Veriko Mirtskhulava, M.D., M.P.H., Ph.D. Candidate

Performance of QuantiFERON-TB Gold In-Tube Test and Tuberculin Skin Test in Assessment of Nosocomial Transmission of Tuberculosis and Infection Control Implementation in Health Care Facilities in Georgia

Advisor: Nino Tabagari, M.D., Ph.D.

AIETI Medical School, Davit Tvildiani Medical University

Co-advisor: Henry Blumberg, M.D.

Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine and Departments of Epidemiology and Global Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA

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Introduction

Introduction

Tuberculosis (TB) has re-emerged as a major public health problem in the country of Georgia following the collapse of the Soviet Union[1, 2]. TB case rates in Georgia increased markedly following the dissolution of the Soviet Union. Following implementation of a national TB program in Georgia, between 2007 and 2013, the annual incidence and prevalence of TB decreased from 149/100,000 to 116/100,000 and from 226/100,000 to 163/100,000, respectively. However, TB and especially highly drug resistant TB, remains a major public health problem in Georgia [3].

The country of Georgia is among the 27 high multidrug resistant TB (MDR-TB) burden countries as designated by the World Health Organization (WHO) [3]. MDR-TB is defined as resistance to isoniazid and rifampicin, with or without resistance to other first-line anti-TB drugs[4, 5]. Among the 27 high MDR-TB burden countries the proportion of MDR-TB cases with extensively drug resistant TB (XDR-TB) was one of the highest (20%) in Georgia in 2013[3]. XDR-TB is defined as resistance to at least isoniazid and rifampicin, and to any fluoroquinolone, and to any of the three second-line injectables (amikacin, capreomycin, and kanamycin) [4, 5]. Currently, 11% of newly diagnosed cases in Georgia and 38% of retreatment cases in Georgia have MDR-TB.

Prior to 2012, in Georgia, as in many other high burden TB countries in Eastern Europe, patients with infectious TB were diagnosed and treated in specialized inpatient and outpatient TB facilities organized by the National Tuberculosis Program (NTP), although persons with undiagnosed TB or suspected cases of TB may have been seen at non-TB health care facilities and referred to a specialized TB facility later[1]. Currently in Georgia, TB care is provided by diverse non-NTP public and private care providers[3].

Nosocomial TB transmission from patients to HCWs has been recognized for many years; the risk of transmission is the greatest in facilities with a high burden of infectious TB cases[6-11]. The XDR-TB strains are posing a major public health threat in contexts characterized by a limited TB IC measures. TB infection control (IC) measures in Georgian health care facilities (HCFs) have been limited and similar challenges have been seen as is the case in most low and middle-income countries (LMICs) which have had very limited introduction of TB IC measures. TB IC measures include administrative, engineering and personal protection controls with administrative controls being most important[9,12].

There are no routine programs in place to screen HCWs for latent tuberculosis infection (LTBI); only ultraviolet (UV) lights and respirators were available in the specialized TB facilities in Georgia[1, 13, 6, 10, 14]. A high prevalence of LTBI among HCWs from specialized TB facilities was found in Georgia in 2006[1]; 77% of HCWs had a positive result for at least one of the two diagnostic tests for LTBI [QuantiFERON-TB Gold In-Tube (QFT-GIT) and tuberculin skin test (TST)] and 50% tested positive for both tests[1].

The goal of this Ph.D. dissertation is to determine the role of TST and QFT-GIT in the assessment of nosocomial TB transmission and implementation of TB IC measures in HCFs in the country of Georgia. Evaluating LTBI test conversion rates provides important information on TB occupational exposure risks among Georgian HCWs. Also, the use of both TST and QFT-GIT LTBI tests allow for the comparison of the two tests. As there is limited data on QFT-GIT use in serial testing in high-burden TB countries (i.e., low and middle-income countries), this dissertation would contribute to the literature in this area.
Specific aims of the dissertation include:

1. To determine prevalence and incidence of LTBI and associated risk factors among Georgian HCWs
2. To evaluated the effect of occupational exposure to TB and BCG vaccination history on the outcome of TST and QFT-GIT positivity at baseline and on the conversion of these tests
3. To assess determinants of TB IC related behaviors among Georgian HCWs

We performed a prospective cohort study among HCWs from TB and non-TB facilities in Georgia using serial testing of health care workers with two diagnostic tests--the TST and the QFT-GIT in 2009 - 2011. The principle hypothesis was that QFT-GIT positivity is more likely to be associated with the well established indicators of occupational TB exposure than TST positivity among HCWs in Georgia. Furthermore, in July – December 2011 we conducted an anonymous survey of Georgian HCWs to provide baseline data on their

knowledge, beliefs, and behaviors related to TB IC. The data will be used to inform the development and implementation of future TB IC interventions/programs at Georgian HCFs.

Literature Review

Nosocomial TB Transmission

TB was recognized as an occupation health hazard for HCWs since the 1950s[15]. Emergence of M/XDR-TB and effect of human immunodeficiency virus (HIV) infection on TB epidemics resulted in reemergence of TB as an occupational health hazard for HCWs in the early 1990s[15]. TB transmission occurs through droplet nuclei aerosolized by patients with TB disease and inhaled by other persons. Transmission is most likely to occur from sputum smear or culture positive TB patients. The magnitude of nosocomial TB transmission varies by setting, occupational group, and TB prevalence in the community, patient population, and effectiveness of TB IC measures. The risk is greater when a larger number of patients with smear-positive TB are managed in the HCF[6, 7, 9-11, 16].

Transmission of TB in health care settings has been reported from virtually every country in the world, regardless of local TB incidence. Most of the studies on nosocmial TB transmission in LMICs published since 1990s reported prevelance of LTBI among HCWs greater than 40%[11]. In the same years, prevalence of postive TST among HCWs from high income countries (HICs) ranged between 1.8% to 46%[17-25]. Risk foactors for the prevalence of LTBI

among HCWs are diverse between LMICs and HICs. In LMICs LTBI is typically associated with markers of occupational exposure. History of contact with TB patients, working in medical wards, and participation in sputum collection and autopsies were independent occupational risk factors for LTBI in several studies conducted in LMIC[11]. Also, markers of cummulative exposure, increasing age and years of employment as a HCW, were associated with higher prevalence of LTBI in most studies[1,11]. Studies from HICs more often reported association of positive TSTs among HCWs with non-occupational factors - older age, foreign birth, bacille Calmette-Guérin (BCG) vaccination, and TB contact outside their work[21,23]. Moreover occupational risk factors including years of employment in health care[1, 17, 23, 25], particularly in internal[21, 22] or respiratory medicine[24], and more direct indicators of TB exposure including working in HCFs with higher mumber of TB admissions[23] and the percentage of patients with TBor HIV[17] were associated with positive TSTs in HCWs from HICs.

Schwartzman et al. reported a significantly and markedly increased infection risk for HCWs[26]. The relative risk estimate for medical personnel is 13.6 (95% CI: 1.4, 132) in the cohort study and 2.6 (95% CI: 1.3, 5.2) in the cross-sectional study[26]. HCWs vs. non-HCWs have 1.5 (95% cinfidence interval (CI): 1.3-1.7) times higher risk of positive TST[25]. Hospital areas with TB patients had 6.3 (95% CI: 0.9–52.8) times higher risk of positive TST compared to the non-exposed departments[27]. Many epidemiological studies reported an association between work on wards with TB patients and TB infection and disease[27,28-30]. Relative risk estimates range from 2.1 [30] to 10.3[28].

