EUROPEAN RESPIRATORY journal

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Research letter

QIAreach™ QuantiFERON®-TB for the diagnosis of *M. tuberculosis* infection

Francesca Saluzzo, Paola Mantegani, Valeria Poletti de Chaurand, Daniela Maria Cirillo

Please cite this article as: Saluzzo F, Mantegani P, Poletti de Chaurand V, *et al*. QIAreach™ QuantiFERON®-TB for the diagnosis of ***M. tuberculosis*** infection. *Eur Respir J* 2021; in press (https://doi.org/10.1183/13993003.02563-2021).

QIAreach™ QuantiFERON®-TB for the diagnosis of *M. tuberculosis* infection

Francesca Saluzzo[1°] & Paola Mantegani[1°], Valeria Poletti de Chaurand[1], Daniela Maria Cirillo[1#]

[1] Emerging Bacterial Pathogens Unit, Division of Immunology, Transplantation and Infectious Diseases, IRCCS San Raffaele Scientific Institute, Via Olgettina 60, 20132, Milan, Italy.

° These authors contributed equally to this work.

#Address correspondence to:

Daniela Maria Cirillo

Div. of Immunology, Transplantation and Infectious Diseases

San Raffaele Scientific Institute

San Gabriele Building

Via Olgettina, 58

20132 Milano, ITALY

Telephone: +39 02 2643 9121

Fax: +39 02 2643 5183

Email: cirillo.daniela@hsr.it

Disclosures: 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.

Key words: Tuberculosis diagnosis, Point Of care Testing, Active tuberculosis, QIAreach(TM) QuantiFERON-TB; QuantiFERON®-TB Gold Plus.

Timely diagnosis and treatment to prevent Tuberculosis (TB) transmission and the consequent replenishment of TB infection (TBI) reservoir, are essential to control and eliminate TB worldwide [1], finally contributing to achieve the World Health Organization's (WHO) End TB goals [2].

Currently, the WHO guidelines recommend the use of either a Tuberculin Skin Test (TST) or an Interferon-gamma release assay (IGRA) to detect TB infection in exposed household members older than 5 years old [3]. The IGRAs 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 4th generation enzyme-linked immunosorbent assay (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 TBI [9], ELISA-based IGRAs are multi-step, time consuming tests, requiring extensive laboratory infrastructure and trained technical personnel [10].

To fulfill the need of granting 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 utilizes 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 minutes[11, 12].

We evaluated the clinical performance of QIAreach QFT in detecting TB infection in a HIV negative population (Table 1A) 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 hours 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 (ATT) and preserved at -80°C were analyzed. This cohort included samples collected at 0, 14, 30, 60, and 180 days from the start of ATT.

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.

To ensure high accuracy in evaluating the test's performances, the sensitivity was calculated by stratifying patients in two groups, (i) patients recruited at baseline and at 14 days from the starting ATT (untreated group), and (ii) patients recruited at 30, 60 and 180 days from the start of ATT (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) vs QFT plus was 99.1% (two-sided 95% CI 95.4.7-99.9%) and a negative percent agreement (specificity) vs QFT Plus was 93.4% (two-sided 95% CI 88.9% to 96.6%). QIAreach QFT overall error rate was 1.3% (4/304).

Thirty-one specimens had uncorrected TB2 values without Nil subtraction below 1 IU/ml (ranging from 0.35 to 0.99) 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 [13]. With an overall sensitivity of 94.6% the QIAreach QFT performances are comparable to those reported here of QFT-plus as well as those 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 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 decentralized 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 1ml. 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 minutes. 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 pediatric 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 TBI [14].

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, still poor in several communities [15]. Therefore, the use of QIAreach QFT in remote settings could favor TB elimination by indirectly potentially reducing the number of need to treat.

Acknowledgements:

QIAGEN (Hilden, Germany) provided the QIAreachTM QuantiFERON-TB test kits free of charge, and provided an unrestricted research grant (ended in 2020) for therapy monitoring to study QTF as marker to IRCCS Ospedale San Raffaele. QIAGEN had no role in study design, collection, analysis and interpretation of data.

A

TB Active cohort comorbities	**+**	**-**	**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**

B

	Active TB (untreated) N 48	**Active TB (treated) N 82**	**Healthy control 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%)**

Table1: (A) Comorbidities of Active TB population; (B) Diagnostic performance of QIAreach QFT assay using QFT-Plus assay as a reference standard

Bibliography

1. Harries A, Kumar A, Satyanarayana S, Takarinda K, Timire C, Dlodlo R. Treatment for latent tuberculosis infection in low- and middle-income countries: progress and challenges with implementation and scale-up. *Expert review of respiratory medicine* Expert Rev Respir Med; 2020; 14: 195–208.

