An Assessment of the Ability of *Mycobacterium tuberculosis* Serology to Detect Active TB Disease.

Short Title: *Mycobacterium tuberculosis* Serology Testing

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ABSTRACT

*Mycobacterium tuberculosis* (*Mtb*) remains a major world disease, with approximately 9 million new cases each year. Identification and treatment of active disease is essential for *Mtb* control. Serology may offer increased detection of active disease in patients with a positive tuberculin skin test (TST) or QuantiFERON®-TB (QFT-G). The InBios Active TbDetect™ IgG ELISA, IBL *Mtb* IgG ELISA, and Anda Biologics TB ELISAs were evaluated for the ability to detect *Mtb* antibodies in patients with active disease. Agreement, sensitivity, and specificity were determined for each ELISA compared to culture or amplified direct detection and *Mtb* low-risk control patients. InBios Active TbDetect™ had an agreement of 96.2%, a sensitivity of 83.3%, and a specificity of 98.9%. The IBL *Mtb* ELISA had an agreement of 84.0%, a sensitivity of 5.6%, and a specificity of 100.0%. The agreement, sensitivity, and specificity of Anda Biologics TB ELISA were 74.2%, 83.3%, and 72.0% respectively. The sensitivity for detecting *Mtb* antibodies in HIV-associated tuberculosis was 50% for both the InBios Active TbDetect™ and the Anda Biologics TB ELISA and 0% for the IBL *Mtb* ELISA. The percent positivity for InBios Active TbDetect™ ELISA, IBL *Mtb* ELISA, and Anda Biologics TB ELISA in latently infected individuals positive by TST and/or QFT-G were 5.1%, 0.0%, and 30.8% respectively. It can be concluded that the InBios Active TbDetect™ IgG ELISA is superior to the other ELISA assays in accurately detecting active *Mtb* disease.
INTRODUCTION

Approximately nine million new cases of disease and over two million deaths result from tuberculosis (TB) each year (29, 38). It is estimated that over one-third of the world’s population is infected, with ~95% of all cases occurring in developing countries. Global measures are currently in place attempting to reduce the transmission of TB.

An essential component of TB control efforts is to identify and treat individuals with active TB disease. The ability to correctly identify individuals with latent TB infection who will progress to active TB disease is vital to this goal (9, 50). Current test procedures are inadequate to accurately detect and identify active TB disease (14, 27, 30, 31, 42, 45). These shortcomings result in the unnecessary treatment of many individuals who may not need it (3, 17, 32, 46). While TST and QFT-G, the traditional methods for latent TB infection screening, rely on the cell mediated response, the humoral response appears to correlate with the progression of the infection to active TB disease (5, 6, 11, 15, 20, 21).

Many studies have been conducted to evaluate the utility of individual specific Mtb antigens for detecting antibodies in patients with active TB disease (1, 7, 10, 11, 20, 21, 25, 26, 29, 39, 40, 46, 47). Several of these antigens have been developed into commercial assays capable of detecting Mtb antibodies (4, 28, 35, 54). This study evaluates three commercially available ELISA assays for their ability to detect IgG antibodies to M. tuberculosis in patients with active TB disease.
MATERIALS AND METHODS

**Human sera.** The procedures followed were in accordance with the ethical standards established by the University of Utah and are in accordance with the Helsinki Declaration of 1975. This study was approved by the Institutional Review Board of the University of Utah, IRB 17152. All patient samples included in this study were de-identified to meet the Health Information Portability and Accountability Act (HIPAA) patient confidentiality guidelines.

Serum samples were stored at -70°C until testing commenced and were then stored at 2-8°C while testing was performed. The whole blood samples were processed immediately after collection. Discrepant samples were repeated on each respective assay to ensure reproducibility. A total of 209 samples were used and divided into four groups.

Risk factors that were evaluated for exposure to TB included work in the health care field, laboratory work especially in mycobacteriology laboratories and specimen processing, immigration from or travel to an endemic country, and exposure to persons with known active disease. Work in a mycobacteriology laboratory, exposure to a known active case or immigration from a country endemic for TB were considered high risk for exposure. Work in a health care field was considered moderate risk.

