AN EVALUATION OF THE QUANTIFERON© GOLD TUBERCULOSIS-SCREENING TEST FOR EMPLOYEES AT THE PORTLAND VETERANS AFFAIRS MEDICAL CENTER

By

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CERTIFICATE OF APPROVAL

This is to certify that the Master's thesis of

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ABSTRACT

Intradermal tuberculin skin test has been the traditional means of screening individuals for tuberculosis (TB). Limitations of the TB skin test have necessitated the creation of alternative TB screening tests for low incidence settings. One such screening test is the QuantiFERON-TB Gold© blood test using the Interferon Gamma Release Assay is now used for screening health care workers at the Portland Veterans Administration Medical Center (PVAMC). The PVAMC and other healthcare facilities using this test for serial testing of healthcare workers (HCW) have reported unexpectedly high numbers of positive tests. Upon retesting, a high proportion of these subjects revert back to negative, suggesting their initial test result was a false positive. In the nearly three years since QuantiFERON-TB (QFT) Gold testing began at the PVAMC, approximately six percent of subjects tested positive. This is at least double the proportion of positive TST tests prior to the change to QFT Gold. We hypothesize that those who reverted back to negative upon retesting had levels of blood interferon gamma close to the manufacturer's cut-off value and did not have TB risk factors.

To test this hypothesis, we retrospectively identified all employees and volunteers who tested TB positive between August 1, 2010 and January 31, 2013. We examined blood interferon gamma values, TB symptoms and factors associated with a positive TB test, to understand which initial tests are likely to revert and which are likely to remain positive. A total of 3114 HCW underwent initial TB screening with the QFT Gold at the PVAMC between August 1, 2010 and January 31, 2013. The initial QFT Gold test found that 6.2% of subjects were positive, 93.5% subjects were negative, and 0.35% subjects were indeterminate. Of the 192 subjects who tested positive, two did not meet the age

requirement of 18 years old and 57 were not retested. The reversion proportion was approximately 36% of those who were initially positive. The odds of reversion were 76% lower when initial blood interferon gamma was equal to or greater than 0.8 IU/mL (p=0.0022; CI 0.091-0.693). Healthy, immunocompetent individuals with positive initial test results between 0.35-0.79 IU/mL should be assumed to be false positive and be retested. Those whose initial test is above 0.8 IU/mL should be considered likely to remain positive, and should be referred to their physician for LTBI evaluation.

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INTRODUCTION

The tuberculin skin test (TST) has been the traditional method of testing for the presence of latent TB infection (LTBI) in HCW in low incidence countries. The TST is subject to bias and errors in placement and reading, and repeated exposure to the antigen in the purified protein derivative may boost future responses to repeat TST (Ringshausen et al. 2010). Those who had non-tuberculous mycobacterial disease (NTM) or bacille Calmette-Guèrin (BCG) vaccinations in other countries may be sensitive to the TST, resulting in false positives (Zwerling et al. 2012). The TST must be read 48-72 hours after intradermal injection, problematic because of low subject compliance for follow-up (Mazurek et al. 2005). To reduce the prevalence of such testing errors, over the past eight years, healthcare institutions in low-incidence countries have employed an alternative to the TST for HCW, a blood test that uses an interferon-gamma release assay (IGRA). The IGRA QuantiFERON© Gold (QFT) blood test for TB (Cellestis Limited, Carnegie, Victoria, Australia) was approved for use in the United States by the FDA in 2005 (Jensen et al. 2005). Because of the drawbacks of the TST and low staff compliance for the two-step process (29% of staff returned for reading), the PVAMC began using QFT Gold in August 2010, a test that requires no subject follow up and whose results are available within 24 hours.

Neither the TST or the QFT can distinguish latent from active TB disease, nor can they determine if TB bacilli are present in the patient (Barry et al. 2009). In fact, these tests can only confirm an immune response to the antigens in the tests. The QFT Gold TB test is a blood test that does not inject antigens into the subject, is not affected by prior BCG vaccination and is less influenced by prior NTM than TST. For testing,

approximately 3mL total whole blood is collected into three heparinized tubes: a Nil (negative control) tube, a TB Antigen (Ag) tube, and a Mitogen (positive control) tube (Cellestis Inc). Samples are mixed with *Mycobacterium tuberculosis*-specific antigens simulating the mycobacterial proteins ESAT-6, CFP-10, and TB7.7, and if TB-positive, stimulate an immune response from antigen-specific CD4+ and CD8+ T-cells in the blood, which then secrete the cytokine interferon gamma (IFN-γ) (Whitworth et al. 2013). Samples are incubated 16-24 hours, and measured for release of IFN-γ using the enzyme linked immune sorbent assay (ELISA) (Gandra et al. 2010). The Nil (negative control) tube contains all the reagents minus the antigens (Jensen et al. 2005). It tests for a possible pre-existing immune response that would generate elevated IFN-γ. The Mitogen (positive control) tube is used to confirm correct handling of blood and proper incubation (if incorrectly handled, the positive control will not produce IFN-γ).

A positive result occurs when the IFN-γ value in the TB Antigen tube is significantly above the IFN-γ value of the Nil Control: TB Ag-nil IFN-γ $\geq$ 0.35 IU/mL (Appendix A: manufacturer's interpretation flow chart). An indeterminate response occurs when the TB Antigen tube and Mitogen tube both have low response to the antigens to which they are exposed (<0.35 and <0.5 IU/mL IFN-γ, respectively). An indeterminate test may be a result of reduced immune function, specimen mishandling or incorrect blood sample acquisition, and may require retesting. Nil value less than 8.0 IU/mL IFN-γ with a TB-Antigen response less than 0.35 IU/mL IFN-γ and a Mitogen response greater or equal to 0.5 IU/mL IFN-γ are considered a negative test (meaning that the Mitogen is responding to the antigens, but the TB-antigen tube secreted little or no

IFN-γ). A single negative test result is considered sufficient for employee TB screening. Additional details on test interpretation provided in Appendix A.

Studies in Japan and Germany have shown that TST and QFT Gold had low agreement in BCG-vaccinated HCW, with QFT Gold being the more accurate test over TST (Nienhaus et al. 2008). Some countries perform both a TST and follow-up IGRA in HCW screening. Results have been mixed on whether TST boosts IGRA values, and currently the CDC recommends either TST or IGRA (not both) for serial TB testing of HCW (Sauzullo et al. 2011; van Zyl-Smit et al. 2009).

Some medical conditions that reduce immune function may result in an indeterminate result, or may make interpretation of a negative TB test (TST or IGRA) difficult, decreasing the predictive value of a negative test. These circumstances may necessitate other means of diagnosis, including chest X-ray, to rule out TB (Mazurek et al. 2005). Conditions that may affect results include HIV/AIDS, use of tumor necrosis factor or immunosuppressive drugs following organ transplantation, diabetes mellitus, silicosis, chronic renal failure, some hematological conditions, and carcinomas of head or neck (Jensen et al. 2005). People with these conditions and LTBI are also at greater risk of progressing to active TB disease.

Subjects with NTM infections from *M. kansasii, M. szulgai,* and *M. marinum* could be responsive to the QFT TB-specific antigens, resulting in a false-positive test (Mazurek et al. 2005). These NTM infections are rare in the general population and occur more frequently in those who are immunocompromised (*M. kansasii, M. szulgai*) and those who are exposed via contact with aquatic species in fish tanks (*M. marinum*) (Griffith et al. 2007; Aubry et al. 2002). Cellestis Inc, the manufacturer of QFT Gold,

recommends the test be used in conjunction with knowledge of the subject's medical history, symptoms, and background.