2. Chakaya JM, Harries AD, Marks GB. Ending tuberculosis by 2030—Pipe dream or reality? *International Journal of Infectious Diseases* Elsevier; 2020; 92: S51–S54.

3. Zhou G, Luo Q, Luo S, Teng Z, Ji Z, Yang J, Wang F, Wen S, Ding Z, Li L, Chen T, Abi M-E, Jian M, Luo L, Liu A, Bao F. Interferon-γ release assays or tuberculin skin test for detection and management of latent tuberculosis infection: a systematic review and meta-analysis. *The Lancet Infectious Diseases* Elsevier; 2020; 20: 1457–1469.

4. Lalvani A. Diagnosing tuberculosis infection in the 21st century: New tools to tackle an old enemy. *Chest* Chest; 2007; 131: 1898–1906.

5. World Health Organization. WHO guidelines on tuberculosis infection prevention and control, 2019 update. 2019.

6. Barcellini L, Borroni E, Brown J, Brunetti E, Campisi D, Castellotti PF, Codecasa LR, Cugnata F, Di Serio C, Ferrarese M, Goletti D, Lipman M, Rancoita PMV, Russo G, Tadolini M, Vanino E, Cirillo DM. First evaluation of QuantiFERON-TB gold plus performance in contact screening. *European Respiratory Journal* 2016; .

7. Petruccioli E, Vanini V, Chiacchio T, Cuzzi G, Cirillo DM, Palmieri F, Ippolito G, Goletti D. Analytical evaluation of QuantiFERON- Plus and QuantiFERON- Gold In-tube assays in subjects with or without tuberculosis. *Tuberculosis* 2017; .

8. Cirillo DM, Barcellini L, Goletti D. Preliminary data on precision of QuantiFERON-TB Plus performance. *European Respiratory Journal* [Internet] European Respiratory Society; 2016 [cited 2021 Sep 23]; 48: 955–956Available from: https://erj.ersjournals.com/content/48/3/955.

9. Sotgiu G, Saderi L, Petruccioli E, Aliberti S, Piana A, Petrone L, Goletti D. QuantiFERON TB Gold Plus for the diagnosis of tuberculosis: a systematic review and meta-analysis. *Journal of Infection* Elsevier; 2019; 79: 444–453.

10. Faust L, Ruhwald M, Schumacher S, Pai M. How are high burden countries implementing policies and tools for latent tuberculosis infection? A survey of current practices and barriers. *Health Science Reports* John Wiley & Sons, Ltd; 2020; 3: e158.

11. Stieber F, Howard J, Manissero D, Boyle J, Ndunda N, Love J, Yang M, Schumacher A, Uchiyama R, Parsons S, Miller C, Douwes H, Mielens Z, Laing T, Nikolayevskyy V. Evaluation of a Novel Lateral Flow Nanoparticle Fluorescence Assay for TB infection diagnosis. *Int J Tuberc Lung Dis.* 2021; in Press.

12. Fukushima K, Akagi K, Kondo A, Kubo T, Sakamoto N, Mukae H. First clinical evaluation of the QIAreachTM QuantiFERON-TB for tuberculosis infection and active pulmonary disease. *Pulmonology* Elsevier Doyma; 2021; .

13. Francis S, Jenny H, Davide M, Jeffrey B, Nduku N, Joanna L, Mei Y, Andrew S, Robin U, Sean P, Chris M, Harmony D, Zoe M, Tim L, Vladyslav N. Evaluation of a Novel Lateral Flow Nanoparticle Fluorescence Assay for TB infection diagnosis. .

14. Roy RB, Thee S, Blázquez-Gamero D, Falcón-Neyra L, Neth O, Noguera-Julian A, Lillo C, Galli L, Venturini E, Buonsenso D, Götzinger F, Martinez-Alier N, Velizarova S, Brinkmann F, Welch SB, Tsolia M, Santiago-Garcia B, Krüger R, Tebruegge M. Performance of immune-based and microbiological tests in children with tuberculosis meningitis in Europe: a multicentre Paediatric Tuberculosis Network European Trials Group (ptbnet) study. *The European Respiratory Journal* [Internet] European Respiratory Society; 2020 [cited 2021 Oct 7]; 56Available from: /pmc/articles/PMC7330130/.

15. WHO. Global Tuberculosis Report 2020. World Health Organization.