# QIAreach QuantiFERON-TB for the diagnosis of *Mycobacterium tuberculosis* infection



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*To the Editor*:

Timely diagnosis and treatment to prevent tuberculosis (TB) transmission and the consequent replenishment of the TB infection reservoir are essential for the control and elimination of TB worldwide [1], ultimately contributing the World Health Organization (WHO)'s End TB goals [2].

Currently, the WHO guidelines recommend the use of either a tuberculin skin test (TST) or an interferon-γ release assay (IGRA) to detect TB infection in exposed household members older than 5 years old [3]. IGRAs have been demonstrated to overcome some drawbacks of TST, leading to advantages for the patient, the physician and the laboratory [4].

Two types of commercial IGRA tests are currently available: T-SPOT®TB (Oxford Immunotec, Abingdon, UK) and QuantiFERON®-TB Gold Plus (QFT-Plus; QIAGEN, Hilden, Germany) [5]. QFT-Plus is a fourth generation ELISA-based IGRA. This test uses antigens able to elicit both CD8 and CD4 T-cell responses, enabling a more comprehensive assessment of cell-mediated immune response to TB infection [6–8]. Although QFT-Plus represents a reliable technology for the diagnosis of TB infection [9], ELISA-based IGRAs are multi-step, time consuming tests, requiring extensive laboratory infrastructure and trained technical personnel [10].

To fulfil the need for access to prompt and reliable diagnosis of TB infection, even in challenging and remote settings, a new lateral flow immunoassay has recently been developed. The QIAreach® QuantiFERON-TB (QIAreach QFT; QIAGEN, Hilden, Germany) is a semi-automated assay that utilises a nanoparticle technology to measure the level of IFN-γ in plasma released by both CD4 and CD8 T-cells using the same TB2 tube of QTFplus. This new technology allows to detect TB infection using only a single blood collection tube (TB2), with single use cartridges (eStick) on a portable platform (eHub), capable of performing up to eight tests and providing a final qualitative result (positive or negative) within 20 min [11, 12].

We evaluated the clinical performance of QIAreach QFT in detecting TB infection in a HIV negative population (table 1) with microbiologically confirmed pulmonary TB, either by nucleic acid amplification or sputum culture, and healthy low-risk volunteers. The total number of specimens tested was 304.

In absence of a gold-standard test for TB infection, we assessed QIAreach QFT test accuracy, sensitivity, and specificity against surrogate reference standards. Sensitivity was estimated in confirmed TB cases, while specificity was assessed in low-risk individuals with no known TB exposure, in a low-incidence setting. From January to May 2021, 174 healthy low-risk individuals accepted to participate to the study at the San Raffaele Research Hospital, Milan, Italy. These were students enrolled in a private medical school in a low incidence setting, without history of exposure to TB or travel to high TB incidence countries. The samples collected were immediately incubated at 37°C for 16–24 h before being tested with both QFT-plus and QIAreach QFT. Moreover, 130 samples previously collected from adult (aged ⩾18 years) active TB cases (defined as specified above) at different time points of anti-TB treatment and preserved at −80°C were analysed. This cohort included samples collected at 0, 14, 30, 60 and 180 days from the start of anti-TB treatment.

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**QIAreach QFT demonstrated a good performance in diagnosing TB infection. This tool may offer technical and operational benefits over more complex IGRA ELISA-based assays, granting timely TB diagnosis in decentralised settings with limited infrastructure.** https://bit.ly/3n6Dxll

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**TABLE 1** Comorbidities of active tuberculosis (TB) population and diagnostic performance of QIAreach QFT assay using QFT-Plus assay as a reference standard

| Comorbities | Positive | Negative | Not known |
|---|---|---|---|
| HIV status | 0 | 130 | 0 |
| Chronic liver failure | 6 | 83 | 41 |
| Chronic renal failure | 1 | 88 | 41 |
| Chronic lung disease | 1 | 88 | 41 |
| Haematological malignancies | 2 | 87 | 41 |
| Diabetes | 6 | 83 | 41 |
| **Diagnostic performance** | **Active TB, untreated (n = 48)** | **Active TB, treated (n = 82)** | **Healthy controls (n = 174)** |
| QIAreach QFT (+) | 45 (93.75%) | 78 (95.12%) | 4 (2.29%) |
| QIAreach QFT (−) | 3 (6.25%) | 4 (4.87%) | 170 (97.70%) |
| QFT-Plus (+) | 43 (89.58%) | 77 (93.91%) | 0 (0%) |
| QFT-Plus (−) | 5 (10.42%) | 5 (6.09%) | 174 (100%) |

Both QFT-plus and QIAreach QFT were performed according to manufacturers' instructions from the same blood sample, QIAreach QFT using plasma harvested from TB2 tube. Specimens with discordant results and errors were re-tested with both tests using different ELISA and eStick batches. Ethical approval was obtained by the institutional review board of San Raffaele Research Hospital in Milan, Italy (protocol number: CE: 142/INT/2016). All patients and healthy controls agreed to the study by signing an informed consent form.