Oregon and the United States as a whole are considered low TB-incidence regions (Center for Disease Control and Prevention, 2013). In 2011, TB incidence in Oregon was 2.0 cases per 100,000 people, the majority of which were found in the Portland area, and 72% of which were in foreign-born residents (Oregon Health Authority, 2012). The 2011 incidence of TB in the United States is 3.4 cases per 100,000 people (Miramontes et al. 2011). Because of low compliance with TST, CDC recommendations, and its low-incidence setting, the PVAMC uses the QFT Gold test for serial TB screening of HCW.

The PVAMC requires all employees and volunteers to complete a health questionnaire concurrent with TB testing to examine medical conditions or symptoms that may affect test results (Appendix B: TB screening questionnaire). It employs approximately 4,000 healthcare and support personnel at its primary medical center and satellite facilities. Prior to August 2010, the PVAMC utilized the TST for employee TB screening. The prevalence of positive tests was approximately 3%, though employee compliance in returning for test reading was only 29%. PVAMC officials believe most people who did not return for test reading likely had no reaction and therefore felt no need to return. Because of this, the actual prevalence of positive tests was likely lower.

Since beginning its use in August 2010, an estimated 6% of employees have tested TB positive using the QFT Gold TB test. When retested, many employees who initially tested positive were anecdotally reported to revert to negative upon retest. This phenomenon has been observed in other health care facilities worldwide, with reversion proportions reported from 40-52.9% (Zwerling et al. 2012). In low-incidence settings

with low-risk subjects, the CDC discourages testing for LTBI (Centers for Disease Control 2013). When used to comply with legal requirements of employment, CDC recommends assuming a positive result is false with no TB risk factors and those subjects be retested rather than being unnecessarily treated for LTBI (Mazurek et al. 2010).

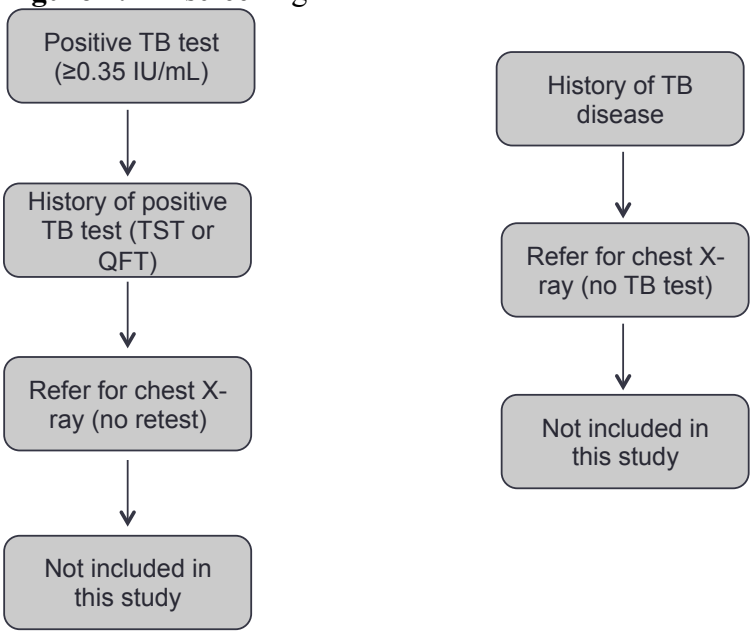
We aimed to identify individuals who tested positive for TB tests with the QFT Gold test, the proportion of those subjects who were retested, and the proportion of those who reverted to negative. Among subjects with a positive initial TB test who were retested, prevalence of TB symptoms and first TB Ag-nil IFN-γ values were compared between subjects who reverted to negative and those who did not. We hypothesized that subjects who reverted had lower first TB Ag-nil IFN-γ values than those who did not, and also had no history of TB or risk factors, and were born in a low TB-incidence country. The goal was to develop a more efficient method for managing positive initial QFT Gold TB tests in PVAMC employees, benefitting both employees and the PVAMC, as cost for an IGRA is approximately $31, nearly three times the cost of TST (Nienhaus et al. 2011).

METHODS

This study was conducted at the PVAMC in Portland, Oregon and was approved as a Quality Improvement/Quality Assurance project by the PVAMC ACOS/Research Department. All new employees and volunteers must undergo TB screening in the Employee Health department, but not everyone undergoes a QFT Gold blood test (Figure 1). Those who have been diagnosed with TB in the past may be required to obtain a chest X-ray without submitting to the QFT Gold test. Following a positive QFT test in employees who have a history of past positive TB test, Employee Health may forego a

second test and instead rely on the symptom screen and/or require a chest X-ray for follow-up (Department of Veterans Affairs), however, during this analysis, it was determined that not every employee fell under these guidelines.

Figure 1: TB screening



Since August 2010, employee screening has been performed using the QFT Gold TB test. Blood is drawn in Employee Health and the PVAMC microbiology lab sends samples to the Oregon State Public Health Laboratory for processing. Results are returned to the PVAMC microbiology lab and are subsequently placed in the employee's record. In March 2012, the PVAMC moved from a shorter questionnaire containing very basic information regarding TB status and symptoms to a lengthier questionnaire that follows CDC guidelines more closely (Appendix B).

Study cohort

Among employees who were tested with QFT Gold, our study cohort included any employee who tested TB positive initially and was retested. As this project examined employees with an initial positive test, we did not consider employees with

indeterminate or negative first TB Ag-nil IFN-γ (negative as defined by manufacturer as TB Ag-nil less than 0.35 IU/mL IFN-γ). On September 27, 2012, the manufacturer of the QFT Gold TB test, Cellestis Inc, recalled QFT Gold collection tubes of a certain lot number. Upon learning of the recall, negative results from these lots were retested by the PVAMC and found to be reliable, however, there were some tubes that produced false positives. The manufacturer could not determine the tubes within affected lots that were prone to false positives and entire lots were recalled. We examined data from the subset of subjects tested during the recall period separately from our study cohort.

The microbiology laboratory at the PVAMC provided an electronic spreadsheet with subject name, last four digits of social security number, and all numeric QFT Gold TB test results from August 1, 2010 – January 31, 2013. For ease of comparison, TB Ag-nil IFN-γ values greater than 10 IU/mL were replaced with a value of 10 IU/mL. Employee Health provided an electronic spreadsheet of employee names with positive QFT TB tests. We merged these files into an electronic spreadsheet of continuous numeric QFT results data for PVAMC employees.

We extracted questionnaire data from electronic medical charts in the PVAMC Computerized Patient Records System database and hard copy charts located in Employee Health. Data acquisition was conducted in accordance with HIPAA regulations and rules set forth by the Chief of Employee Health. Following data gathering, a random number generator was employed to create a four-digit case number for each subject and identifiers including name and social security number were removed, to prevent identifiable protected health information from leaving Employee Health.

Demographic variables collected for subjects included sex, age, country of birth, and U.S. military veteran status (yes or no). Employee Health does not track race or ethnicity. To better assess past TB history, we created the dichotomous variable "History of LTBI" by combining variables past positive TB test, past TB diagnosis, past use of INH, and past chest X-ray because of TB diagnosis. A subject was considered to have a history of TB with a "yes" response to at least one variable. To examine an association between birth in a high-TB incidence country and reversion, we created the dichotomous variable "foreign born." A subject was considered born in a high TB-incidence country if they were born outside of the U.S.A., Canada, or Western Europe.