According to a systematic review of TB among HCWs in LMICs, the annual risk of TB infection (ARI) ranged from 3.9% to 14.3% among HCWs.[10] Another review by Menzies et al. published in 2007 reported the median ARI attributable to health care work of 5.8% (range 0–11%) in LMICs and of 1.1% (0.2–12%) in HICs.

Rates of active TB in HCWs were consistently higher than in the general population in all LMICs, although findings are variable in HICs[11, 31, 32] . In facilities with fewer HCWs per a TB patient cared for reported a higher incidence of TB disease. Furthermore, HCWs from TB inpatient facilities, general medicine wards, laboratories and emergency rooms had higher incidence of TB compared to the general population. HCWs from surgery and obstetrics and gynecology departments had a lower active TB incidence. The incidence was lowest in administrative staff[11].

TB transmission in HCFs can be significantly reduced with the implementation of effective TB IC measures[7, 9, 12, 33, 34]. The nosocomial transmission of MDR-TB and extensively drug resistant TB (XDR-TB) further highlights the need for effective TB IC measures[35-37]. Dramatic nosocomial outbreaks of MDR-TB in HIV infected populations in the ealry 1990s in the US fostered further strengthening of administrative, personal and engineering IC measures in many hospitals in HICs[11], although since the first recognition of nosocomial TB transmission in the 1950s, effective IC measures have been implemented in resource-rich countris.[9]

Most HICs screen HCWs periodically for LTBI as part of their TB IC programs[9, 38] but this practice is unusual in most LMICs[9, 34].

WHO policy on TB IC identifies a set of activities for national and subnational TB IC. These activites include: identification and strengthening of a coordinating body for TB IC, development of a comprehensive budgeted plan including budget for human resource requirements, monitoring and evaluation of TB IC measures including supervision activities and enabling to conduct operational research. The national and sub-national managerial activities listed above provide the managerial framework for the implementation of TB IC in HCFs. TB IC elements at the facility level are generally implemented based on risk assessment and informed by climatic, cultural, cost and programmatic factors. The measures at this level also include administrative and environmental controls, and personal protective equipment[12]. These types of control should be implemented together because they complement one another.

While most HICs have successfully implemented TB IC measures[9], TB IC measures are limited or virtually non-existent in most resource-limited TB endemic countries [1, 10, 14, 39, 40].

As it is mentioned in the End TB draft strategy developed by the WHO, "Regulatory mechanisms essential to ensure effective IC, rational use of tuberculosis diagnostics and medicines, mandatory disease notification, functioning vital registration systems, and protection of the legal rights of people with tuberculosis remain weak"[41].

Multiple studies suggest that the decline in nosocomial TB transmission observed in specific institutions is associated with the rigorous implementation of IC measures [33, 42-45]. Reports of increased implementation of recommended TB ICs combined with decreased reports of outbreaks of TB disease in health-care settings suggest that the recommended controls are

effective in reducing and preventing health-care–associated transmission of *M. tuberculosis* [46]. Administrative IC measures had a modest impact in LMICs, yet seemed the most effective in HICs.[11]

HCW training and education regarding LTBI and TB disease is an essential part of managerial controls in a TB surveillance and IC program[9, 12].

Latent Tuberculosis Infection

Ending the tuberculosis epidemic will entail early diagnosis and proper treatment of all cases of active tuberculosis as well as a gradual removal of the pool of LTBI in some 2000 million people.[41] LTBI is defined as a state of persistent immune response to stimulation by *Mycobacterium tuberculosis* antigens without evidence of clinical and radiographic findings active TB. A direct measurement tool for LTBI in humans is currently unavailable. The vast majority of infected persons has no signs or symptoms of TB.[47,48]

In general, tubercle bacilli cannot be recovered from sputum or other sites in latently infected persons,[49] indicating a low bacillary burden. Based on human pathology data and emerging data from nonhuman primates, LTBI appears to represent a spectrum of microbiological and pathological states in which the organisms are viable but fail to produce respiratory or constitutional symptoms.[50]

However, persons with LTBI can progress to active TB at any time, often many years or even decades after initial infection,[51] thereby serving as a source of new infections. Although

identification and treatment of infectious persons are paramount, global TB eradication efforts must also focus on detecting and treating cases of LTBI[41]. According to the Institute of Medicine, "to make significant progress toward the elimination of tuberculosis in the United States, efforts to prevent cases from occurring must be amplified".[52] Current diagnostic tests do not discriminate between LTBI and active TB, and treatment for LTBI requires prolonged administration of antibiotics.[53] An improved understanding of the host and pathogen mechanisms underlying LTBI may yield novel assays which can identify persons at increased risk for progression to active disease,[54] as well as new drugs to shorten the duration of LTBI treatment.[55] Active TB can occur soon after initial exposure and infection (i.e., primary disease) or after a period of LTBI (reactivation disease). Although reactivation TB cannot be differentiated from primary disease on clinical or laboratory grounds, epidemiological studies have demonstrated that the majority of active TB cases in the United States and other countries with low TB prevalence occur as a result of reactivation of LTBI. The goal of LTBI treatment is to prevent reactivation, and this is especially recommended for persons who are at increased risk for progression from LTBI to disease.[56] Persons at increased risk of reactivation of LTBI include those with HIV/AIDS, those receiving immunosuppressive treatment, including cancer chemotherapy, systemic steroids, and anti-TNF agents, and those with chronic systemic diseases, such as end-stage renal disease, rheumatic disorders, and diabetes mellitus.[57] Identification and successful treatment of persons with LTBI at risk for reactivation are important components of global TB elimination efforts[41].

LTBI Diagnostic Tests

Tuberculin Skin Test

The tuberculin skin test is one of the few tests developed in the 19th century that is still in present use in clinical medicine. The first tuberculin test material was prepared by Robert Koch[58]; The TST was introduced in 1910 by Mantoux.[59] Several factors others than LTBI can influence TST's positivity. Inter subject variability in biological response to tuberculin[60], interreader variability[60], the booster effect [61-64], immune response to nontuberculous mycobacterial antigens [65, 66], and previous vaccination with bacillus Calmette-Guerin (BCG) can all be responsible for a positive TST result [67-73]. BCG vaccination is the main problem in interpreting TST results, in particular in countries where the rate of vaccination is high and the prevalence of tuberculosis is low[59]. One must be cautious in interpreting TST reactions because of the potential implications associated with a positive result, such as the need for chest radiography and 6- to 9-month preventive chemotherapy, the risk of treatment hepatotoxicity, and the anxiety generated in the patient.

Tuberculin reactivity after BCG vaccination for adults in western countries with a low prevalence of TB could therefore differ from that experienced in countries with moderate or high prevalence rates,[74] The proportion of individuals with a prior BCG vaccination who have a positive TST result has been reported to vary from 0% to 90%. Subsequent reactivity

of TST can vary depending on BCG dose, manufacturer of the vaccine, age when vaccinated, and the interval between vaccination and testing[71].

Several reports from Quebec suggest that BCG vaccination in infancy does not contribute to a subsequent positive PPD response, whereas BCG given in childhood or at an older age may result in a positive TST[71].

Given such a long history of TST use, it may seem surprising that aspects of interpretation of this test remain controversial. However, this reflects changes in the populations affected with tuberculosis and their relative frequency of true positive tests from TB infection, and false-positive tests associated with bacillus Calmette-Guèrin (BCG) vaccination, or nontuberculous mycobacteria, as well as the recent human immunodeficiency virus (HIV) epidemic[60].