Group I consisted of 88 samples from healthy, U.S. born individuals that tested negative by QuantiFERON®-TB Gold (QFT-G) and had no risk factors for *M. tuberculosis* infection. All serum samples from group I were tested on each of three commercially available ELISA assays.
Group II included serum samples from 18 *M. tuberculosis* culture positive and/or Amplified Direct Detection (ADD) positive patients. The samples in group II were tested for *Mtb* antibodies using the three commercial ELISA kits.

Group III serum samples were collected from 25 individuals who had received the Bacillus Calmette-Guérin (BCG) vaccination. The majority of individuals received the BCG were vaccinated in infancy or early childhood and obtained the vaccinations in Western and Eastern Europe, Central and South America and Asia. All samples from group III were tested by the three antibody detection assays.

Group IV was comprised of 78 individuals who were diagnosed with latent *Mtb* infection by tuberculin skin test (TST) and/or QFT-G. Tuberculin testing in the study subjects were performed within one year to one month before blood samples were drawn. Fifty samples were positive for TST, 14 were positive for QFT, and 14 were positive for both TST and QFT. All 78 samples were assessed for anti-*Mtb* antibodies using the three commercial ELISA assays.

*M. tuberculosis culture and Amplified Direct Detection.* Serum samples from group II were obtained from individuals found to be culture or ADD positive for *Mtb* through testing at ARUP Laboratories (Salt Lake City, UT).

**Tuberculin skin test (TST).** Some participants received a standard dose of 5 Tuberculin units intradermally. Trained health care professionals interpreted the results 48-72 hours after application according to the American Thoracic Society (ATS)/Centers for Disease Control and Prevention guidelines (8). The positive interpretation of a TST is an induration area ≥10mm in individuals with increased risk factors and ≥15mm in
individuals without known risk factors for *M. tuberculosis* infection (16). Other participants were not tested due to a history of a positive TST in the past.

**QuantiFERON®-TB Gold (QFT-G) testing.** Sodium heparin blood tubes were collected from groups I and IV and tested using QuantiFERON®-TB Gold (Cellestis Inc. Victoria, Australia) according to the manufacturers guidelines. Results were interpreted as positive, negative, or indeterminate.

**M. tuberculosis antibody detection by commercial ELISA.** Anti-*Mtb* antibody levels were assessed using three commercially available ELISA assays. The three kits evaluated included InBios Active TbDetect™ IgG ELISA (InBios International, Seattle, WA), IBL *Mtb* IgG ELISA (IBL-Hamburg, Hamburg, Germany), and the Anda Biologics TB ELISA (Anda Biologics, Strasbourg, France). Each assay follows standard ELISA format. For the InBios Active TbDetect™ ELISA, serum samples were tested according to manufacturers’ guidelines. The assay utilizes several antigens including Mtb81, Mtb8, Mtb48, DPEP(MPT32), 38kDa protein, and two additional proprietary antigens. Results are expressed in optical density (OD) with an OD ≥0.500 indicating a positive result.

The IBL *Mtb* IgG ELISA was evaluated using the protocol supplied by the manufacturer. The microtiter wells are coated with 18, 36, and 40kDa recombinant antigens. The results are expressed in U/ml and are calculated in reference to a cut-off standard provided by the manufacturer. A value of >12 U/ml is representative of a positive result. Because of the initial low sensitivity observed with the IBL kit, samples were also tested with a second different lot number of the IBL kit to control for the
possibility that the low sensitivity was due to lot to lot variability. The outcomes were identical with the second lot.

The Anda Biologicals TB ELISA was evaluated using A60 antigen sensitized microtiter wells obtained from the manufacturer following the recommended protocol. Serum samples were diluted 1:100 for testing. 100µl of each diluted sample was added to the sensitized microtiter well and incubated for 60 minutes at room temperature (18-24°C). The wells were washed and 100µl of horseradish peroxidase-conjugated goat anti-human IgG solution was added and incubated for 30 minutes at room temperature. Following another wash step, tetramethylbenzidine (TMB) was added and incubated at room temperature for 20 minutes. The enzymatic reaction results in the formation of a blue color which intensifies in proportion to the amount of anti-\textit{Mtb} antibodies that is present. The reaction was stopped by adding 1N H$_2$SO$_4$ which results in a color change from blue to yellow and the absorbance was read. Positive and negative control sera were analyzed with each run to ensure test function. Sample results were compared to a reference standard and reported in U/ml. A value $\geq$1.2 U/ml was considered a positive value.