Statistical Analysis

Reversion was the dichotomous outcome measure examined in this analysis. Because we were examining continuous and categorical variables that could predict reversion to TB negative, t-tests, Chi square tests, and logistic regression were planned for these data. We calculated summary data including frequency and percent of total for all dichotomous and nominal categorical variables; and mean, median, standard deviation, and range for continuous variables age and TB Ag-nil IFN-γ values. Chi square tests or Fisher's exact test were conducted with individual categorical variables as predictors for reversion. First TB Ag-nil IFN-γ was categorized at different levels, and chi square tests were conducted with each of these variables as predictors of reversion. Distribution of first TB Ag-nil IFN-γ and age variables was examined. First TB Ag-nil IFN-γ was skewed, and was log-transformed to determine if this transformation would provide a better fit for our model than the raw untransformed variable (Appendix C). Coding of all variables is shown in Appendix D.

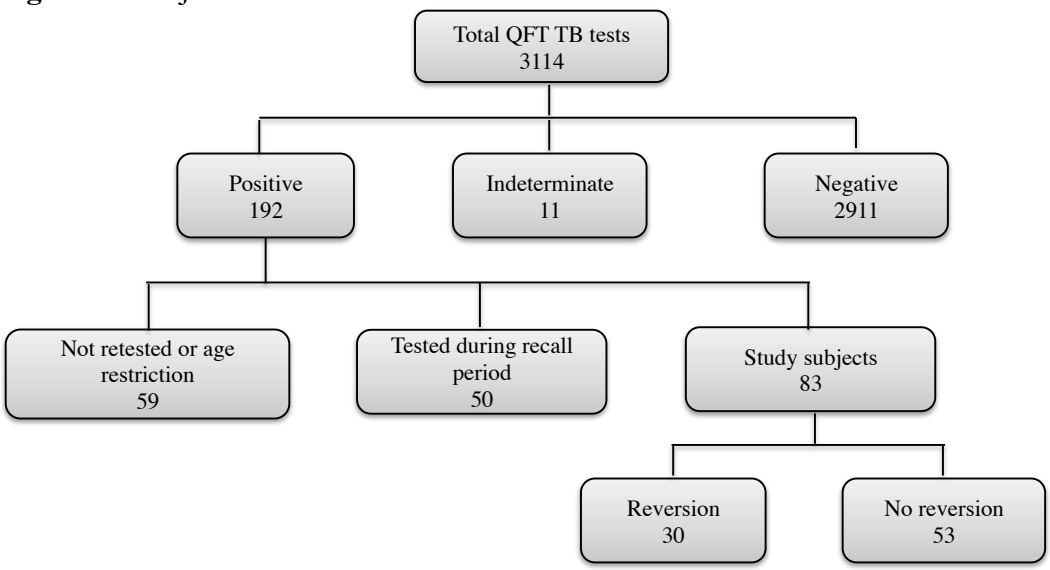
To determine an appropriate model that predicts test reversion, we performed univariate and multivariate logistic regression and examined a possible interaction between first TB Ag-nil IFN-γ values and age. We included in a multivariate model all covariates with univariate significance less than 0.25. Variables that were not significant in the multivariate model and did not change the odds ratio of the primary predictor variable 10% or more were removed from the model. Odds ratios and their 95% confidence intervals were estimated from the logistic regression models. Four models with the best predictive value were selected. We assessed the fit of these potential models using the Hosmer-Lemeshow goodness of fit test. Strength of each model was assessed using the Akaike Information Criterion (AIC), which compares models based on -2 Log likelihood, and the model with the lowest AIC value was selected.

Torres-Costa et al. (2012) found that 49% of 55 subjects whose initial positive QFT Gold test was 0.35-0.7 IU/mL reverted, while just 12.4% of 153 subjects whose initial positive QFT Gold test was greater than 0.7 IU/mL reverted. A second study with a sample size of 42 had similar reversion proportions with the low first TB Ag-nil IFN-γ, but the reversion proportion for the higher first TB Ag-nil IFN-γ was 19% (Schablon et al. 2010). As this sample was quite small, we used the Torres-Costa study for power calculations. The estimated sample size required for a two-sample comparison of proportions is 48, where p1=0.49 and p2=0.12, with 80% power and $\alpha = 0.05$. Analyses were performed with Stata, version12 (Stata Corp, TX).

RESULTS

A total of 3114 HCW underwent initial TB screening with the QFT Gold at the PVAMC between August 1, 2010 and January 31, 2013. The initial QFT Gold test found that 6.2% of subjects were positive, 93.5% were negative, and 0.35% subjects were indeterminate (Figure 2). Of the 192 subjects who tested positive, two did not meet the age requirement of 18 years old and 57 were not retested. Employee Health did not retest these subjects because they had a high likelihood of second positive test due to past positive TB test, and most were referred to their physician for chest X-ray. Up to 50 tests may have been affected by product recall May 1 - October 4, 2012, which could have falsely increased the proportion of positive tests. Subjects tested during the recall period had significantly higher proportion of reversion than those tested outside the recall period ($X^2_1 = 12.68$; $p<0.0001$) (Appendix G). Out study cohort consisted of 83 subjects who tested positive with the non-recalled QFT Gold TB test and were retested with non-recalled QFT Gold TB test.

Figure 2: Subject selection



The mean age (± SD) of all subjects was 47.6 years (±16), and they ranged in age from 21 to 79 years old (Table 1). Those who reverted were significantly younger than those who did not (t=2.192; p=0.0313). Forty percent of subjects were women and 41% were military veterans. Twenty percent of reversions and 16% of non-reversions had history of TB (past positive TB test, past TB diagnosis, past use of INH, and/or past chest X-ray because of TB diagnosis). One subject (1.6%) had prior BCG vaccination. Foreign birth was reported for 17% of reversions and 18% of non-reversions.

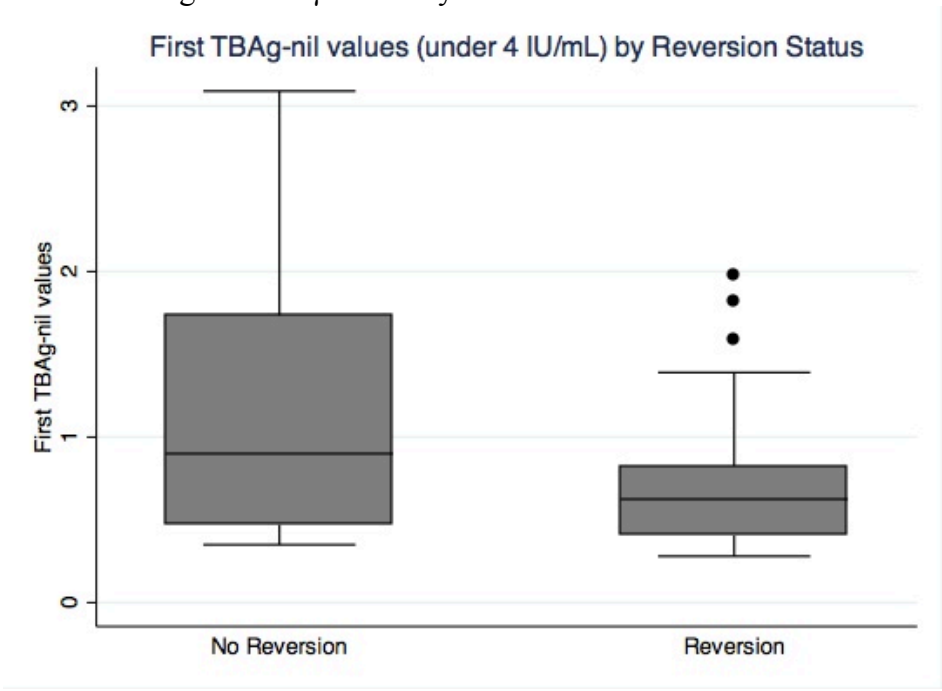
Symptoms and risk factors for TB were also assessed with the TB-screening questionnaire. One subject (2%) reported a recent history of night sweats. No positive responses were given for inappetence, cough lasting 2 weeks, weight loss, fever, or hemoptysis. Five subjects who did not revert (6.1%) reported a TB exposure within the past two years. Five subjects who did not revert (6.1%) reported a prior TB diagnosis; three confirmed a diagnosis of latent TB, and two did not report their type of TB diagnosis. Five subjects who did not revert (6.2%) reported past use of INH. No subjects who reverted answered positive to any of these questions.