Interferon-gamma Release Assays

For many years the tuberculin skin test (TST) was the only test available for diagnosis of LTBI; however, the interferon-gamma release assays (IGRAs), T-cell based assays, have become available and provide alternative diagnostic test for LTBI.[75] Two commercially available IGRAs have been approved for use by the U.S. FDA—the Quanti- FERON-TB Gold In-Tube (QFT-GIT) assay (Cellestis Inc., Valencia CA) and the T-SPOT.TB assay (Oxford Immunotec, Abingdon, UK). QuantiFERON®-TB Gold In-Tube (IT) is an in vitro diagnostic test using a peptidecocktail simulating ESAT-6, CFP-10 and TB7.7(p4) proteins to stimulate

cells in heparinised whole blood. Detection of interferon-γ (IFN-γ) by Enzyme-Linked Immunosorbent Assay (ELISA) is used to identify in vitro responses to these peptide antigens that are associated with Mycobacterium tuberculosis infection. [76]

QuantiFERON®-TB Gold IT is an indirect test for M. tuberculosis infection (including disease) and is intended for use in conjunction with risk assessment, radiography and other medical and diagnostic evaluations.[76]

IGRAs have several advantages over the TST: they require only one visit, are not affected by BCG vaccination, have less cross-reaction with non-tuberculous mycobacteria, are less subjective in measuring results, and can be repeated without boosting. However, there is a lack of data on how IGRAs perform when used for serial testing, especially in low and middle-income countries. In 2005, the U.S. Centers for Disease Control (CDC) recommended that IGRAs can be used in all settings where the TST has been used, including the serial testing of HCWs[77]. The updated 2010 CDC guidelines caution that ‘‘lenient criterion to define IGRA conversion might produce more conversions than are observed with the more stringent criteria applied to TSTs. Furthermore, an association between an IGRA conversion and subsequent disease risk has not been demonstrated.

The criteria for interpreting changes in an IGRA that identify new infections remain uncertain’.[75] Guidelines from Australia advise caution when using IGRAs for HCW screening,[78] and Canadian guidelines do not recommend the use of IGRAs for serial testing of HCWs,[79] citing a lack of available data. A World Health Organization (WHO) policy statement on the use of IGRAs in low- and middle-income countries indicates that ‘‘data on

serial testing and reproducibility of IGRAs, as well as evidence on the predictive value of IGRAs in HCWs, are still absent for high-incidence settings".[80]

IGRAs are in vitro blood tests that detect immunologic responses to *Mycobacterium tuberculosis* complex antigens, and have potential for improving LTBI testing.[75, 81] IGRAs require one patient–provider interaction to obtain results, which can be available within 1 day and are not affected by prior BCG vaccination.[82, 83] The Centers for Disease Control and Prevention (CDC, Atlanta, GA) has issued guidance that, for detection of TB infection, IGRAs may be used in place of a TST, and IGRAs are preferred for testing BCG-vaccinated persons.[75] The QuantiFERON-TB Gold In-Tube (QFT-GIT; Cellestis [a Qiagen company], Valencia, CA) and T-SPOT.TB (T-SPOT; Oxford Immunotec Ltd., Abingdon, UK) tests are U.S. Food and Drug Administration–approved IGRAs for diagnosing TB infection. IGRAs perform well for detection of TB infection among contacts of individuals with active pulmonary TB.[84, 85] However, with the introduction of IGRAs into clinical practice, a broader population is being tested including individuals undergoing serial testing in the absence of known exposure. Published experience, much of which is from settings of routine clinical use with potential selection bias with respect to individuals selected for testing or repeat testing, indicates unexpectedly high rates of IGRA positivity, conversion (change from a negative to positive), and reversion (change from positive to negative)[86-91 20].

Methods

Study Setting and Population

This dissertation included two studies that examined a subset of specific aims. The first study - a prospective cohort study was conducted from 2009 – 2011. HCWs from the Georgian National TB Program (NTP), including the National Center for Tuberculosis and Lung Diseases (NCTLD) in Tbilisi, its affiliated TB outpatient clinics from whole country, as well as HCWs from non-TB primary health care centers (PHC) were eligible to enroll. An HCW was defined as anyone working in a health care setting, regardless of direct patient contact. The PHCs are not specialized in TB patient care but commonly refer TB suspects to the NTP. Inclusion criteria were age >18 years old, HCW in the country of Georgia, and provision of written informed consent. Exclusion criteria were history of active TB and allergy to the purified protein derivative used in the TST. Our target population consisted of 4,485 HCWs including physicians, nurses, and administrative and technical staff. One-thousand-four-hundred HCWs worked for the NTP and 3,085 HCWs were from PHCs. To estimate association between indicators of occupational TB exposure and positive results of the LTBI dianostic tests (TST and QFT-GIT) 95% confidence level and 80% power was used. Sample size was calculated by EpiInfo Version 6 Statcalc. This was a voluntary study. A convenience sampling method was used. HCWs were approached with information about the study at their place of work and were enrolled if they agreed to participate and provided informed consent. Initially, HCWs completed a questionnaire with demographic information, medical

history, and employment history, and then were tested for LTBI with QFT-GIT and TST. HCWs who tested positive for LTBI by either test were referred to the NCTLD for evaluation to rule out active TB. This evaluation included chest x-ray and symptoms screening. As it is not the standard of care in Georgia, no HCWs were treated for latent TB infection.

The second study - a population-based HCWs survey about TB and TB IC was conducted between July-December 2011 among HCWs in Georgia. Target population for this survey was the same as for the prior study, HCWs from the Georgian NTP, including NCTLD in Tbilisi, its affiliated TB outpatient clinics from whole country, as well as HCWs from non-TB PHCs were eligible to enroll. Inclusion criteria were age $\geq$18 years old and being a HCW. Sample size was calculated accounting for 95% confidence level, 5% margin of error, and estimated 70% of a particular response to the survey questionnaire. HCWs were approached with information about the survey at their place of work and were enrolled if they agreed to participate

Ethics Statement

The study was approved by the Emory University Institutional Review Board and Georgian NCTLD Ethics Committee. For the prospective cohort study HCWs enrolled into the study provided written informed consent in their native Georgian language, but for the anonymous survey only oral consent was provided.

Data Collection

Data on potential risk factors for and prevalence and incidence of LTBI were collected using a questionnaire and a data collection form. Data on determinants of HCWs behaviors related to TB IC were collected via an anonymous questionnaire.

As part of the prospective cohort study on rates and risk factors for LTBI among Georgian HCWs, HCWs and medical students enrolled into the study completed a questionnaire. The questionnaire included questions regarding demographic information (date of birth, gender, country of birth, race, ethnicity), medical history (history of BCG vaccination, information about community TB exposure, history of tuberculin skin testing, history of TB disease), employment history (occupation, number of years employed as a HCW, or number of years as a medical student, job title). The questionnaires were available for participants to fill out in their native Georgian language of Kartuli (Appendix 1).

After completing the questionnaire, two diagnostic tests for LTBI were performed: the TST and the QFT-3G test. The tests results were recorded in a data collection form (Appendix 2).

As part of the population based HCWs survey about TB and TB IC, an anonymous self-administered 55-question survey was provided to all participants in the Georgian language (Kartuli) (Appendix 3). The survey was piloted with 10 HCWs from the NCTLD; these HCWs were not included in the final sample. The survey was developed based on the Health

Belief Model (HBM) conceptual framework.[92-95] The survey collected information about respondents' TB knowledge, their health-related behaviors, and willingness to engage in health-related behavioral change with respect to the following: respirator use, UV lights, willingness to be annually screened for LTBI, and willingness to be treated for LTBI if tested positive by LTBI diagnostic tests. In addition, the survey measured the following HBM constructs: perceived susceptibility to and perceived severity of LTBI and TB disease including multi and extensively drug-resistant (M/XDR) TB, perceived benefits of IC measures, perceived barriers to implementing IC measures, and cues to action such as availability of respirators and instructions from managers related to using the respirators. We also asked various socio-demographic questions in order to further characterize the study population.