To ensure high accuracy in evaluating the test's performances, the sensitivity was calculated by stratifying patients into two groups: 1) patients recruited at baseline and at 14 days from starting anti-TB treatment (untreated group); and 2) patients recruited at 30, 60 and 180 days from the start of anti-TB treatment (treated group).

Sensitivity of QIAreach QFT for detection of TB infection was 93.7% (two-sided 95% CI 82.2–98.7%) and 95.1% (two-sided 95% CI 88–98.7%), respectively for the untreated and treated groups. The specificity was 97.7% (two-sided 95% CI 94.2–99.4%). The overall percentage agreement with QFT-Plus was 95.7% (two-sided 95% CI 92.8.3–97.7%) with a Cohen's κ of 0.96. The positive percent agreement (sensitivity) *versus* QFT plus was 99.1% (two-sided 95% CI 95.4.7–99.9%) and a negative percent agreement (specificity) *versus* QFT Plus was 93.4% (two-sided 95% CI 88.9–96.6%). The QIAreach QFT overall error rate was 1.3% (4/304).

31 specimens had uncorrected TB2 values without nil subtraction below 1 IU·mL$^{-1}$ (ranging from 0.35 to 0.99 IU·mL$^{-1}$) on QFT-plus and all tested positive on QIAreach QFT, while 97% of specimens that tested negative on QIAreach QFT had TB1-nil and/or TB2-nil values below 0.28 on QFT-plus.

This study is one of the first conducted on a large cohort and supports earlier reports of a good clinical performance of QIAreach QFT in diagnosing TB infection [11]. With an overall sensitivity of 94.6%, QIAreach QFT performance is comparable to that reported here of QFT-plus as well as that previously described in the most recent meta-analysis [9]. Agreement rates with the established QFT-Plus were remarkably high.

Four cases from healthy low-TB risk individuals group resulted positive on QIAreach QFT. One of them had a TB2-nil value close to cut-off point of 0.35 IU·mL$^{-1}$ on QFT-Plus. The individuals from which they were collected presented a normal white blood cell count when sampled and did not report a history of autoimmune diseases or of any condition that could cause false-positive or false-negative results according to QFT Plus and QIAreach QFT information for use.

QIAreach QFT may offer several technical and operational benefits over more complex IGRA ELISA-based assays. First, it could simplify the overall workflow, also allowing the implementation of the test with minimal training in decentralised settings with limited infrastructure, where ELISA may not be readily available. In addition, this test addresses one of the main limitations to the wide implementation of IGRA in infants and young children, reducing the volume of blood required to perform the test from

4 mL to 1 mL. Finally, this assay can be rapidly performed without need for sample batching, since each eStick can be run independently with a capacity of up to eight samples per eHub, providing a digital readout of the results in a maximum of 20 min. This could significantly improve the number of samples that can be tested per day in comparison to ELISA-based assays.

Further studies are needed to accurately evaluate the assay performances in different settings and study populations, including immunocompromised patients, people living with HIV and children. The analysis of QIAreach QFT performance in a cohort of paediatric patients with central nervous system tuberculosis would also be relevant, as in this population IGRA tests have been found to be less sensitive in identifying TB infection [13].

Nonetheless, these technical characteristics combined with the identified clinical performance make this technology of relevance in areas where the low level of infrastructure and the limited number of skilled laboratory technicians constitute major barriers for the implementation of IGRA technologies.

Improving accessibility to TBI testing could increase acceptability of preventive therapy, which is still poor in several communities [14]. Therefore, the use of QIAreach QFT in remote settings could potentially favour TB elimination by indirectly reducing the number needed to treat.

**Francesca Saluzzo[1,2], Paola Mantegani[1,2], Valeria Poletti de Chaurand[1] and Daniela Maria Cirillo[1]**

[1]Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy. [2]These authors contributed equally to this work.

Corresponding author: Daniela Maria Cirillo (cirillo.daniela@hsr.it)

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Conflict of interest: All authors declare donation of free QIAreach tests to the unit. In addition, D.M. Cirillo declares an unrestricted research grant from Qiagen, which ended in 2020, for therapy monitoring to study QTF as a marker. The grant (22000 E) was awarded to IRCCS Ospedale San Raffaele. The company had no role in the study design.

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