In March 2012, Employee Health expanded their questionnaire to contain CDC-recommended questions regarding immunity-related conditions that might affect the test results. The employees administered these questions, therefore, were limited to those tested March 2012 and after. Seven (24.1%) of 29 subjects reported a past chest X-ray because of a positive test; six of those did not revert. One subject who did not revert (3.9%) reported having close contact with someone with tuberculosis. One subject who did not revert (4%) reported being a smoker. No subjects reported AIDS, renal failure, diabetes mellitus, use of glucocorticoids, cancer of the head or neck, a TB test conversion

in the past two years, abnormal chest X-ray from fibronodular disease or granulomatous disease, treatment with tumor necrosis factors, silicosis, HIV, use of immunosuppressants following organ transplantation, or being less than 90% of ideal weight.

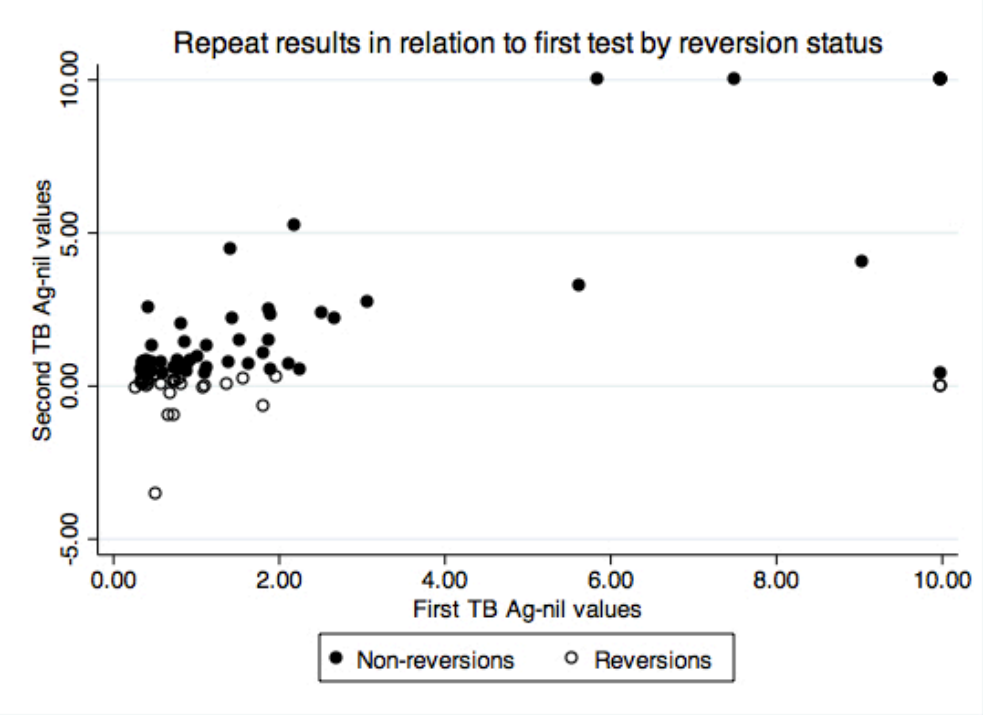
Of 83 subjects who initially tested TB positive with QFT-Gold, 30 (36.1%) reverted to negative upon repeat testing (Table 1). The mean and median of the first test value (first TB Ag-nil IFN-γ) for those who reverted were 1.36 and 0.69 IU/mL IFN-γ, respectively (range 0.35-10 IU/mL); and for those who did not revert mean and median were 2.42 and 1.15 IU/mL IFN-γ (range 0.35-10 IU/mL) (Figure 3). Distribution of the variable first TB Ag-nil IFN-γ was skewed and upon log-transformation, the relationship between ln-first TB Ag-nil IFN-γ and reversion status was significant (t=2.5; p=0.0146). The mean and median of the second TB Ag-nil IFN-γ for reversions was -0.028 IU/mL and 0.05 IU/mL IFN-γ respectively (range -3.56 to 0.34 IU/mL); and for non-reversions were 2.29 IU/mL and 0.94 IU/mL IFN-γ respectively (range 0.36-10 IU/mL).

Figure 3: First TB Ag-nil IFN-γ values by reversion status



The mean and median difference between the first and second TB Ag-nil IFN-γ values for reversions were -1.07 IU/mL and -0.655 IU/mL IFN-γ respectively (range -0.1 IU/mL to -10.01 IU/mL); and for non-reversions were -0.17 IU/mL and -0.03 IU/mL IFN-γ respectively (range -9.64 IU/mL to 4.34 IU/mL) (Figure 4).

Figure 4: First and second TB Ag-nil IFN-γ values by reversion status



Univariate logistic regression models with "reversion" as the dichotomous outcome variable were examined (Table 1). Sex and veteran status were not significantly associated with reversion, nor were past positive TB test and night sweats. The log-transformed variable "first TB Ag-nil" was significant in predicting reversion, as was age, when incorporated individually as the single predictor in a simple logistic regression model.

Prior studies have suggested a grey area above and close to the manufacturer's cut point of 0.35 IU/mL IFN-γ consistent with false positives. To examine this possibility, the "First TB Ag-nil" IFN-γ variable was converted into three categorical measures, and

subjects with first TB Ag-nil values below each of three cut-points (0.7, 0.8, and 0.9 IU/mL IFN-γ) were significantly more likely to revert. Table 2 (below) contains summary data for those who reverted and those who did not at these cut points.

Table 1: Characteristics of subjects who tested TB positive with QFT-Gold test, by reversion status; and univariate analysis comparing reversions and non-reversions

Univariate analysis				
Variable	**Reversions n=30**	**Non-reversions n=53**	**OR (95% CI)**	**p**
ln-First TB* mean (SD)	-0.26 (0.87)	0.3 (1.04)	0.523 (0.303-0.902)	0.0108
First TB Ag-nil ≥ 0.7	15 (50)	38 (72)	0.395 (0.155-1.003)	0.0494
First TB Ag-nil ≥ 0.8	10 (33)	36 (68)	0.236 (0.091-0.693)	0.0022
First TB Ag-nil ≥ 0.9	8 (27)	32 (60)	0.239 (0.090-0.635)	0.0027
Age mean (SD)	42.7 (14.6)	50.4 (15.8)	0.968 (0.939-0.998)	0.0293
Female	11 (37)	22 (41.5)	0.816 (0.324-2.051)	0.6642
Veteran	10 (33)	24 (45)	0.604 (0.238-1.534)	0.2847
History TB	6 (20)	8 (16)	1.344 (0.417-4.331)	0.6226
Foreign born	5 (17)	9 (18)	0.972 (0.292-3.237)	0.9634

*log-transformed first TB Ag-nil values (IU/mL) (addressing non-normal distribution of variable)

Table 2: summary data for 3 cut points based on reversion status

First TB Ag-nil value	Reversion n (%)	Non-reversion n (%)	Total n (%)
<0.7 IU/mL	15 (50)	15 (50)	30 (100)
≥0.7 IU/mL	15 (28.3)	38 (71.7)	53 (100)
<0.8 IU/mL	20 (54)	17 (46)	37 (100)
≥0.8 IU/mL	10 (21.7)	36 (78.3)	46 (100)
<0.9 IU/mL	22 (51.2)	21 (48.8)	43 (100)
≥0.9 IU/mL	8 (20)	32 (80)	40 (100)

A multivariable logistic regression model relating all variables with univariate significance less than 0.25 was created (Appendix E). As individual predictors, only the ln-first TB Ag-nil IFN-γ values, each of the three dichotomous first TB Ag-nil IFN-γ variables, and age were significant in a simple logistic regression model. Variables that might predict LTBI, history of TB (past positive TB test, past TB diagnosis, past use of INH, and/or past chest X-ray because of TB diagnosis), and foreign birth were of no

significance in the model, as no added variable changed the odds ratio of the primary predictor by greater than 10%. The possibility that advanced age could lead to decreased immune function for some subjects, affecting TB Ag-nil IFN-γ values, was considered, and an interaction term between the two was examined, though it was not significant in any of the models examined. Multiple logistic regression determined that, after adjusting for age, ln-first TB Ag-nil IFN-γ was significant in predicting reversion, as were each of the dichotomous first TB Ag-nil IFN-γ variables.

