



Short Communication

Sample Collection Procedures and Impact on QuantiFERON®-Gold in Tube Results

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Introduction

Accurate diagnosis of infection with *Mycobacterium tuberculosis* is important in low prevalence populations, such as healthcare workers, subjected to serial- or mass entry screen testing. There is no gold standard laboratory test for detecting latent tuberculosis infections (LTBI). Interferon- γ (IFN- γ) release assays (IGRAs) are in vitro immunodiagnostic tests that measure the effector T cell-mediated IFN- γ response to synthetic *Mycobacterium tuberculosis*-specific polypeptides. When compared to the tuberculosis skin test (TST) IGRAs, including the QuantiFERON-TB Gold In-Tube (QFT-GIT, QIAGEN GmbH, GER), have been shown to reduce the number of false positive LTBI diagnoses and thus substantially lower follow-up costs [1,2].

Recent experience with the QFT-GIT test has shown that despite a high negative predictive value (NPV), a low positive test result (<1 IU/ml) is not necessarily a reliable indication of LTBI [3]. It has been shown that certain pre-analytical factors influence IGRA results [4,5]. For example, incubation delays have been shown to be of particular concern in regard to the sensitivity of QFT-GIT [6]. Thus, it is critical that the pre-analytical variables of IGRAs be understood and are highly standardized as possible. To further improve the knowledge and thereby improve interpretation of QFT-GIT results, particularly results near the cut-off value for a positive test, we compared two different methods of patient blood sampling in parallel. We studied a total of 107 persons who were screened for LTBI using QFT-GIT sampled directly at point of care (PoC) and compared the results with a laboratory based QFT-GIT blood transfer method. We show the degree to which the results obtained from the two different methods can differ. We demonstrate that pre-analytical treatment of PoC-QFT blood sampling can result in significantly higher values for individual tubes, and hence, we provide evidence that pre-analytical factors do have an impact on results. Hence they should be considered when evaluating/validating the sampling and transportation procedures for the QFT-GIT test.

Methods

The results of PoC-drawn QFT-tubes and QFT-tubes obtained by transferring blood collected in lithium-heparin (Li-H) monovettes to QFT-tubes in the laboratory were compared.

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Study subjects

107 healthy healthcare workers (HCWs) were screened for LTBI as part of their compulsory initial medical examination. After consent was obtained, a secondary blood Li-H sample was taken to be included in this study. Each subject underwent consequently parallel QFT-GIT testing, with 2 sets of blood collection tubes drawn simultaneously: One set was drawn at the PoC directly into QFT-GIT tubes (PoC-QFT) and immediately transported via the hospital pneumatic delivery system or the hospital manual transportation service to the laboratory. The secondary blood sample was simultaneously drawn into a single Li-H tube and transported via the hospital pneumatic delivery system to the laboratory.

Experimental procedure

All tests were performed according to the QFT-GIT package insert. In summary: One milliliter of blood was drawn at PoC directly into each of the QFT-GIT blood collection tubes. The QFT-GIT tubes are either coated with saline (negative control, nil), the mitogen phytohemagglutinin (positive control, mitogen) or ESAT-6/CFP-10/TB 7.7 peptides (*M. tuberculosis*-specific antigens, MTB-ag).

Additionally, blood was collected in a 7.5-ml S-Monovette® 7.5ml LH (Sarstedt, Germany). Immediately upon arrival in the laboratory, 1 ml was transferred to each QFT-GIT tube by a laboratory technician. LT-QFT tubes were shaken gently by inverting 10 times according to the manufacturer's package insert and were immediately incubated at 37°C for 16-24 h. Plasma was separated and stored at room temperature for up to 1 h before a quantitative enzyme-linked immunosorbent assay (ELISA) for IFN- γ (measured in International Units (IU) IFN- γ /ml) was performed on a DSX automated system (Dynex Technologies, Chantilly, VA). Interpretation of results was done by the software provided by the QFT-GIT manufacturer. According to the manufacturer, a positive result was defined as a TB response (TB antigen minus nil) value of ≥ 0.35 IU/ml and $\geq 25\%$ of the nil value.

Statistics

The agreement between the two sets of results obtained with the two procedures was quantified by using Cohen's kappa coefficient. The differences in means were compared by using the paired student t-test.

Results

In 98% (105/107) of the subjects, the qualitative results were congruent between PoC-QFT and LT-QFT (kappa=0.79). For 1.9% (2/107) discordant results were obtained. In both instances the PoC-QFT gave a positive result while the correspondent LT-QFT remained negative. Repeated testing one week later with the LT-QFT method and an additional IGRA test, T.SPOT-TB (Oxford Immunotec, UK) yielded both negative results for both subjects, thus indicating false positive results from the original PoC-QFT.