Laboratory Methods

QuantiFERON-TB Gold In-Tube Test

Three ml of blood was drawn (Appendix 4) for the QFT-GIT test, which was performed according to the manufacturer's instructions[76] and as previously described[96]. The assay involved 2 stages: incubation of whole blood with antigens, and measurement of IFN-γ

production in harvested plasma by enzyme-linked immunosorbent assay [ELISA]. Venous blood was directly collected into three 1-mL heparin-containing tubes. One tube contained only heparin as negative control, another also contained the T-cell mitogen phytohemagglutinin as positive control, and the third tube had overlapping peptides representing the entire sequences of ESAT-6 and CFP-10, and another peptide representing a portion of TB7.7. [20] Within 1 to 12 hours of blood draw, the tubes were incubated at 37°C. After 24 hours of incubation, the tubes were centrifuged and plasma harvested and stored at 4°C for two weeks or frozen at –70°C until the ELISA is performed. The amount of IFN-γ was quantified using an ELISA. The IFN-γ values (IU/mL) for tuberculosis-specific antigens and mitogen was corrected for background by subtracting the value obtained for the respective negative control. The entire QuantiFERON-TB 3G assay is described in Appendix 5. IFN-γ values > 10 IU/ml were treated as 10 IU/ml. Repeat QFT-GIT testing was performed on participants 6–26 months after baseline testing. QFT-GIT was performed on all participants who underwent repeat testing. As recommended by the manufacturer[76] and the CDC,[75] the QFT-GIT result was defined as positive if the response to the TB antigens minus the negative control was ≥0.35 IU/ml and >25% of the negative control, negative if these criteria were not met, and indeterminate if either the negative control had a result of >8 IU/ml or if the positive control had a result of, < 0.5 IU/ml[75]. According to CDC guidelines, a QFT-GIT conversion was defined as a baseline interferon-gamma (IFN-γ), 0.35 IU/ml and a follow-up IFN-γ level ≥0.35 IU/ml, without any consideration of the magnitude in change of the IFN-γ

response [75]. A QFT-GIT reversion was defined as a baseline IFN-γ ≥0.35 IU/ ml and a follow-up IFN-γ level <0.35 IU/ml.

Tuberculin Skin Testing

The TST was performed using the Mantoux method[97,96] and read 48–72 hours after placement. The TST was placed intradermally in the volar aspect of the left forearm using a sterile tuberculin syringe using 5 tuberculin units (TU) or 0.1 ml of PPD (Tubersol H, Connaught; Swiftwater, PA, USA). Study participants were instructed to return to have the TST read 48 to 72 hours after placement. The amount of induration (in mm) was recorded on the data collection form. Readings were recorded in whole numbers and the reading was rounded up to the next whole number (e.g., for a reading between 15 and 16 mm of induration, 16 mm of induration will be recorded). The research staff was trained on tuberculin skin testing. According to the American Thoracic Society (ATS) and CDC guidelines, a TST was defined as positive if the induration in HCWs was ≥10 mm, and a TST conversion was defined as a change in induration from <10 mm to ≥10 mm, with an increase of ≥10 mm within 2 years [75, 97]. Only patients with a negative baseline TST had repeat TST testing performed at follow up. Repeat testing was performed over a range of 6–26 months due to limited research study staff and inability to test large numbers of HCWs

simultaneously. Due to limited research study staff and limited resources, not all HCWs were offered repeat testing. Repeat testing was performed by convenience sampling.

Study Measures and Definitions

For determination of the prevalence of a positive TST result, we included participants who had TST performed in our study or reported prior history of positive TST (n= 308). Tuberculin skin testing was performed using the Mantoux method[98,97]. A positive TST was defined as induration ≥10 mm[97,75]. Once a health care worker had a positive TST (induration of ≥10 mm), further testing using the TST was no performed. [60]. Georgian HCWs were assumed to stay TST positive once tested positive with TST (induration ≥10 mm) due to steady risk of occupational TB exposure and nonexistence of LTBI preventive therapy for HCWs in Georgia[1].

For determination of prevalence of positive QFT-GIT, we included participants who had QFT-GIT measured (n= 319). A positive QFT-GIT result was defined based on manufacturer recommendations and as previously published[99]. A result was considered positive if the response (interferon-gamma release) to the TB antigens minus the negative control was ≥0.35 IU/ml and >25% of the negative control, negative if these criteria were not met, and indeterminate if either the negative control had a result of >8 IU/ml or if the positive control had a result of, 0.5 IU/ml[75].

Occupational TB exposure frequency was categorized as daily (contact ≥ 5 days per week), frequent (contact < 5 days per week and ≥ twice per month), rare (contact < twice per month and ≥ once per 3 months), and very rare (contact < once per 3 months). For multivariate logistic regression analysis the occupational TB exposure variable was later dichotomized in two ways: frequent occupational TB exposure (defined as contact ≥ twice per month) opposed to rare occupational TB exposure (defined as contact < twice per month and daily occupational TB exposure (defined as contact ≥ 5 days per week) opposed to less than daily occupational TB exposure (defined as contact < 5 days per week).

Five-point Likert-type scales were used to assess HCWs' beliefs and behaviors.[100, 101] Perceived susceptibility to TB infection was measured using a five-level variable where 1 indicated no perceived possibility and 5 indicated very good chance of being infected with TB. Perceived severity of TB infection was also assessed using a five-level variable where 1 indicated strong agreement and 5 indicated strong disagreement with the statements of concerns about acquiring LTBI and TB disease.

Statistical Considerations

Data were collected and entered into a REDCap database. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies[102]. Statistical analysis was performed in IBM SPSS Statistics version 19.

Products of the analysis included prevalence estimates of LTBI among HCWs enrolled, estimates of the LTBI diagnoistic test (TST and QFT-3G) results and conversion rates (from a negative test result to a positive test result), agreement between the diagnostic tests (TST and QFT-3G), and comparison of the tests (TST and QFT-3G) results with respect to their association with risk factors. Furthermore, results of the analysis included estimates of knowledge of TB, beliefs about TB and TB IC and IC releated behaiviours, and determinats of TB IC realted behaiviors among Georgian HCWs.

Agreement between the two diagnostic tests for LTBI (TST and QFT-GIT) was determined using the kappa (κ), where $\kappa > 0.75$ represents excellent agreement, $\kappa = 0.4\text{-}0.75$ represents fair to good agreement, and $\kappa < 0.4$ represents poor agreement [103].

Multivariate analysis was performed using logistic regression modeling with outcomes of TST positivity, QFT-GIT positivity, and discordant LTBI test results TST positive / QFT-GIT negative group. Participants were included in these models if they had measured TST (or history of positive TST) and measured QFT-GIT. The same participants were included in the models for QFT-GIT positive and TST positive. The purpose of the multivariate model was to estimate relationship between well established indicators of occupational TB exposure and positive results of the LTBI diagnostic tests (TST and QFT-GIT) among HCWs in Georgian HCFs. Demographic information, BCG vaccination history, and the set of indicators of TB exposure at work and outside the work were defined in the multivariate model to provide the largest model to be initially considered. Collinearity was assessed for multivariable models, variables with significant collinearity were removed from final models. Colliniarity

was assessed using the "/statistics=defaults tol" subcommand in the IBM SPSS Statistics version 19. Variables with the "tolerance" values < 0.10 and the the variance inflation factor (VIF) values 10 < were excluded from the final model. The "tolerance" is an indication of the percent of variance in the predictor that cannot be accounted for by the other predictors, hence very small values indicate that a predictor is redundant. The VIF is (1 / tolerance)[104]. Interaction terms were created based on biologic plausibility and were tested individually for significance with the Likelihood Ratio Test[105].