The goodness of fit of each of these four models was assessed, and the high p-value was an indication that the observed and expected values were close, and the models fit the data. The area under a ROC curve plot of each model indicated they all had acceptable discriminative ability.

When adjusting for age and TB risk factors, ln-first TB Ag-nil IFN-γ value was significant in predicting reversion ($X^2_2 = 10.07$; p=0.0065). After adjusting for age and TB risk factors, the odds of reversion were 55% lower for subjects whose first TB Ag-nil IFN-γ was equal to or greater than 0.7 IU/mL ($X^2_2 = 7.47$; p=0.0239); 75% lower for subjects whose first TB Ag-nil IFN-γ was equal to or greater than 0.8 IU/mL ($X^2_2 = 13.02$; p=0.0015); and 74% lower for subjects whose first TB Ag-nil IFN-γ was equal to or greater than 0.9 IU/mL ($X^2_2 = 12.27$; p=0.0022).

After assessing the fit of the four prospective models, they were compared against one another using the AIC method. The strongest model with the lowest AIC value was the model categorizing TB Ag-nil into a dichotomous measure of +/- 0.8 IU/mL IFN-γ while adjusting for age. Below this cut point in our study cohort, 54% of 37 subjects reverted to negative, but above the cut point, just 22% of 46 subjects reverted (Table 2).

The predicted probability of reversion for subjects with first TB Ag-nil IFN-γ less than 0.8 IU/mL is 0.54, or 54%. For those with first TB Ag-nil IFN-γ equal to or greater than 0.8 IU/mL the predicted probability of reversion is 0.22, or 22%.

The predicted probability of reversion was 50% or higher for 24 of 37 (65%) subjects when age-adjusted first TB Ag-nil IFN-γ was less than 0.8 IU/mL. No subjects with age-adjusted first TB Ag-nil IFN-γ greater than 0.8 IU/mL had a predicted probability of reversion over 38%.

Recall subjects

The removal of 50 subjects tested during the 2012 recall window decreased our sample size to 83. The proportion of reversion for the 50 subjects testing positive in the recall was 72%, significantly higher than the reversion proportion of the group tested with non-recalled tests ($X^2_1 = 12.68$; $p<0.0001$; Appendix F, G). A model with a dichotomous cut-off of 0.6 IU/mL IFN-γ (not significant in our cohort) was highly significant when the recall subjects were included. The influence of incorporating the recall group into our study cohort (increasing the sample size to 133) would have greatly increased the significance of our model, and likely would have changed it altogether.

DISCUSSION

We examined characteristics and test results of subjects who initially tested TB positive using the QFT Gold test at the PVAMC, in order to determine what, if any factors predict test reversion from positive to negative upon repeat testing. Subjects with first TB Ag-nil IFN-γ values equal to or greater than 0.8 IU/mL were significantly less likely to revert to negative upon retesting using the QFT Gold TB test. Past history of TB

(past positive TB test, past TB diagnosis, past use of INH, and/or past chest X-ray because of TB diagnosis), country of birth, and symptoms associated with TB were not significantly associated with reversion in this study. Age was not a significant confounder in our model. Our hypothesis that those who reverted back to negative upon retesting had levels of blood interferon gamma closer to the manufacturer's cut-off was supported. The hypothesis that those who reverted were born in low TB-incidence countries and had no history of TB was not supported, however.

The lack of a gold standard for diagnosing LTBI means that determining the sensitivity and specificity of the QFT Gold test is difficult at best, and some have questioned its value in low-incidence settings (Kleinert et al. 2012). There is disagreement regarding appropriate blood IFN-γ cut-offs using QFT Gold for serial testing of HCW in low-incidence settings (Zwerling et al. 2012). Torres-Costa et al. (2011) found that 49% of Portuguese HCW with first TB Ag-Nil IFN-γ levels 0.35-0.70 IU/mL reverted to negative when retested, and subsequently recommended that healthy, immunocompetent individuals in low-incidence settings with initial blood IFN-γ values between 0.2-0.7 IU/mL should be retested prior to treatment for LTBI. Schablon et al. (2010) examined four different definitions for conversion and reversion in German HCW. They found that the use of a simple cut-point of 0.35 IU/mL TB Ag-Nil IFN-γ levels for positive tests had the highest proportion of reversion, and recommended considering retesting test results in an "uncertainty zone" around the manufacturer's cut-point of TB Ag-nil IFN-γ levels 0.2-0.7 IU/mL.

Little is known about the biological basis for conversions and subsequent reversions with QFT Gold TB test. Pai (2010) argues that, rather than 2 definitions for

TB disease (active vs. latent), TB disease falls along a continuous spectrum and that conversions and reversions with QFT might be indicative of transitions occurring within this spectrum, further questioning the value of a strict cut-point.

Past history of TB, country of birth, symptoms associated with TB and an interaction between age and first TB Ag-nil value were not significant in this study. This may be due to the lack of retest of subjects who tested positive who were deemed to have a high likelihood of remaining TB positive because of TB risk factors, to the generally good health of the employee subjects in this study, and the low incidence of TB in the Portland metro area. Prior studies indicated that many covariates result in a positive QFT Gold test in HCW, including age, sex, type of job, travel outside the country, timing of BCG, and being a HCW in a foreign country (Zwerling et al. 2012).

The proportion of positive initial QFT Gold TB screening tests in HCW at the PVAMC does not correspond with the fact that positive TST prevalence was less than half that prior to switching to QFT Gold, though positive QFT Gold prevalence is consistent with other studies. A strong association was found between first TB Ag-nil value and reversion. This study showed that subjects whose initial test result was within 0.35-0.79 IU/mL IFN-γ should be retested. Subjects whose initial QFT Gold test results are above this level are significantly less likely to revert to negative.

Sensitivity, specificity, PPV, and NPV of QFT Gold

The gold standard for diagnosing active TB is culture of *M. tuberculosis*, but there is no gold standard for diagnosing LTBI. This makes assessing the utility of using the QFT Gold TB test for diagnosing LTBI impossible. The European Centre for Disease Prevention and Control (2011) conducted a meta-analysis with studies using newly

diagnosed active TB patients as surrogates for LTBI subjects. They found that the pooled sensitivity of QFT Gold across studies was 67%, and specificity was 99.4%. Pooled positive predictive value of QFT Gold for progression of active TB was 2.8-14.6%, while negative predictive value of QFT Gold for no progression to active TB was 88%. We do not have information tracking the subjects with LTBI that converted to active TB at the PVAMC, and cannot make any conclusions about the PPV of QFT Gold.