Overall, when comparing the IFN- γ values of the mitogen tubes no significant difference between the results of PoC-QFT tube and LT-QFT-tube was detected. However, when comparing the results between PoC- and LT-QFT for the nil and MTB-ag tubes, a

statistically significant difference ($p < 0.05$) could be observed (Figure 1). In general, the PoC-QFT assays resulted in higher IFN- γ values than their corresponding LT-QFT assays. Although this difference is mostly equalized in the final QFT-GIT result (MTB-ag minus nil) it demonstrates that -probably pre-analytic- treatment of PoC-QFT tubes can result in significantly higher values for the individual tubes.

Discussion

In this study, we investigated the role of a QFT-PoC blood sampling procedure with subsequent transportation to the laboratory and a laboratory-based QFT-tube blood transfer procedure in the reproducibility of the overall QFT-GIT assay. We found that the final QFT-GIT result agreement (MTB-ag minus nil) is only little (2/107) affected by the differential approaches. However, we found statistically significant differences when comparing the IFN- γ values of individual tubes. A recent study by Gaur and colleagues [7] demonstrated the influence of blood volume and tube shaking on QFT-GIT results. Compared with gentle shaking, the authors showed a significant increase in median IFN- γ levels in the nil and MTB-ag tubes with vigorous shaking. Our finding that PoC-drawn QFT-GIT also showed significantly increased nil and MTB-ag values might represent the influence of the transportation system (pneumatic delivery) that most probably inflicts also rather vigorous additional shaking to the delivered tubes. The final impact on the final result (MTB-ag minus nil) of such an additional tube shaking on QFT-GIT results would strongly depend on whether the MTB-ag and nil tubes were shaken identically. Our results suggest that there is a higher possibility of PoC-QFT tests to result in either a false-positive or false-negative QFT-GIT result due to the higher possibility of differential pre-analytical treatment of the nil and TB antigen tubes, especially when the result is near the assay cutoff value of 0.35 IU/ml.

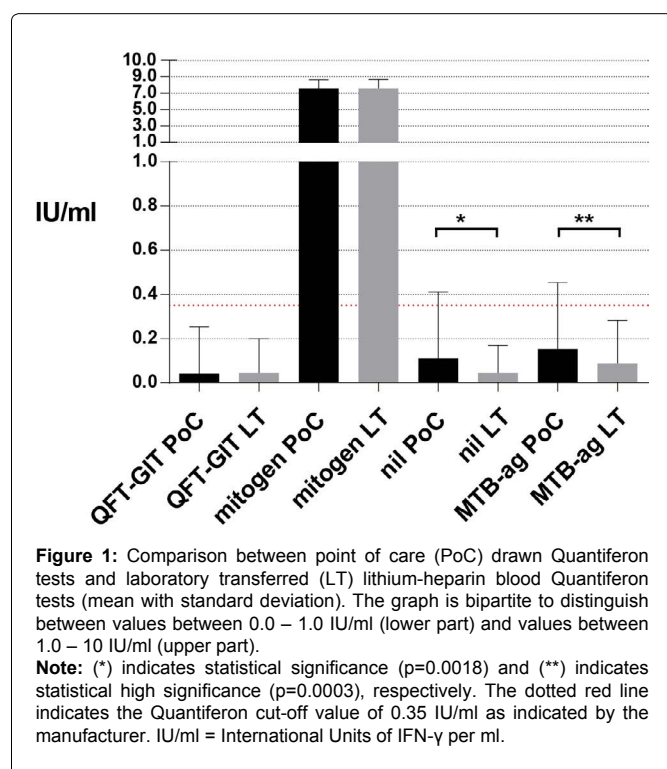
Together with recent reports of suspicious low positive results or unexplained variability (conversions/reversions) around the QFT-GIT's cut-off value (0.35 IU/ml) [5], this study provides further data regarding the importance of pre-analytical factors for the QFT-GIT assay. However, the contribution of pre-analytical variables to IGRA variability has to be further assessed. A minimization of pre-analytical variables is definitely worthwhile. Our study helps defining such variables and might be useful to improve the process for LTBI screening from patient to laboratory. We therefore believe that the LT-QFT method can eliminate pre-analytical variables such as unequal filling, shaking and uncontrollable transportation effects on the QFT-tubes and thereby improve the reproducibility of the QFT-GIT assay. The LT-QFT method also prevents both the inadvertent use of expired QFT-tubes by PoC personnel. Our study gains further relevance with regard to the introduction of the new QuantiFERON-TB Gold Plus (QFT-Plus, QIAGEN GmbH, Ger) which contains besides the nil tube, the mitogen tube and the MTB-ag tube (detecting CD4+ IFN- γ production) an additional fourth tube, MTB-ag2, detecting IFN- γ production of CD8+ cytotoxic T lymphocytes. ESAT-6 and CFP-10 specific CD8+ T lymphocytes are described as being more frequently detected in subjects with active TB disease versus LTBI, and may be associated with a recent MTB exposure [8]. The use of four instead of three individual QFT-tubes and especially the direct comparison of two tubes (MTB-ag vs. MTB-ag2) in this new assay format requires consequently a further minimization of possible pre-analytical biases through unequal handling of the individual tubes.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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