We used a backward elimination procedure for removing variables. Variables included in the final multivariate models were chosen on the basis of biologic plausibility and statistical significance of their association with the outcomes. Variables with potential confounding effect were also kept in the final model. A p-value ≤ 0.05 was defined as statistically significant. To analyze how the final model predicted the categorical outcomes we used the Hosmer and Lemeshow goodness of fit test[106]. Cases with studentized residual values greater than 2.5 were inspected in further detail to determine why these cases were outliers and were removed from the analysis if this was deemed necessary[106].

Incidence rates for TST and QFT-GIT conversion (in 100 person/years) were determined by dividing the number of events by the total amount of person-time contributed by those who were negative at time of first testing and accounting for the time to follow-up testing. Risk factors for TST and QFT-GIT conversion were determined by univariate logistic regression analysis and multivariate logistic regression analysis.

Proportions of concordant results of the two diagnostic tests were compared between HCWs with a bacillus Calmette-Guerin (BCG) scar vs. no BCG scar and between HCWs with self-reported frequent (≥ twice a month) occupational TB exposure vs. those who saw TB patients rarely (< twice a month). The proportions were compared by two-proportion z-test[20]. Proportions of positive QFT-GIT at baseline and repeated testing were also compared among HCWs with self reported frequent occupation TB exposure vs. those who reported rare TB exposure at work. The proportions were compared by McNemar's test[20].

For determination of estimates of HCWs knowledge of TB, their beliefs about TB and TB IC, and TB IC related behaviors we first calculated frequency distributions; if < 10% of participants responded to a question item, that item was excluded from further analysis. Five-level variables measuring HCWs beliefs about TB IC measures were reduced to three-level variables for multivariate analysis. We used binomial logistic regression to estimate the association between HCW demographic characteristics and knowledge of TB; ordinal (when proportional odds assumption was met) or multinomial logistic regression were used to estimate the association between HCW's beliefs and their IC related behaviors.[105] In multivariable models we adjusted for variables that met statistical and epidemiological criteria[105] and were congruent with the HBM framework. Initially the largest multivariate model was reduced to the final multivariate model by a backward elimination procedure. Collinearity was assessed for multivariable models, variables with significant collinearity were removed from final models. Colliniarity was assessed using the "/statistics=defaults tol" subcommand in the IBM SPSS Statistics version 19. We used the Mann-Whitney U-test

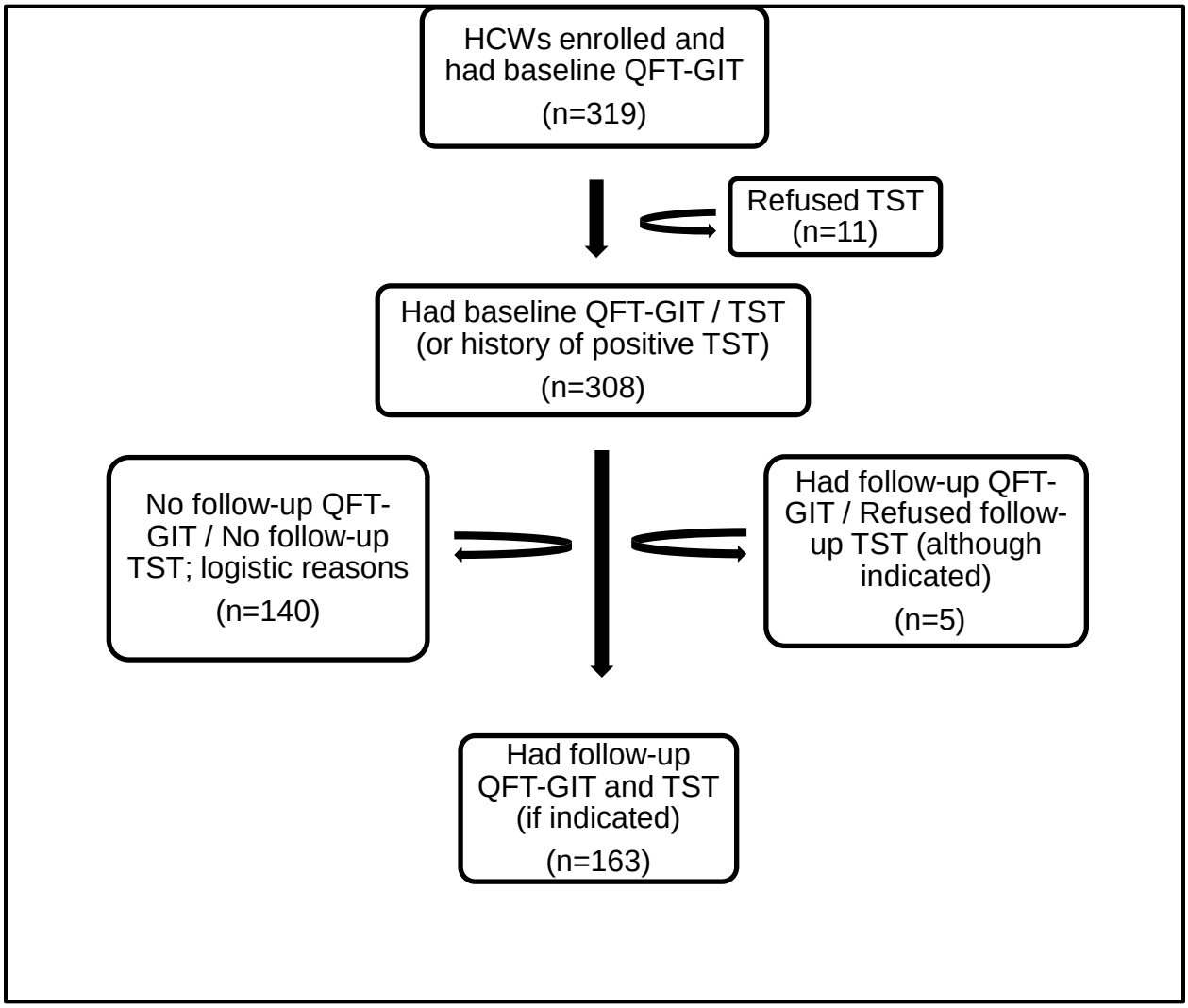
to compare the median scores of HCWs' beliefs among two independent groups – HCWs who answered a TB related knowledge question correctly and HCWs who answered the question incorrectly.[107]

Results

Study Population

Three-hundred-nineteen Georgian HCWs were enrolled in the prospective study (Figure 1); all enrolled had a QFT-GIT performed. Fifty-nine HCWs did not have a TST performed (48 participants reported a prior positive TST in the past and 11 refused to have a TST done).