**HIV Serology.** HIV status was determined for serum samples from group II by serology testing performed at ARUP Laboratories. Samples were screened using the Bio-Rad GS HIV-1/HIV-2 PLUS O EIA (Hercules, CA) with positive samples undergoing confirmation by Bio-Rad GS HIV-1 Western Blot Kit (Hercules, CA).

**Statistical Analysis.** The data was analyzed for agreement, sensitivity, and specificity with 95% confidence intervals (CI) using a two-way contingency table analysis with Yates-corrected Chi-squared test (19). Equivocal results were excluded
from the calculations. Receiver operator characteristic curves were generated using R version 2.7.2 (R Development Core Team). Area under the curve analysis was performed using Statistical Analysis Software version 9.1.3 (SAS Institute Inc., Cary, NC). Scatter plots were generated using GraphPad Prism version 5.01 (GraphPad Software, Inc., La Jolla, CA).
RESULTS

Comparison of InBios Active TbDetect™ IgG ELISA against culture/ADD and a healthy control group. When serum samples from group I (healthy normal controls) and group II (culture positive or ADD positive) were evaluated by the InBios Active TbDetect™ IgG ELISA, an overall agreement of 96.2% (agreement with positive and negative results) was observed. The clinical sensitivity and clinical specificity was 83.3% (CI, 68.6-87.8%), and 98.9% (CI, 95.8-99.8%) respectively (Table 1). One sample from group I tested positive, while 87 tested negative on the InBios assay. No clinical history could be collected on the positive individual to explain the presence of Mtb antibodies. Fifteen samples tested positive and 3 tested negative from group II.

Because of previous reports of poor sensitivity of tuberculosis antibody tests in HIV-associated tuberculosis, HIV status was determined for the group II culture positive/ADD patients (49). Of the eighteen samples from group II, six were HIV positive. The three samples that tested negative on the InBios assay were from patients with HIV-associated tuberculosis. If the HIV-associated tuberculosis samples are removed from analysis, the clinical sensitivity is 100% (12 out of 12). The sensitivity of the InBios antibody test for HIV-associated tuberculosis is 50.0% (3 out of 6).

Comparison of IBL Mtb IgG ELISA to culture/ADD and a healthy control group. The IBL Mtb IgG ELISA assay was evaluated using serum samples from groups I and II. The agreement, sensitivity, and specificity were determined to be 84.0%, 5.6% (CI, 1.2-5.6%), and 100.0% (99.1-100.0%) respectively (Table 1). All 88 samples from group I tested negative and 17 of the 18 samples from group II also tested negative on the IBL assay. All six of the sera from HIV-associated tuberculosis patients were negative.
for tuberculosis antibodies by the IBL assay; the remaining eleven negatives were HIV negative.

Comparison of Anda Biologicals TB ELISA against culture/ADD and a healthy control group. Serum samples from group I and group II were analyzed using Anda Biologicals TB ELISA assay. The overall agreement was found to be 74.2%, the clinical sensitivity was 83.3% (CI, 63.5-94.0%), and the clinical specificity was 72.0% (CI, 67.2-74.6%) (Table 1). Twenty-one of 88 samples from group I were positive for a positivity rate of 24% in healthy QFT-G negative individuals. Three of the 18 samples from group II were negative. All three of the negative samples from group II were from patients with HIV-associated tuberculosis. The sensitivity of the Anda assay for HIV-associated tuberculosis was 50.0% (3 out of 6).

