QUANTIFERON-TB: AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) PROCEDURE THAT AIDS IN THE DETECTION OF THE *MYCOBACTERIUM* TUBERCULOSIS INFECTION

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In past decades, the *in vivo* tuberculin skin test (TST) was the procedure performed to inform patients of their tuberculosis infection. This test confirmed their exposure and production of antibodies. However, the TST did not determine whether the patients were positive for being infected with tuberculosis. The results of this skin test were most often verified by a microbiological culture test that usually took up to 2–3 weeks to determine the status of the patients in question. Unfortunately, the variability and inconsistency of its results demanded a more sensitive and non-invasive test.

The QuantiFERON-TB, (QF-TB) is a sensitive assay, introduced by Cellestis from Australia, that detects the cell-mediated immune responses to tuberculosis infection by measuring the elevation of the gamma interferon, (IFN-γ), produced by the macrophages or antigen specific T-cells. By incubating whole patient blood with purified protein derivatives (PPD), which was derived from the *M. tuberculosis* and *M. avium* strains, this conventional method not only diagnoses a patient with the disease but can also assess the immunological responses to multiple antigens within several hours.

Our team tested numerous patient samples to determine whether they were positive for tuberculosis. Upon completing the experiment, we measured the absorbency values, optical densities of the patients using the Precision Microplate Reader and analyzed our findings with Cellestis' QF-TB software. We were able to develop a standard curve from the mean optical densities of the IFN-γ standards. While the QF-TB experiment found 43.7% of our patients testing positive, 36.7% negative, 15.6% indeterminate, and 3.2% conditional positive, previous TST/QF-TB studies have proven 88% compatibility between the two testing methods where the QF-TB test favored the more reliable and consistent results.

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INTRODUCTION

Beginning in 1940, streptomycin, an anti-TB drug that proved to be less toxic than actinomycin, was the antibiotic used to kill microorganisms. Unfortunately, resistant mutants resulted several months into their rapid recoveries. To account for the bacteria that were resistant to the original drug, antibiotic therapy devised a four-drug regimen that would aid in the killing of resistant bacteria, most commonly, with isoniazid, rifampin, pyrazinamide and ethanbutol. While wealthier nations have the resources available to them to control and treat patients with tuberculosis, developing countries are still struggling with this epidemic. If better diagnostic testing, research towards a vaccine, and more effective anti-TB drugs are provided by the advanced healthcare systems, then a brighter future would be in store.

METHODS

When we received the heparinized tubes, we stimulated the whole blood with four purified protein derivatives to determine their immunological responses to these antigens. The green-tubed blood samples were then gently rocked on the Bellco Biotechnology Rocker Platform for several hours prior to aliquoting 1 mL of each sample into a Sarstedt 24-well plate, depending on when the samples were received. After three droplets (100μL) of the Nil Control, Human PPD, Avium PPD, and the Mitogen were added to the blood samples, the plasmas were then thoroughly mixed on a QuantiFERON Microplate Shaker for one minute at an amplitude of nine and a waveform of 20. These plasmas were then incubated overnight in a 5% carbon dioxide NAPCO 6000 incubator. The following morning the blood would have settled to the bottom of each well with approximately 400μL of the stimulated plasma on top. 300μL of the original 400μL were then pipetted and stored in individually marked tubes for the second phase of the experiment.

In the morning, all the reagents, except for the conjugate that remained in the refrigerator, equilibrated for 60 minutes prior to beginning the second phase. Using either a Costar Multichannel pipette or a Gilson Pipetman single pipette, the vortexed (Thermolyne Maxi Mix Plus™) 50μL of the stimulated plasmas and the 50μL of the mixed green diluent (6mL) and peroxidase conjugate (60μL), were then incubated for an hour. After the first incubation period, each well was washed six times by a Coulter Microplate Washer to remove extraneous material while the bounded antigen-antibody complex remained. Assuming a full plate was used, 120μL of the chromogen100× concentrate and 12mL of the enzyme substrate buffer mixture was added to each well and was left to incubate for 30 minutes. An enzyme stopping solution was added after this incubation period. Within five minutes of adding the stopping solution, the 96-well plate was placed on the Precision Microplate Reader (Molecular Devices) to be analyzed by Cellestis' QF-TB Analysis Software. Once the optical densities of the patient samples were read, they were compared to a standard curve that was derived from the mean absorbencies of the Human IFN-γ Standards. These

standards, which were provided by Cellestis, were generated from their comparison with the National Institute of Health IFN-γ standards. The calculated %Human Response and the %Avian Response, which were derived from measuring the elevation in the IFN-γ production, determined their status.

Data Analysis

The data indicated that out of the 32 patients, 14 tested positive (43.7%), 12 tested negative (37.5%), 5 tested indeterminate (15.6%) and 1 tested conditional positive (3.2%) with the QF-TB test. Given the 88% compatibility, extrapolated results would show 16 patients tested positive, 14 patients negative, and 2 patients undetermined.

RESULTS

The results presented above were determined through a series of mathematical steps. First, we found the IFN-γ concentrations from our standard curve by using the patient optical densities. We were then able to compile the Human and Avian Response percentages with the N, H, A, and M as the IFN-γ concentrations.

The Human and Avian Response percentages were then measured against designated cut-offs to determine the status of our patients. While a %Human Response ≥15% and a %Avian Response ≤10% indicate TB infection in patients with a recognized risk of TB exposure, a %Human Response ≥30% and a %Avian Response ≤10% represent TB infection in patients with no identified risk factor to TB exposure. When the Human Response is between 15% and 30% and the Avian Response is ≤10% then the patient is deemed conditional positive, either unlikely for low-risk patients or likely for patients with identified risks. When the difference between the Mitogen and Nil Control is less than 1.5 IU/mL, the results are indeterminate, meaning that feasible data was not obtained. When the %Avian Response is greater than 10%, the patient's response to the PPD is predominately directed toward the *M. Avium* as opposed to the *M. tuberculosis,* meaning the patient tested negative for the *M. tuberculosis* infection.

CONCLUSION

In our experiment we tested the compatibility of the QF-TB and the tuberculin skin tests (TST). The QF-TB assay works in conjunction with the TST in hopes to become FDA-approved as the official diagnostic test in determining TB infection. The QF-TB was shown to be more reliable and compatible with the chest radiograph in comparison to the TST. The TST's false-positives and false-negatives limit the verity of its results while the QF-TB's sensitivity eliminates the false-positives brought about in the TST from the BCG vaccine. Although the TST parallels this testing procedure, its refined techniques, procedures, timeliness, and recombinant antigens optimize the operation and its use in the future.

Although the QF-TB test is more reliable and consistent in comparison to the TST, the QF-TB also has its share of limitations. While its purpose is to detect the latent tuberculosis infection, its use is not recommended when dealing with patients that are TB suspects or individuals with an increased risk of progression to active TB. When someone has tuberculosis or is immuno-compromised his or her immune system produces less antibodies and IFN-γ. Since the elevated production of proteins is the marker for the experiment, the QF-TB test is not suggested when at-risk patients for TB are involved. Although there are second and third generation tests that are more invasive, in terms of differentiating LTBI and TB, and disregarding the BCG vaccine, the QF-TB is limited in what it will be able to identify. Such measures should be improved upon so that this test can be routinely used throughout the world, or the second and third generation tests should continue to be reworked and fine-tuned so that it can receive its FDA approval.

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