Figure 1. Enrollment and follow-up of participants



NOTE. HCW, HCWs; QFT-GIT, QuantiFERON-TB Gold In-tube assay; TST, tuberculin skin test

The characteristics of the study population (n=319) are described in Table 1. The majority of the participants were female (81%), reflecting makeup of HCWs at the NTP and affiliated institutions. The mean age was 41 years (standard deviation [SD], 11.4 years). The majority of HCWs in our study were from Tbilisi (86%), the capital of Georgia. One hundred ninety three (60%) participants worked in specialized TB facilities, and 116 (39%) worked in non-TB facilities. Fifty percent of the HCWs reported frequent TB exposure at work (contact ≥

twice per month) as opposed to rare TB exposure at work (contact ≤ once a month). The mean number of years in health care was 17.0 (SD, 12.6).

Table 1. Characteristics of the study population (N=319)	
Characteristic	**No. (%)**
Demographic Characteristic	
Age, y	
18-32	84 (26 %)
33-41	76 (24 %)
42-49	81 (25 %)
≥ 50	78 (25 %)
Female Gender	**259 (81 %)**
Georgian ethnicity	**305 (96 %)**
Education	
Graduate school	230 (72 %)
Undergraduate	68 (21%)
Secondary school or less	21 (4 %)
Self reported positive history of BCG vaccination	**285 (89 %)**
BCG Scar by inspection	**244 (77 %)**
Employment Characteristics	
HCW Employment Location	

Tbilisi	274 (86 %)
Other Locations	45 (14 %)
Health care facility	
TB inpatient facility	121 (38 %)
TB outpatient facility	72 (23 %)
Non-TB health facility	126 (39 %)
Occupation	
Administrative staff	92 (29 %)
Laboratory Worker	22 (7 %)
Medical students	14 (4 %)
Nurses	51 (16 %)
Physicians	116 (36 %)
Other	24 (8 %)
Years working in health care	
0-4	71 (22 %)
5-14	81 (25 %)
15-24	72 (23 %)
>25	95 (30 %)
Occupational TB exposure frequency	
Daily (≥ 5 days a week)	101 (32%)
Frequent (< 5 days a week and ≥ twice a month)	58 (18 %)

Rare (≤ once a month and ≥ once a quarter)	61 (19 %)
Very rare (< once a quarter)	99 (31 %)
Positive history of TB contact outside their work	74 (23 %)

Note. BCG, HCW, health care worker; TB, tuberculosis

For assessment of determinants of TB IC related behaviors among Georgian HCWs a total of 298 HCWs were approached in the population based survey to enroll in the study with 58 (19 %) refusing to participate. The characteristics of the study population (n=240) are described in Table 2. The mean age of HCWs who participated was 44.3 years (standard deviation (SD) 11.4 years). The majority of the participants were female (90%) again reflecting the gender distribution of HCWs at the NTP and affiliated institutions. Nearly half (54%) HCWs were from the capital city, Tbilisi. Fifty-seven percent of the HCWs worked at specialized TB facilities. Respirators were available most of the time for only 65% of HCWs. Forty-eight percent were physicians and 39% were nurses. The mean number of years in health care was 19.7 (SD 10.9 years).

Table 2. Characteristics of the study population (N=240)	
Characteristic	No. (%)
Demographic Characteristic	
Age, y	

≤ 25	59 (25 %)
36 – 44	59 (25 %)
45 – 51	59 (25 %)
> 60	57 (24 %)
Data missing	6 (2 %)
Female Gender	**216 (90 %)**
Employment Characteristics	
Location of HCW employment	
Tbilisi	130 (54 %)
Other Locations	110 (46 %)
Health Facility	

TB Facility	136 (57 %)
Non-TB health facility	104 (43 %)
Respirator Is available (most of the time)	
Inpatient TB facility	35 (92 %)
Outpatient TB facility	77 (79 %)
Non-TB health facility	45 (45 %)
Works primarily with TB patients	136 (57 %)
Occupation	
Physician	114 (48 %)
Nurse	94 (39 %)
Other	27 (11 %)
Missing	5 (2 %)
Years working in health care	
≤ 5	26 (11 %)
6-20	98 (41 %)
21-34	80 (33 %)
35 ≤	22 (9 %)
Data missing	14 (6 %)

Note. BCG, HCW, health care worker; TB, tuberculosis

Prevalence of TST and QFT-GIT Positivity

The prevalence of a positive TST at baseline was significantly higher among health care worker than the prevalence of a positive QFT-GIT: 63% (193/308) for TST vs. 46% (147/319) for the QFT-GIT (OR =1.84, 95% CI 1.33-2.53, p<0.001). The prevalence having both diagnostic tests positive was 39% (121/308). The prevalence of LTBI by any of the two diagnostic tests being positive was 69% (219/319). Among HCWs who worked in TB facilities, 107 of 193 (55%) had a positive QFT-GIT vs. 40 of 126 (32%) of HCWs working in non-TB facilities (OR =2.68, 95% CI 1.67-4.28, p<0.0001). Among HCWs working in TB facilities, 128 of 188 (68%) had positive TST vs. 65 of 120 (54%) of those working in non-TB facilities (OR =1.8, 95% CI 1.13-2.90, p<0.02).

Risk factors for LTBI prevalence

In univariate analysis, risk factors for a positive diagnostic test for LTBI included: frequent (contact ≥ twice per month) occupational TB exposure (TST: OR 1.6, 95% CI 1.01-2.56, QFT-GIT: OR 3.1, 95% CI 1.95-4.87), increasing age (TST: OR 1.28, 95% CI 1.04-1.57, QFT-GIT: OR 1.39, 95% CI 1.14-1.70), and working in TB HCF (TST: OR 1.8, 95% CI 1.13-2.89, QFT-GIT OR 2.68, 95% CI 1.67-4.28) (Table 3).

Table 3. Univariate analysis for risk factors for a positive TST and QFT-GIT among Georgian HCWs		
Characteristic	**Positive TST (n=305)[a]**	**Positive QFT-GIT (n=317)[a]**
	aOR (95% CI)	aOR (95% CI)
Frequent vs. rare contact with TB patients	1.6 (1.01-2.56) [b]	3.1 (1.95-4.87) [b]
Age, years	1.28 (1.04-1.57) [b]	1.39 (1.14-1.70) [b]
TB HCF vs. Non-TB HCF	1.8 (1.13-2.89) [b]	2.68 (1.67-4.28) [b]

NOTE. TST, Tuberculin skin test; QFT-GIT, QuantiFERON-TB Gold In-tube assay; Frequent, contact with TB patients ≥ twice per month; Rare, contact with TB patients < twice per month; [a] Outlier cases were removed from the analysis; [b] Statistically significant effect

In multivariate analysis, increasing age was associated with a positive TST result. HCWs in age group of 33-41 years (aOR 3.63, 95% CI 1.65-7.97), HCWs in age group of 42-49 years (aOR 2.77, 95% CI 1.29-5.95) and HCWs in age group of ≥ 50 years (aOR 3.91, 95% CI 1.69-9.04) were more likely to have positive TST at baseline compared to HCWs in age group of 18-32 year. (Table 3). In multivariate analysis to independent risk factors associated with a positive QFT-GIT, HCWs who reported frequent (≥ twice per month) contact with TB patients (aOR 3.53; 95% CI 1.55-8.06) compared to HCWs with uncommon (< twice per month) contact with TB patients were more likely to have positive QFT-GIT at baseline.

Also, HCWs in age group of 33-41 years (aOR 3.36; 95% CI 1.47-7.68), HCWs in age group of 42-49 years (aOR 4.26; 95% CI 1.73-10.52), and HCWs with age ≥ 50 years (aOR 5.25, 95% CI 1.67-16.45) compared to HCWs in age group of 18-32 years were more likely to have positive QFT-GIT at baseline (Table 4).