Comparison of the ELISA tests by scatter plot. Quantitative ELISA results (OD$_{450}$ readings or U/mL values) of healthy patients and active TB disease patients were graphed in a scatter plot (Fig. 1). The Anda ELISA showed the greatest range of results for active TB disease patients, from 0.2 to 4.2 U/mL. The range of results of values for healthy patients for the Anda ELISA overlapped into the positive range to 2.4 with one result at 4.3. The InBios ELISA showed a range of results for active TB disease patients, from 0.144 to 2.582 OD$_{450}$ with a tight distribution of OD$_{450}$ values for the healthy patients below the cutoff value. Most of the IBL ELISA values were in the negative range for both healthy and active TB disease patients with the only positive having a result just above the cutoff at 12U/mL.

Receiver operator characteristic curves (ROC). ROC curves for InBios, IBL and Anda were constructed by plotting the true-positive rate and false-positive rate with
each unique value of the indicator variable (i.e. commercial test kit) for healthy and
active TB disease patients (Fig. 2). An indicator variable with high discriminatory ability
will have a curve with an area under the curve (AUC) near 1, and an indicator variable
with low discriminatory ability will have an AUC near 0.5. InBios demonstrated a
significantly higher discriminating power than the IBL and the Anda tests with a high
AUC value of 0.911 close to a value of 1. The Anda test had very poor discriminatory
ability with an AUC of 0.528 close to a value of 0.5.

**Measurement of *Mycobacterium tuberculosis* antibodies in individuals**

receiving the Bacillus Calmette-Guérin (BCG) vaccine. Twenty-five serum samples
from group III (BCG-vaccinated individuals) were evaluated for the presence of *M.
tuberculosis* antibodies by each ELISA (Table 2). Antibodies were detected in one
sample by the InBios Active TbDetect™ ELISA giving a percent positivity of 4.0%. The
IBL *Mtb* IgG ELISA detected no antibodies in this group. The Anda Biologicals TB
ELISA detected antibodies in 14 samples resulting in a 56.0% positivity rate.

**Presence of *M. tuberculosis* antibodies in patients with evidence of latent *Mtb*
infection.** Seventy-eight patients that were positive by the QFT and/or TST were
evaluated for the presence of *M. tuberculosis* antibodies (Table 2). Fourteen positive
samples were positive by QFT (17.9%), 50 samples were positive by TST (64.1%), and
14 samples were tested by both TST and QFT positive for both (17.9%). All samples
were evaluated using each of the three commercial ELISA assays evaluated in this study
for the presence of antibodies to *M. tuberculosis*.

Testing on the InBios Active TbDetect™ IgG ELISA yielded four of the 78
samples from group IV being positive, resulting in a percent positivity of 5.1% (Table 2).
Three of the four samples that were positive InBios Active TbDetect™ IgG ELISA were from patients who had moderate to high risk of exposure to tuberculosis (Table 3). One of the positive serum samples was from a medical technician with diabetes who worked in the specimen processing department of a large diagnostic laboratory. The patient was negative in the past with TST, but received 6 months of INH prophylaxis following his recent positive QFT-G result. The second positive serum sample was from a medical technologist who was a long-term employee in a mycobacteriology laboratory. This technologist was given 6 months of INH prophylaxis in 1988 for conversion to a positive TST and is positive by QFT-G. The third positive serum sample was from a patient who had emigrated from Bolivia and had both a positive TST and QFT-G result. The fourth patient had no known risk factors for exposure to TB and was positive by TST but negative by the QFT-G.

The IBL Mtb IgG ELISA failed to detect M. tuberculosis antibodies in any of the serum samples from this group. The Anda Biologicals TB ELISA detected 24 positive samples from this group giving a percent positivity of 30.8%, about 10 times higher than the expected conversion rate (3-5%) from latent TB infection to active TB disease that has been cited in several studies on tuberculosis infection rates (3, 24).
DISCUSSION

