



Impact of blood volume, tube shaking, and incubation time on the reproducibility of QuantiFERON-TB Gold In-Tube assay



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Abstract

Serial testing studies with interferon gamma release assays (IGRAs) are reporting challenges with reproducibility of results in health care workers (HCWs). We hypothesized the existence of unrecognized preanalytical sources of variability that contribute to irreproducible results. In this study we investigated the effect of blood volume (0.8, 1.0, and 1.2 ml), tube shaking (gentle versus vigorous) and duration of incubation (16, 20, and 24 hr) on the QuantiFERON-TB gold in-tube (QFT-GIT) results in 48 (15 infected and 33 uninfected) donors. The median TB response (TB Ag - Nil) was significantly higher with 0.8 ml blood (0.87 IU/ml) compared to 1.0 ml (0.84 IU/ml; $P = 0.002$) and 1.2 ml (0.44 IU/ml; $P = 0.0007$) in subjects with TB infection. Compared to 0.8 ml, there was a higher rate of false negative results with 1.0 ml (33.3%; $P = 0.15$) and 1.2 ml (46.7%; $P = 0.02$) blood in infected subjects. Blood volume did not significantly change positivity rates in uninfected controls ($P > 0.05$). Compared to gentle shaking, vigorous shaking increased the median IFN- γ response in Nil (0.04 vs. 0.05 IU/ml; $P = 0.007$) and TB Ag (0.06 vs. 0.11 IU/ml; $P = 0.05$ value) tube but there was no significant change in TB responses (0.01 vs. 0.01, $P = 0.7$). Except for TB response at 16 hr vs. 24 hr in uninfected donors ($P = 0.04$), the duration of incubation did not have a significant impact on TB response or the proportion of positive results in uninfected and infected donors ($P > 0.05$). This study identifies blood volume and tube shaking as preanalytical sources of variability which require standardization in order to improve the quality of QFT-GIT results.

Methods

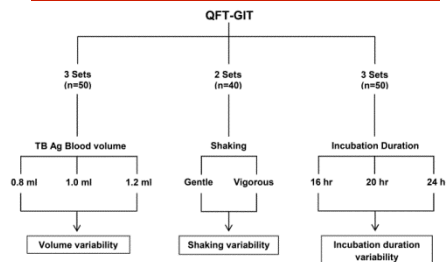


Fig 1. Schematic representation of experimental design. Tests were performed as per manufacturer's instruction except the modification tested in present study.

Results

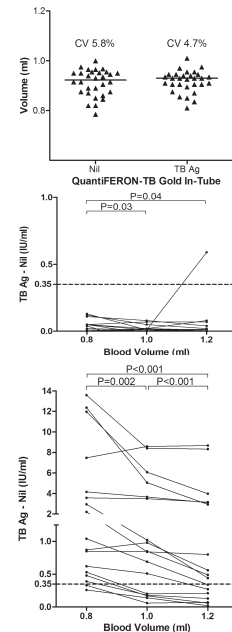


Fig 2. Effect of variable blood volume on QuantiFERON-TB Gold In-Tube (QFT-GIT) results. (A) Distribution of blood volume drawn in Nil and TB Ag tube (n=30). TB response (TB Ag-Nil) values for 33 uninfected (B) and 17 infected (C)

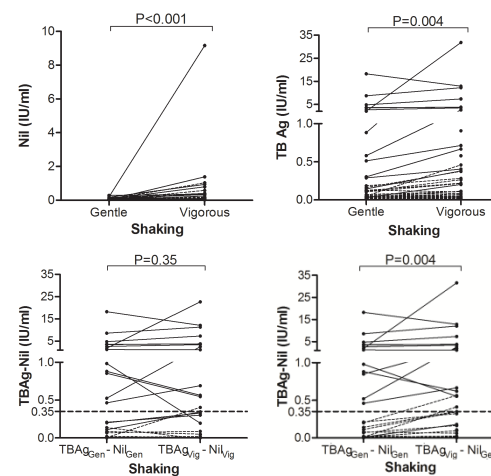


Fig 3. Effect of variable tube shaking on QuantiFERON-TB Gold In-Tube (QFT-GIT) assay. (A and B) IFN- γ release in Nil (A) and TB Ag tube (B) with gentle and vigorous shaking. (C and D) TB response (TB Ag - Nil) of gentle versus vigorous shaking (C) and versus vigorous TB Ag only (D).

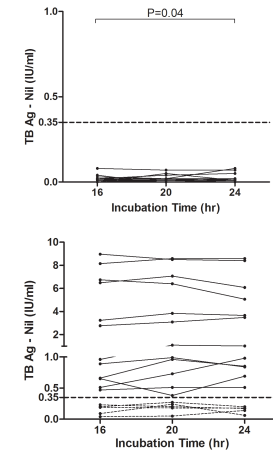


Figure 4. Effect of variable incubation duration on QFT-GIT results. TB response (TB Ag-Nil) values for 33 uninfected (A) and 17 infected (B) subjects tested with indicated duration of incubation.

Conclusions

1. Blood volume and shaking are identified as previously unrecognized sources of variability.
2. Incubation duration does not alter the proportion of positive and negative results in infected and uninfected subjects, respectively.
3. Lower blood volume with 0.8 ml blood increased the sensitivity of QFT-GIT in subjected with latent TB.
4. Elimination of systematic sources of variability may improve the reproducibility of high-risk subjects undergoing serial testing.