Table 4. Multivariate analysis for risk factors for a positive TST and QFT-GIT among Georgian HCWs

Characteristic	Positive TST (n=305)[a]	Positive QFT-GIT (n=317)[a]
	aOR (95% CI)	aOR (95% CI)
Frequent vs. rare contact with TB patients	1.03 (0.43 - 2.46)	**3.53 (1.55 – 8.06)[b]**
Age, years		
33-41 vs. 18-32	**3.63 (1.65-7.97)[b]**	**3.36 (1.47 – 7.68) [b]**
42-49 vs. 18-32	**2.77 (1.29-5.95) [b]**	**4.26 (1.73 – 10.52) [b]**
≥ 50 vs. 18-32	**3.91(1.69-9.04) [b]**	**5.25 (1.67 – 16.45) [b]**

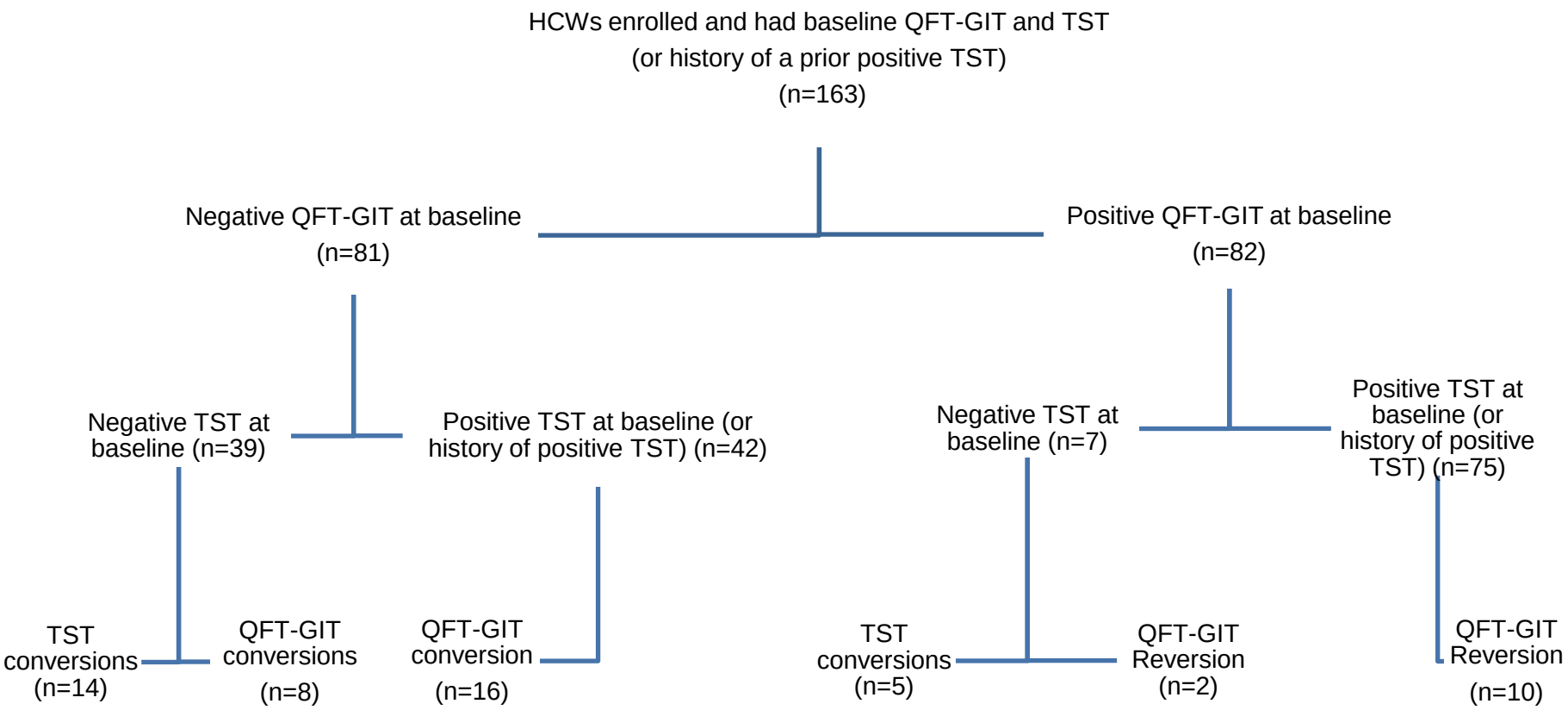
NOTE. TST, Tuberculin skin test; QFT-GIT, QuantiFERON-TB Gold In-tube assay; Frequent, contact with TB patients ≥ twice per month; Rare, contact with TB patients < twice per month; [a] Outlier cases were removed from the analysis; [b] Statistically significant effect

TST and QFT-GIT Conversion Rates

Among the 163 HCWs who had QFT-GIT and TST (or positive history of TST) performed at baseline and had repeated testing, 81 (49.7%) were susceptible to QFT-GIT conversion (negative QFT-GIT at baseline) and 46 (28.2%) were susceptible to TST conversion (negative TST at baseline) (Figure 2). The median time from baseline to repeat LTBI testing was 69 weeks (range 10-112 weeks). QFT-GIT conversions were documented among 24 (29.6%) of 81 HCWs. TST conversions occurred in 19 (41.3%) of 46 HCWs (Figure 2).

The conversion rate by QFT-GIT regardless of baseline TST result was 23.0/100 person-years. The conversion rate by TST regardless of baseline QFT-GIT result was 31.2/100 person-years. The conversion rate by either test among those who had concordantly negative TST and QFT-GIT results at baseline (n=39) was 28.6/100 person-years (26.7/100 person-years for TST conversion and 15.3/100 person-years for QFT-GIT conversion). QFT-GIT reversion occurred in 12 (14.6%) of 82 HCWs with positive QFT-GIT at baseline, and a reversion rate by QFT-GIT was 11.1/100 person-years (Figure 2).

Figure 2. Results of Diagnostic tests for Latent TB Infection among HCWs, who underwent serial testing: Conversions and Reversions



HCW, health care worker; QFT-GIT, QuantiFERON-TB Gold In-tube assay; TST, tuberculin skin test

Risk factors for TST and QFT-GIT conversion

Sixteen (84%) of 19 TST conversions and 19 (79%) of 24 QFT-GIT conversions occurred among HCWs, who worked at TB facilities. In univariate analysis, there were no variables that were significantly associated with a TST conversion (Table 5). Age per year (OR=1.09, 95% CI 1.03-1.16) and BCG scar (OR=0.31, 95% CI 0.10-0.96) were associated with QFT-GIT conversion, in univariate analysis (Table 5).

Table 5. Univariate analysis for latent tuberculosis infection (LTBI) diagnostic test (TST and QFT-GIT) conversion.

Characteristic	TST conversion (regardless of QFT-GIT) (18/44) [a]	QFT-GIT conversion (regardless of TST) (23/80) [a]
	Adjusted OR (95% CI)	Adjusted OR (95% CI)
Frequent vs. rare contact with TB patients	3.0 (0.78-11.60)	1.2 (0.45-3.26)
Age in years	1.0 (0.97-1.10)	**1.1 (1.03-1.16)** [b]
TB HCF vs. non-TB HCF	3.6 (0.66 - 19.26)	1.5 (0.49-4.79)
BCG Scare	3.0 (0.54-16.24)	**0.3 (0.10-0.96)** [b]

NOTE. Frequent contact is contact with TB patients ≥ twice per month; Rare contact is contact with TB patients < twice per month; TST, tuberculin skin test; QFT-GIT,

QuantiFERRON TB Gold In-tube test; [an] Outlier cases were removed from the analysis; [b] Statistically significant effect

In multivariate analyzes, there were no variables that were significantly associated with a TST conversion (Table 6). Increasing age per year (OR 1.14, 95% CI 1.02-1.27) was an independent risk factor associated with QFT-GIT conversion; BCG vaccination scar (OR 0.16, 95% CI 1.03-0.79) was associated with a decreased risk of conversion, in multivariate analysis (Table 6).