Assays for the serodiagnosis of tuberculosis have been regularly evaluated with greatly varying results (1, 2, 12, 39, 44, 46, 52). Rarely is an acceptable sensitivity and specificity from a commercially available kit for routine laboratory testing described. The increased availability, decreased price, and rapid reporting ability of serology testing have prompted continued investigation into these options. Each of the three commercial ELISA assays evaluated showed significant differences in their ability to detect *Mtb* infection. InBios demonstrated a significantly higher discriminating power than the IBL and the Anda tests by both R.O.C. and scatter plot. Most studies indicate a combination of several specific antigens to be the most effective way to increase sensitivity while not significantly decreasing specificity (1, 36, 39, 52, 55). The Anda Biologicals TB ELISA relies on the use of only a single (A-60) antigen for antibody detection. While the sensitivity (83.3%) of the assay appears to be acceptable, the 24% positivity rate in healthy low risk individuals makes this kit unacceptable for use as a screen for active TB disease. Although the IBL *Mtb* IgG ELISA employs three separate antigens for detection and had excellent specificity (100%), it performed with a very poor sensitivity (5.6%) which also eliminates it as a viable tool for detecting active TB disease. The InBios Active TbDetect™ IgG ELISA uses seven separate antigens. It performed well in both sensitivity (83.3%) and specificity (98.9%). The InBios kit, however, was insensitive (50% sensitivity) in detecting active TB disease in HIV-positive patients.

The ability of an assay to differentiate between latent TB infection and active TB disease offers a significant improvement to current routine test methods. Studies on
tuberculosis infection indicate an expected conversion rate from latent TB infection to active TB disease at 3-5% within two years of exposure (3, 24). The InBios kit positivity rate in the latently infected individuals in our study (5.1%) was significantly closer to the expected percent positivity rate than Anda (30.8%) or IBL (0.0%). Three of the four patients that tested positive for TB antibodies by the InBios kit indeed had a moderate to high risk of exposure to tuberculosis. The increased reactivity of the Anda assay is most likely due to the cross-reactivity of the A-60 antigen used in the assay with many environmental strains of *Mycobacterium*. Additionally, the administration of the TST test to some of our study patients may even have had a booster effect on the antibody response to the A-60 antigens used in the Anda ELISA. In this study, InBios assay appeared to offer reliable data to aid differentiation between active TB disease and latent TB infection in HIV-negative individuals.

One obstacle in tuberculosis testing is the high positivity rate for TST in BCG vaccinated individuals (13, 22, 51). As observed in other studies, the high reactivity (56.0%) of the Anda Biologicals assay in the BCG vaccinated group gives the test little clinical utility in this population (12, 34).

A significant problem for effective tuberculosis disease detection is the increasing prevalence of HIV-associated tuberculosis (18, 41, 48, 56). Although some *Mtb* antigens have shown a propensity for detecting TB in HIV/AIDS infected individuals, a reliable and effective assay has not been developed (1, 23, 25, 36, 53). Our study showed that the InBios and Anda ELISA assays both showed reactivity with sera from only 3 of 6 HIV-associated tuberculosis patients. Both assays failed to detect antibodies in the same three samples.
Considering the stage of HIV/AIDS infection at the time of testing and exposure to *Mtb*, several significant differences in antibody detection may be observed (33, 37, 43). While reports vary greatly on the effectiveness of various antigens, it is clear that additional work is necessary before accurate claims about the effectiveness of *Mtb* serology in HIV patients can be made.

In conclusion, of the three commercial antibody detection ELISA assays we evaluated, the InBios Active TbDetect™ was found to be the best overall assay for detecting active Mtb infection. The application of this assay in various populations and with a range of TB disease states should be further studied. The good sensitivity and specificity of the InBios Assay in the detection of active *Mtb* suggests that it may be a valuable additional diagnostic tool in the diagnosis of *Mtb* infection when used in conjunction with clinical findings and additional *Mtb* testing.
ACKNOWLEDGEMENTS

This study was supported by the ARUP Institute for Clinical and Experimental Pathology®. A special thanks to InBios International, Inc., IBL-Hamburg, and Anda Biologicals for supplying reagents for this study. We also wish to thank Andrew Wilson from ARUP for his aid in the ROC analysis.
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FIGURE LEGENDS

Figure 1. Scatter plots of healthy patients (solid circles) and patients with active TB disease (solid triangles) on each of the tested ELISA kits. The dotted line on each graph represents the cutoff value for the respective kit (0.500 OD$_{450}$ for InBios, 12 U/mL for IBL and 1.2 U/mL for Anda).