Strengths and Limitations

Our study design was simple and low cost yet provided a great deal of information about its subjects. The sample consisted of healthy, immunocompetent HCW, and could be generalizable to HCW without TB risk factors in other healthcare facilities in the U.S. The results of this study are consistent with results from prior studies, in that we determined a grey area above the manufacturer's cut-off that is suspect for false positives. The study, however, may have been limited by the small sample size. Because there is no gold standard for confirming LTBI, we could not confirm that the persistent positive subjects were LTBI-positive.

It is unlikely this association is due to chance, as the QFT Gold TB test uses specific TB antigens that would be extremely unlikely to pick up other NTM infection. All employees and volunteers are subject to TB screening protocols at the PVAMC. On that level, selection bias is not a concern. However, not all positive QFT Gold TB tests are retested – in our population, over 25% were not retested. Those who were not retested were generally subjects with TB risk factors and high likelihood of a repeat positive TB test. This could be selection bias as these subjects were considered by Employee Health to be unlikely to revert upon retesting and were excluded from further

testing, therefore not included in our study. The removal of these potential retest subjects could have led to a falsely higher proportion of reversion. In fact, if these 57 subjects were retested and did not revert, the reversion proportion would have been 21.4% rather than 36.1%. When 43 subjects with reported TB Ag-nil values were included in the predictive model, the predictive model using the cutoff of 0.8 IU/mL was highly significant. The removal of these subjects who were at a higher risk for LTBI left a subject pool that largely did not have TB risk factors. The conclusions and recommendations, subsequently, are meant for subjects who are healthy and immunocompetent, with no TB risk factors.

Recall bias may be of concern in this study. In examining questionnaires, there were several instances in which a subject responded "yes" to a question about something related to a positive TB test (e.g. past INH use or past chest X-ray due to TB diagnosis), but answered "no" to the question about ever having a positive TB test. The creation of the variable "History of LTBI" took that into account by compiling all the questions related to a positive TB test.

Human error may have caused errors in sampling and processing, and misclassification of some blood test results. Blood is drawn by Employee Health nurses, and is transferred to an off-campus site for processing. Two instances of repeat tests labeled as "negative" by the lab were actually positive (TB Ag-nil $\geq$0.35 IU/mL IFN-γ), and were adjusted in the data prior to analysis. These were only discovered because they had an initially positive test. It is unknown if any initial positive results were mistakenly classified as negative, as negative results were not examined in this analysis.

Some complex medical terminology on the questionnaire may be challenging for those whose native language is not English, which could have underestimated positive responses. An underestimation of responses to covariates may have kept potential confounders or effect modification from being understood and may explain in part the simplicity of the model. Another potential limitation is the lack of race and ethnicity data. These demographic variables could be associated with LTBI, but PVAMC does not track this information. Finally, though it was adequately powered, the small sample size may have been a limitation in this analysis and a study with a larger sample size may have led to a more complex model.

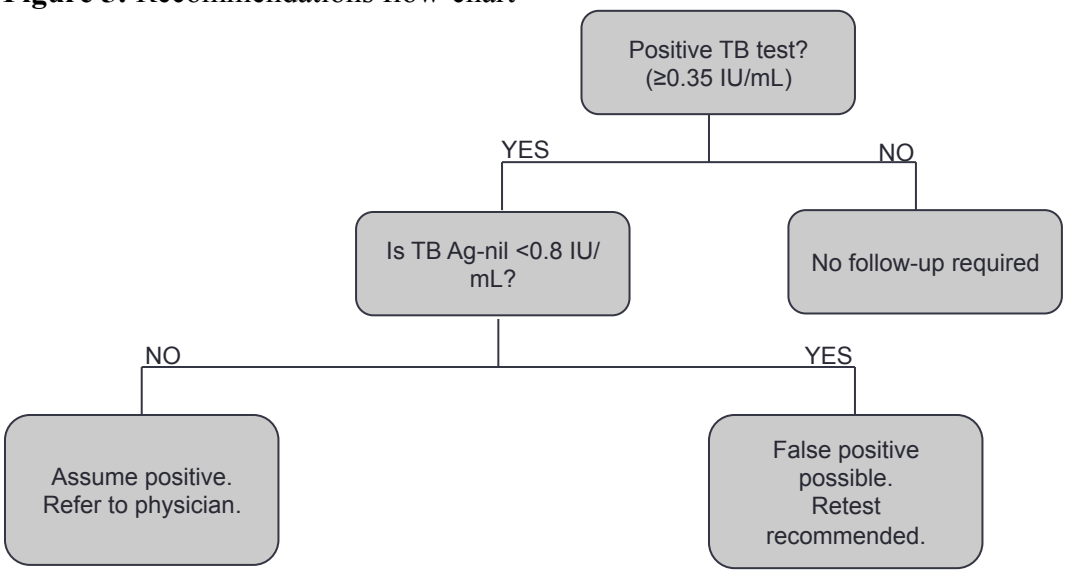
RECOMMENDATIONS

The manufacturer of QFT-Gold TB test (Cellestis Inc) recommends that any value above 0.35 IU/mL blood IFN-γ be considered a positive test. Our study found that subjects with positive results between 0.35-0.79 IU/mL IFN-γ are highly likely to revert to negative upon retesting. In fact, 67% of subjects who reverted had a first TB Ag-nil IFN-γ value less than 0.8 IU/mL, while 32% of subjects who did not revert had a first TB Ag-nil IFN-γ value less than 0.8 IU/mL.

We recommend considering healthy, immunocompetent employees with no TB risk factors whose positive first TB Ag-nil IFN-γ value is less than 0.8 IU/mL likely false positive (Figure 5). These subjects should be retested, as 46% of subjects in our study who tested within this frame did not revert upon retest. Those subjects whose first TB Ag-nil IFN-γ value is greater than 0.8 IU/mL IFN-γ were significantly less likely to revert upon retest and should be referred to a physician for evaluation of LTBI. In our

study, 78% of those whose first QFT TB test was over 0.8 IU/mL IFN-γ did not revert upon retest.

Figure 5: Recommendations flow-chart



For the PVAMC in particular, if a veteran employee tests positive, knowledge of deployment locations would be useful to determine if the subject was in a high TB-incidence location, though this is not currently tracked on the TB-screening questionnaire. Human resources at the PVAMC are striving to reach 35% veteran employment, and as conflicts overseas wind down and veterans return to the U.S., more may be seeking employment in the VA system. In our study, 34 (41%) of the subjects in this study were veterans and 24 (71%) of those veterans did not revert, and deployment information in the context of TB screening may be useful information to consider.

An alternative to the recommendations is a calculator for predicted probability of reversion based on either the cut point of 0.8 IU/mL or ln-first TB Ag-nil IFN-γ values (Appendix H). This might offer an advantage if Employee Health has a specific predicted probability of reversion above which they would decide to retest subjects. The predicted probability of reversion when using the cut point of 0.8 IU/mL IFN-γ is 22% if

over the cut point and 54% if under the cut point. Use of the continuous ln-first TB Ag-nil IFN-γ variable in the predicted probability of reversion calculator could provide a more accurate predicted probability of reversion for each individual subject, but would be more complicated to determine mathematically.

SUMMARY AND CONCLUSION

In our study, healthy, immunocompetent PVAMC employees with no TB history or risk factors whose first TB Ag-nil IFN-γ values using the QFT Gold TB test were 0.35-0.79 IU/mL had high proportion of reversion (indicating false positive tests), and should be retested prior to referral for LTBI evaluation. Employees with first TB Ag-nil IFN-γ higher than 0.8 IU/mL have significantly lower proportion of reversion. These subjects should be referred to their physician for LTBI evaluation.

