Lower Indeterminate Rates Using Single Lithium-heparin Tube Blood Collection For the QuantiFERON®-TB Gold Plus (QFT®-Plus) Test

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IDWeek 2020
October 21-25, 2020

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Introduction

- Detection of latent tuberculosis infection (LTBI) is critical to prevent emergence of *Mycobacterium tuberculosis* (MTB) clinical disease.[1]
- The QuantiFERON®-TB Gold Plus (QFT®-Plus) test is an assay for detecting a cell-mediated immune response to MTB. It has been approved by the US Food and Drug Administration since 2017.[2]
- The QFT-Plus test measures the *in vitro* quantitative IFN-γ responses to MTB or control antigens, in separate reaction tubes, from an incubated blood sample. The test results can be indeterminate (IND) if one or both controls yield unacceptable results.
- The QFT-Plus test has 2 options for blood specimen collection:
  - Collection into a single lithium-heparin transport tube, then aliquot into 4 QFT-Plus blood collection tubes (1-tube QFT-Plus).
  - Collection directly into 4 QFT-Plus blood collection tubes (4-tube QFT-Plus).
- In this study, we retrospectively analyzed the performance of QFT-Plus tests from >1.8 million specimens to examine the relationship of IND results to factors including blood specimen collection method.

Methods

- QFT-Plus ELISA testing was performed as specified in the package insert at various Quest Diagnostics locations. Either 1-tube and 4-tube specimen collection methods were used; 1-tube QFT-Plus specimens were aliquoted within 48 hours.
- De-identified laboratory results from QFT-Plus tests from 2018 and 2019 from US patients were extracted from the Quest database. IND results were compared between the 2 specimen collection methods and among age groups.
- For individuals with multiple QFT-Plus tests performed, results of follow-up QFT-Plus tests on subsequent specimens were also evaluated, stratified by the time between tests.
- Comparisons of IND rates between groups were performed using the Z-test to compare proportions. *P*-values <.05 or non-overlapping confidence intervals were considered statistically significant.

Results

IND Results by Specimen Collection Method

- Over 1.8 million QFT-Plus tests were performed using the 1-tube blood collection method, with an IND rate of 0.83% (**Figure 1**).
- Over 0.3 million QFT-Plus tests were performed using the 4-tube blood collection method, with an IND rate of 4.2% (**Figure 1**).

Results (continued)

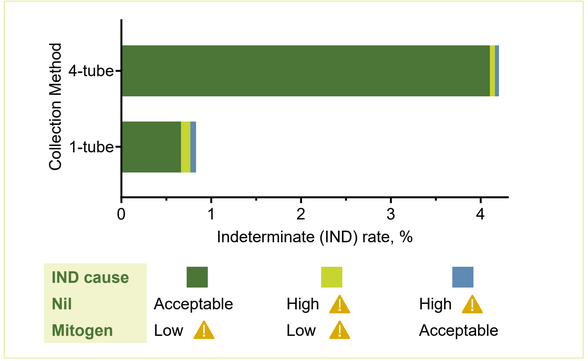

Figure 1. Rates of indeterminate QFT-Plus results by specimen collection method (1-tube versus 4-tube). Nil is the negative control and mitogen is the positive control for the assay.

- The rates of IND results from 1-tube and 4-tube QFT-Plus tests were significantly different (Z-test, *P* <.0001).
- Almost 20% of the 1-tube QFT-Plus IND results were associated with high background levels (>8 IU/ml) in the Nil control (negative control), while only 2.4% of the 4-tube QFT-Plus IND results were associated with this Nil result (Z-test, *P* <.0001) (**Figure 1**).

IND Results by Age

- IND results were also significantly more frequent among certain age groups of pediatric (0 to 2 and 3 to 5 years of age) and elderly (>65 years of age) patients (**Figure 2**).

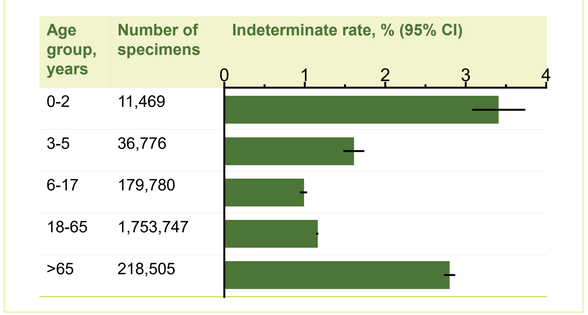

Figure 2. Rates of indeterminate QFT-Plus results by age group. Results from both collection methods are included. Error bars indicate 95% CI.

Results (continued)

Repeat QFT-Plus Testing

- A subset of patients had repeated QFT-Plus tests performed, including some with IND results of the initial test.
- Results of the initial tests were compared to those of the subsequent tests for tests performed <1 month apart and tests performed ≥1 month apart (**Figure 3**).
- For repeat tests <1 month apart, 64% of initial IND results were resolved as negative (50%) or positive (14%).
- For repeat tests ≥1 month apart, 77% of initial IND results were resolved as negative (67%) or positive (10%).

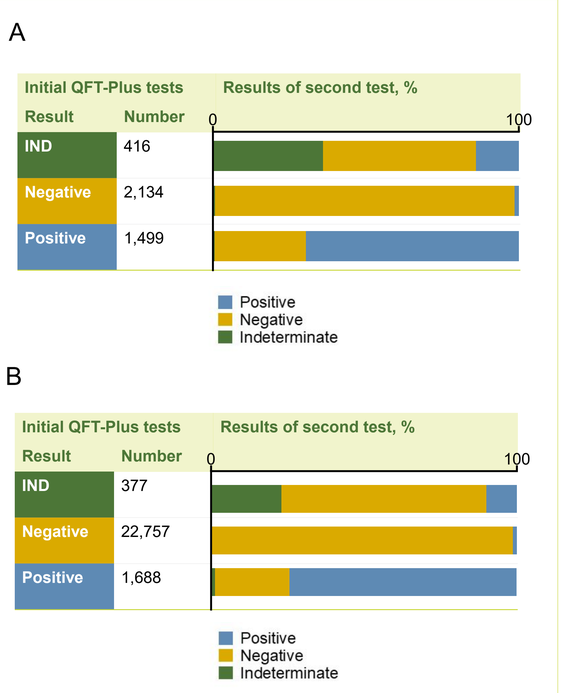

Figure 3. Results of repeat QFT-Plus tests by initial test result. (A) Tests performed <1 month apart; (B) tests performed ≥1 month apart. Results from both collection methods are included.

Conclusions and Discussion

- In this large national study, we found that the rate of IND results from QFT-Plus tests was 4-fold lower when the 1-tube specimen collection method was used compared to the 4-tube method.
- In instances of IND results (from both 1- and 4-tube QFT-Plus tests), the Nil (negative control) and mitogen (positive control) results were assessed.
  - The primary reason for initial IND results was a low (false-negative) result from the mitogen.
- A higher proportion of IND QFT-Plus results from the 1-tube method (12%) was attributed to high background levels in the Nil control compared to the 4-tube method (1.4%).
  - These results could indicate the presence of heterophile antibodies or increased intrinsic IFN-γ secretion by the lymphocytes in the lithium-heparin blood collection tube.
- When QFT-Plus tests were repeated on a second specimen within 1 month of an initial IND result, 64% of the initial IND results were resolved (to either a positive or negative result). Waiting at least 1 month to collect the second specimen increased the proportion of resolved IND results to 77%.

References

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Acknowledgments

The authors thank Allison TerBush and Quest Global Scientific Publications for assistance preparing the poster.

Disclosure

All authors are employees of Quest Diagnostics.