Figure 2. The receiver operating characteristic (ROC) curves of InBios (solid), IBL (dashed) and Anda (dotted) for healthy and active TB disease patients. The areas under the curve (AUC) for InBios, IBL and Anda are 0.911, 0.528 and 0.797 respectively. When compared to InBios the p values for IBL and Anda are both < 0.0001 indicating a significant difference between InBios and both the IBL and the Anda kits.
TABLE 1. Summary of results comparing InBios Active TbDetect™ IgG ELISA, IBL *Mtb* IgG ELISA, and Anda Biologicals TB ELISA to Culture/ADD and a healthy control group.

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>InBios Active TbDetect™ IgG ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>86</td>
</tr>
<tr>
<td>Equivocal</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>88</td>
</tr>
<tr>
<td>Agreement</td>
<td>96.2%</td>
<td>(C.I. 91.1-97.7)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>83.3%</td>
<td>(C.I. 68.6-87.8)</td>
</tr>
<tr>
<td>Specificity</td>
<td>98.9</td>
<td>(C.I. 95.8-99.8)</td>
</tr>
<tr>
<td>IBL <em>Mtb</em> IgG ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>17</td>
<td>88</td>
</tr>
<tr>
<td>Equivocal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>88</td>
</tr>
<tr>
<td>Agreement</td>
<td>84.0%</td>
<td>(C.I. 82.5-84.0)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>5.6%</td>
<td>(C.I. 1.2-5.6)</td>
</tr>
<tr>
<td>Anda Biologicals TB ELISA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Samples are positive by culture and/or ADD. The eighteen positive samples are comprised of 12 that are confirmed serologically to be HIV negative and 6 that are serologically HIV positive.

Negative samples are from low risk U.S. born individuals (group I).

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<table>
<thead>
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<tbody>
<tr>
<td>Positive</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>54</td>
</tr>
<tr>
<td>Equivocal</td>
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<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>88</td>
</tr>
</tbody>
</table>

Agreement 74.2% (C.I. 65.5-78.3)

Sensitivity 83.3% (C.I. 63.5-94.0)

Specificity 72.0 (C.I. 67.2-74.6)
TABLE 2. Summary of positive results of InBios Active TbDetect™ IgG ELISA, IBL, Mtb IgG ELISA, and Anda Biologicals TB ELISA in Latent Infections (n=78), BCG vaccinated (n=25)

<table>
<thead>
<tr>
<th>Commercial kit</th>
<th>Latent infections</th>
<th>BCG vaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>InBios Active TbDetect™</td>
<td>4 (5.1%*)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>IBL Mtb IgG ELISA</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Anda Biologicals TB ELISA</td>
<td>24 (30.8%)</td>
<td>14 (56.0%)</td>
</tr>
</tbody>
</table>

* percent positive
TABLE 3. Antibody positive patients with expected latent TB infection

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>QFT-G&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TST&lt;sup&gt;b&lt;/sup&gt;</th>
<th>InBios&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Travel or Risk Factors</th>
<th>Testing</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>M</td>
<td>Pos&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Neg&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Pos</td>
<td>Medical Technician</td>
<td>Neg chest x-ray</td>
<td>Treated for 6 months in 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Diabetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>M</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Mycobacterium Lab Technician</td>
<td>Neg chest x-ray</td>
<td>Treated in 1988</td>
</tr>
<tr>
<td>40</td>
<td>M</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Immigrated from Bolivia in 2000</td>
<td>Neg chest x-ray</td>
<td>No history of treatment</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>No known travel or risk factors</td>
<td>Neg chest x-ray</td>
<td>No history of treatment</td>
</tr>
</tbody>
</table>

<sup>a</sup> Samples were tested using QuantiFERON®-TB Gold.

<sup>b</sup> Tuberculin skin test (TST).

<sup>c</sup> InBios Active TbDetect<sup>™</sup> IgG ELISA.

<sup>d</sup> Positive

<sup>e</sup> Negative