PUBLIC HEALTH IMPLICATIONS

A positive TB test is distressing for an employee and expensive for employee and employer. Management of positive tests by using these recommendations could streamline the TB screening process; potentially reducing unnecessary retesting on those likely to stay positive, and reducing LTBI treatment on those likely to revert.

FUTURE STUDIES

This study can serve as preliminary work for the PVAMC and potentially other healthcare facilities that use the QFT Gold TB test for employee TB screening. Veterans may have a higher probability of being exposed to TB if deployed to high-incidence locations, and potentially an increased risk of contracting LTBI. This study did not find an association between veteran employees and reversion, but this may be an area of study worth further examination with a larger sample, particularly with information about deployments.

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Appendix A: QuantiFERON-TB Gold interpretation flow chart (Cellestis Inc)

Nil (IU/mL)*	TB Antigen minus Nil (IU/mL)	Mitogen minus Nil** (IU/mL)	QFT result	Interpretation
≤8.0	<0.35	≥0.5	Negative	MTB infection unlikely
	≥0.35 & <25% of Nil	≥0.5		
	≥0.35 & ≥25% of Nil	Any	Positive	MTB infection likely
	<0.35	<0.5	Indeterminate	Indeterminate for TB antigen responsiveness
	≥0.35 & <25% of Nil	<0.5		
>8.0	Any	Any		

* Nil - negative control (no antigens)
** Mitogen - positive control

Cellestis, Inc

Appendix B: PVAMC Employee Health TB questionnaire

Portland VA Medical Center Tb Screening

Please return this form to Employee Health in person or send it through Inter-Office mail (mail code P5OCCH) using a confidential envelope. If you do not understand what the form is asking, please speak with an Employee Health Nurse, Bldg. 101 Room 127 or Ext. 55165.

Applicant Name: _____ Date: _____

Date of Birth: _____ Last 4 digits of SS#_____

Job/Work Title: _____

Part I: Tb Symptom Assessment

Have you ever had a positive Tb test? ☐ YES ☐ NO

If yes, when? _____

Do you currently have any of the following which are UNEXPLAINED by other causes?

Tiredness lasting more than 3 weeks?	☐ YES	☐ NO
Loss of appetite (not being hungry)?	☐ YES	☐ NO
Cough lasting for more than 2 weeks?	☐ YES	☐ NO
Night sweats?	☐ YES	☐ NO
Weight loss?	☐ YES	☐ NO
Fever (usually at night)?	☐ YES	☐ NO
Coughing up blood?	☐ YES	☐ NO

Part II: Tb Risk Assessment

1. What is your country of birth? _____
2. If not born in U.S., how old were you when you moved to this country? _____
   Have you received a BCG vaccination?_____
3. Have you had a possible or known exposure to Tb in the last 2 years? ☐ YES ☐ NO

   If yes, date? _____

   Was your exposure at ☐work ☐home ☐military service ☐other
4. Have you ever been told you have Tb? ☐ YES ☐ NO

   If yes, date? _____

   Were you told your Tb was ☐active ☐latent ☐don't know

5. Have you ever taken a medication called INH? ☐ YES ☐ NO

If yes, when? _____ For how long? _____

Name and location of prescribing provider: _____

6. Have you ever had a chest x-ray because of a positive Tb test?* ☐ YES ☐ NO

If yes, what is the date of your last chest x-ray? _____

Have you had any major health changes (see #8) since your last chest x-ray? ☐ YES ☐ NO

Can you provide us with a copy of your Tb test and/or chest x-ray?

☐YES ☐NO ☐Already on file with PVAMC Employee Health

7. In the last **TWO** years, have you had **CLOSE** contact with anyone with active Tb? ☐ YES ☐ NO

Close contact is defined as someone with active Tb living in the household, or a non-household member with whom there is regular contact (more than 4 hours per week).

8. Please select all the conditions below that apply to you. If none of the conditions apply, please check the box at the bottom that says, "None of these."

☐ AIDS

☐ Treatment with glucocorticoids (a type of steroid)

☐ Chronic renal failure requiring dialysis

☐ Any type of Diabetes Mellitus

☐ A Tb test (skin or blood test) that converted to positive in the last 2 years

☐ Abnormal chest x-ray showing fibronodular disease

☐ Treatment with Tumor Necrosis Factor (TNF) – alpha inhibitors such as Infliximab or Etanercept

☐ Silicosis

☐ Infected with Tb at a young age (0-4 years old)

☐ Cancer of the head or neck

☐ Cigarette smoker (greater than 1 pack/day)

☐ HIV infection

☐ Organ or tissue transplant requiring suppression of your immune system

☐ Abnormal chest x-ray showing granuloma or old granulomatous disease

☐ Underweight (less than 90% of ideal body weight or body mass index (BMI) leess than or equal to 20

☐ None of these

**EH Staff, do not order Quantiferon testing for applicants with YES responses to these questions. Request copy of last positive Tb test and subsequent chest x-ray. If applicant is not able to provide, please order a chest x-ray for the applicant. Review SF 93 Health History for information r/t immunosuppressive medications or diseases or discuss with applicant/employee. Make OHP-Tb note documenting findings and scan this form to that note.*

Appendix C: stem and leaf plot showing distribution of first TB Ag-nil values

```
Stem-and-leaf plot for stTBAgnil (1st TBAg-nil)

stTBAgnil rounded to nearest multiple of .01
plot in units of .01

   0** | 28,35,35,36,36,36,36,36,36,37,38,38,39,39,40,41,42,43,44,44,47,47,48
   0** | 50,52,52,58,59,62,67,71,74,74,74,75,77,78,81,81,84,84,85,87,90,90,95
   1** | 02,10,11,11,15,15,39,41,43,44
   1** | 53,58,65,82,82,88,89,92,92,97
   2** | 14,20,27
   2** | 53,69
   3** | 09
   3** |
   4** |
   4** |
   5** |
   5** | 64,86
   6** |
   6** |
   7** |
   7** | 51
   8** |
   8** |
   9** | 04
   9** |
  10** | 00,00,00,00,00,00,00
```

