0.80)
EPTB	40	0	5 (12.5)	35 (87.5)	34	35	2.29 (1.23–10)	2.95 (1.15–10)	0.06 (−0.17–0.46)
BCG									
Negative	6	0	2 (33.33)	4 (66.67)	4	4	1.15 (0.33–1.86)	1.47 (0.4–2.75)	0.07 (0.01–0.44)
Positive	54	0	4 (7.41)	50 (92.59)	44	50	2.01 (0.9–6.57)	2.79 (1.02–8.2)	0.14 (−0.20–0.65)

Data are presented as n, n (%) or median (interquartile range). IFN: interferon; TB: tuberculosis; PTB: pulmonary tuberculosis; EPTB: extrapulmonary tuberculosis; BCG: bacille Calmette–Guérin. #: percentages are based on the total number of valid tests, excluding indeterminate results.

a patient with meningeal tuberculosis receiving prednisone treatment. The sensitivity of the test slightly increases to 88.79% (95% CI 81.41–93.47%), when excluding immunocompromised patients from the data.

Six patients had a positive result by TB2 only. All these patients had smear-positive pulmonary tuberculosis (PTB) and four of them had more than three cavities. Moreover, we found that the difference between TB2 and TB1 was higher in smear-positive than smear-negative patients. Considering the difference between the two antigen tubes as a surrogate marker of the magnitude of CD8^{+} T-cell responses, the last finding is in agreement with what observed in flow-cytometry studies. *M. tuberculosis*-specific CD8^{+} T-cells correlate with an increased mycobacterial load and are more frequently found in smear-positive PTB individuals [11].

Finally, in the QFT-Plus test, we observed a significant difference in interferon-γ response between the two tubes; however, whether this difference is due to the additional CD8^{+} T-cell stimulation has not yet been proven. A previous study analysed the contribution of CD4^{+} and CD8^{+} antigen-specific responses to novel peptides with promiscuous human leukocyte antigen II binding capabilities derived from *M. tuberculosis* genes over-expressed in an *in vitro* macrophage model [12]. In the study by Losi *et al.* [12], a small subgroup of active tuberculosis patients had a significantly higher frequency of peptide-specific interferon-γ CD69^{+} CD4^{+} and interferon-γ CD69^{+} CD8^{+} T-cells compared to controls. Flow-cytometry studies in a larger population need to be carried out to confirm the presence of CD8^{+} response in the newly added tube.

Despite progress being made in global tuberculosis control, the current incidence decline is far from what is needed to achieve tuberculosis elimination by the year 2050. Global tuberculosis control could be enhanced by the identification and treatment of symptom-free people who are latently infected with *M. tuberculosis* [1, 2]. Of particular relevance for low-incidence countries would be the development of rapid and accurate tests able to detect, among subjects with LTBI, those at higher risk of developing active tuberculosis disease who would most benefit from preventive treatment [13].

QFT-Plus is a new IGRA that includes a set of peptides designed to stimulate *M. tuberculosis*-specific CD8^{+} T-cells. The new test shows a high specificity and retains the same sensitivity as the previous version. In addition, the increased interferon-γ release by combined stimulation of CD4^{+} and CD8^{+} T-cells observed in the newly added antigen tube (TB2) might be advantageous for improving the assay's accuracy in patients with low CD4^{+} T-cell counts.

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QuantiFERON-TB Plus improves sensitivity for active TB and maintains high specificity among unvaccinated controls http://ow.ly/XjYPK

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