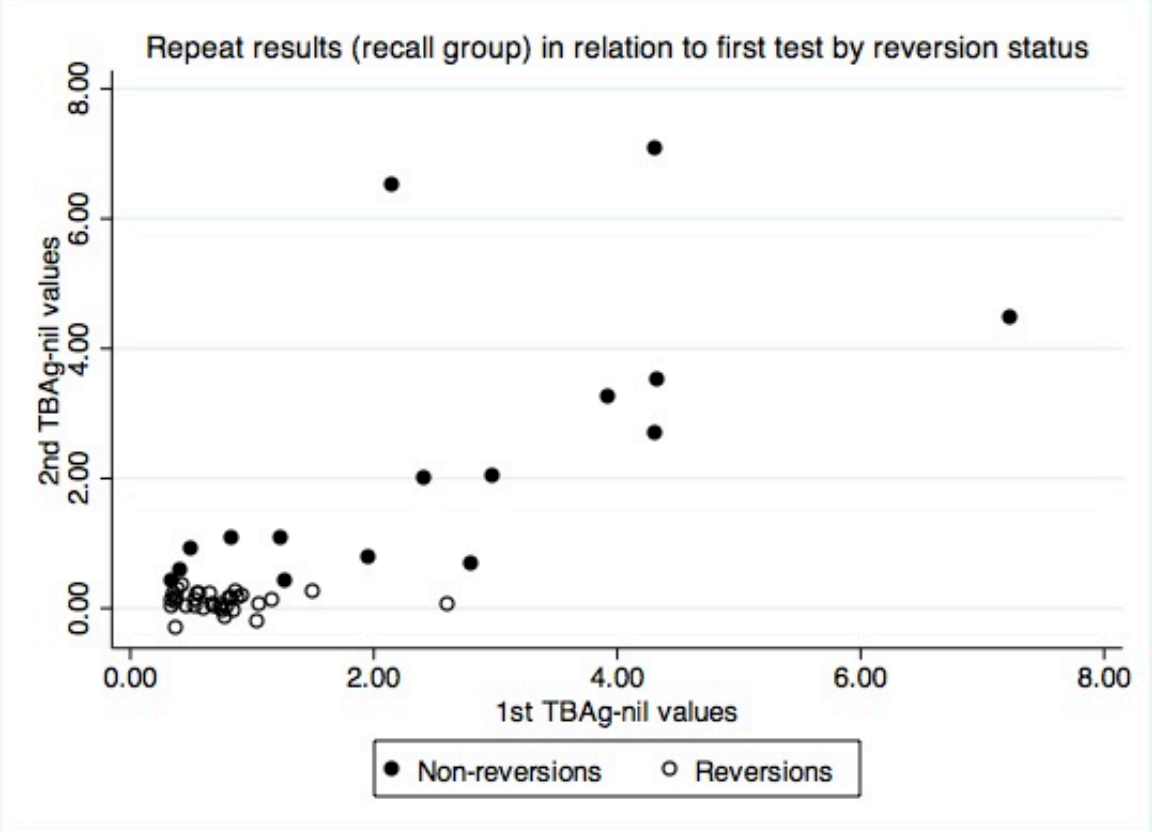
Appendix D: Variable coding

Variable coding		
Measure	**Possible responses**	**Coding for analysis**
Reversion	Y/N	0=No 1=Yes
First TBAg-nil values	Continuous	Continuous
First TBAg-nil value >= 0.7 IU/mL	Y/N	0=No 1=Yes
First TBAg-nil value >= 0.8 IU/mL	Y/N	0=No 1=Yes
First TBAg-nil value >= 0.9 IU/mL	Y/N	0=No 1=Yes
Age	Discrete numerical	Continuous
Sex	M/F	0=Male 1=Female
Veteran	Y/N	0=No 1=Yes
Past Positive TB test	Y/N	0=No 1=Yes
BCG vaccination	Y/N	0=No 1=Yes
Country of Birth	Name	Nominal
Tired > 3 weeks	Y/N	0=No 1=Yes
Poor appetite	Y/N	0=No 1=Yes
>2 week cough	Y/N	0=No 1=Yes
Night sweats	Y/N	0=No 1=Yes
Fever	Y/N	0=No 1=Yes
Weight loss	Y/N	0=No 1=Yes
Coughing up blood	Y/N	0=No 1=Yes
TB exposure within 2 yrs	Y/N	0=No 1=Yes
Prior TB diagnosis	Y/N	0=No 1=Yes
Past use of INH	Y/N	0=No 1=Yes
Past chest X-ray due to positive test	Y/N	0=No 1=Yes
Close contact with TB	Y/N	0=No 1=Yes
Use of glucocorticoids	Y/N	0=No 1=Yes
Cancer of head or neck	Y/N	0=No 1=Yes
Smoker	Y/N	0=No 1=Yes
AIDS	Y/N	0=No 1=Yes
Renal failure	Y/N	0=No 1=Yes
Diabetes Mellitus	Y/N	0=No 1=Yes
TB conversion within 2 years	Y/N	0=No 1=Yes
Abnormal chest X-ray-fibronodular disease	Y/N	0=No 1=Yes
Abnormal chest X-ray-granulomatous disease	Y/N	0=No 1=Yes
Treatment with TNF	Y/N	0=No 1=Yes
Silicosis	Y/N	0=No 1=Yes
HIV	Y/N	0=No 1=Yes
Use of immunosuppressants	Y/N	0=No 1=Yes
Underweight	Y/N	0=No 1=Yes
History of LTBI	Y/N	0=No 1=Yes
Foreign born	Y/N	0=No 1=Yes

Appendix E: Multiple regression models examined

Logistic Regression Models									
Models with Reversion outcome	**N**	**Chi2(1)**	**P-value**	**OR**	**SE**	**z**	**p-value**	**CI**	**AIC value**
Overall Model	83	10.07	0.0065						104.54
ln-1st TB Ag-nil				0.548	0.154	-2.14	0.032	0.316-0.951	
Age				0.971	0.016	-1.85	0.064	0.941-1.002	
Overall Model	83	7.47	0.0239						107.14
1st TB ≥ 0.7 IU/mL				0.446	0.218	-1.65	0.099	0.171-1.165	
Age				0.971	0.015	-1.86	0.063	0.942-1.002	
Overall Model	83	13.02	0.0015						101.59
1st TB ≥ 0.8 IU/mL				0.249	0.124	-2.8	0.005	0.094-0.659	
Age				0.97	0.016	-1.87	0.062	0.94-1.002	
Overall Model	83	12.27	0.0022						102.34
1st TB ≥ 0.9 IU/mL				0.26	0.132	-2.65	0.008	0.096-0.705	
Age				0.971	0.016	-1.78	0.075	0.941-1.003	

Appendix F: Recall group (n=50) first and second TB Ag-nil IFN-γ values by reversion status



Appendix G: Comparison of 3 sample populations

	Sample minus recalls (n=83)	Recall group (n=50)	Full sample (n=133)
Reversion (%)	30 (36.1)	34 (68)	64 (48.1)
Females (%)	33 (39.8)	19 (38)	52 (39.1)
Veterans (%)	34 (41)	14 (28)	48 (36.1)
History of TB (%)	14 (17)	5 (10)	20 (15)
Mean age (SD) (yr)	47.6 (15.7)	42.9 (16.1)	45.8 (15.9)
Median (range) first TB Ag-nil (IU/mL): reversions	0.69 (0.35-10.0)	0.70 (0.35-2.62)	0.70 (0.35-10.0)
Median (range) first TB Ag-nil (IU/mL): non-reversions	1.15 (0.35-10.0)	2.30 (0.35-7.24)	1.41 (0.35-10.0)
t-test p-values: ln-first TB Ag-nil by reversion status	0.0146	<0.0001	<0.0001

Appendix H:
Logit equation and predicted probability of reversion with a cut-point of 0.8 IU/mL.

Without age adjustment:
Logit: g(first TB=*) = 0.163 – 1.443(first TB) =
Predicted probability of reversion: π(first TB) = $e^{g(\text{First TB})}/1+e^{g(\text{First TB})}$ =

* Where first TB <0.8 IU/mL, first TB = 0; first TB ≥0.8 IU/mL, first TB = 1

Example: Subject #1022: TB = 1.88

Logit: g(first TB =1) = 0.163 – 1.443(1) = -1.28
Predicted probability of reversion: π(first TB =1) = $e^{-1.28}/1+e^{-1.28}$ = ***0.22***
22% probability of reversion

Logit equation and predicted probability of reversion with continuous ln_TB Ag-nil.

Logit: g(ln_firstTB Ag-nil) = -.575 – 0.649(ln_first TB Ag-nil)
Predicted probability of reversion: π(ln_first TB Ag-nil) = $e^{g(\text{ln\_first TB})}/1+e^{g(\text{ln\_first TB})}$ =

Example: Subject #1022: TB = 1.88

Logit: g(ln_firstTBAg-nil=0.631) = -0.575 - 0.649(.631) = -0.985
Predicted probability of reversion for first TBAg-nil=1.88:
π(ln_firstTBAg-nil=0.631) = $e^{-0.985}/1+e^{-0.985}$ = ***0.272***
27.2% probability of reversion