Table 6. Multivariate analysis for latent tuberculosis infection (LTBI) diagnostic test (QFT-GIT and TST) conversion.

Characteristic	TST conversion (regardless of QFT-GIT) (19/44) [a]	QFT-GIT conversion (regardless of TST) (23/80) [a]
	Adjusted OR (95% CI)	Adjusted OR (95% CI)
Frequent vs. rare contact with TB patients	3.07(0.21-43.43)	1.12 (0.17-7.18)
Age in years	0.98 (0.85-1.12)	**1.14 (1.02-1.27)[b]**
BCG Scare	8.29 (0.60-114.03)	**0.16 (0.03-0.79) [b]**

NOTE. Frequent contact is contact with TB patients ≥ twice per month; Rare contact is contact with TB patients < twice per month; TST, tuberculin skin test; QFT-GIT,

QuantiFERRON TB Gold In-tube test; [a] Outlier cases were removed from the analysis; [b] Statistically significant effect

Consistently Positive QFT-GIT

Among 163 HCWs, who underwent serial testing, 70 (43%) had positive QFT-GIT results both at baseline and repeated testing. The proportion of HCWs with consistently positive QFT-GIT results on both rounds of LTBI testing was higher among HCWs with frequent (≥ twice per month) TB exposure at work compared to HCWs with rare (< twice per month) occupation TB exposure (48% vs. 34%, p<0.001) (Table 7).

Table 7. Consistently Positive QFT-GIT

Characteristic	Frequent Contact (n=107)	Rare Contact (n=56)
	No. (%)	No. (%)
Consistently Positive QFT-GIT	51 (47.7)	19 (33.9)

NOTE. Frequent contact is contact with TB patients ≥ twice per month; Rare contact is contact with TB patients < twice per month; QFT-GIT, QuantiFERRON TB Gold In-tube test

Agreement between the Diagnostic Tests for Latent TB Infection (LTBI)

At baseline there was fair concordance between the TST and QFT-GIT [κ] =0.40, p<0.01 Agreement between the two diagnostic tests for LTBI was 70% (214/308); with 30% (93/308) of tests concordantly negative, 39% (121/308) tests concordantly positive. At repeated testing, there was poor concordance between the TST and QFT-GIT [κ] =0.37, p<0.01. Agreement between the two diagnostic tests for LTBI was 71.8% (117/163); with 15% (25/163) of tests concordantly negative, 56% (92/163) tests concordantly positive.

We found higher proportion of concordant results between the two diagnostic tests for LTBI among HCWs with BCG vaccination (documented by the presence of a BCG scare) compared to HCWs who did not have the presence of a BCG vaccination scar at baseline (66% vs. 81%, p<0.02, n=308). We also found high proportion of concordant results between two diagnostic tests for LTBI among HCWs with BCG vaccination (documented by the presence of a BCG scare) compared to HCWs who did not have the presence of a BCG vaccination scar at repeated testing (79% vs. 70%, p<0.27, n=163).

There was no significant differences between the results of the two diagnostic tests for LTBI among HCWs with frequent (≥ twice a month) occupational TB exposure compared to those HCWs who saw TB patients rarely (< twice a month) both at baseline (74% vs. 65%, p<0.08, n=308) and repeated testing (72% vs. 71%, p<0.94, n=163).

Risk factors for discordant results between TST and QFT-GIT

At baseline, discordant resutls between TST and QFT-GIT was 30% (94/308); with 23% (72/308) TST positive and QFT-GIT negative, and 7% (22/308) QFT-GIT positive and TST negative. At repeated testing, discordant resutls between TST and QFT-GIT was 28% (46/163); with 27% (44/163) TST positive and QFT-GIT negative, and 1% (2/163) QFT-GIT positive and TST negative.

In multivariate analysis, we found that the HCWs with discordant LTBI test results TST positive / QFT-GIT negative group, were less likely to report frequent (≥ twice per month) occupational TB exposure (aOR: 0.3, 95% CI: 0.12-0.85) and were more likely to have BCG vaccination scar found by inspection (aOR: 2.6, 95% CI: 1.12-5.83) compared to the HCWs with concordant LTBI test results (n=214) at baseline LTBI screening.

In multivariate analysis, only increasing age was in association with discordant LTBI diagnostic test results at repeated testing; risk of TST-positive / QFT-GIT negative results compared to concordant results the LTBI diagnostic tests (n=117) was lower among HCWs in age group 42-49 years vs. HCWs in age group 18-32 years (aOR: 0.24, 95% CI: 0.07-0.84) and among HCWs with age ≥ 50 years vs. HCWs in age group 18-32 years (aOR: 0.04, 95% CI: 0.01-0.26).

Active TB Disease

Only one HCW was diagnosed with active TB disease after symptom screen and chest x-ray at time of LTBI testing. Three HCWs did develop active disease during this study. These HCWs had tested positive both by TST and QFT within 12 months before being diagnosed with active TB. It is expected that TB cases are under-reported among HCWs to the NCTLD/NTP TB surveillance department due to the stigma associated with having TB disease. It is expected that all that all diagnosed TB cases are notified to the NCTBLD/NTP TB surveillance department.

HCWs Knowledge about TB

The HCW overall average knowledge score was 61%. HCWs, who worked with TB patients, knew more about TB (69% overall average score) compared with HCWs, who did not (49.16% overall average score; $P < .01$). Nearly all HCWs (98%) knew that TB is transmitted by an airborne route, and 70% of HCWs knew epidemiological, clinical, and laboratory characteristics of LTBI. However, only 43% of HCWs knew the risk of LTBI progression to TB disease, and only 30% were able to identify correctly high-risk groups for LTBI progression to TB disease. The majority of HCWs (85%) knew the preferred regimen for LTBI treatment, but fewer (66%) knew the justification for latent TB therapy.

HCWs Beliefs about LTBI and TB IC

With respect to HCWs, perceived threat of TB infection and perceived benefits and barriers of TB IC, 53% of HCWs in this study thought that they were at risk of having LTBI at some point in the future; 36% of the study participants were concerned about acquiring LTBI with MDR-TB strains; 48% thought of LTBI as a serious health condition; but 43% of HCWs did not want to receive treatment for LTBI because they believed that they would be exposed to TB again (Table 8).

Table 8. Health care Worker Beliefs about Latent Tuberculosis Infection and Tuberculosis IC (N=240)

Characteristic	No Chance (1), no. (%)	Little Chance (2), no. (%)	No Opinion (3), no. (%)	Some Chance (4). no. (%)	Very Good Chance
Perceived Susceptibility					
Have LTBI now	48 (20.0)	71 (29.6)	11 (4.6)	72 (30.0)	38 (15.8)
Will test positive for LTBI in the future	22 (9.2)	65 (27.1)	25 (10.4)	99 (41.3)	29 (12.1)
Will be diagnosed with TB	35 (14.6)	75 (31.3)	14 (5.8)	104 (43.3)	12 (5.0)

in the future									
Characteristic	**Strongly Agree**	**(1), no. (%)**	**Agree**	**(1), no. (%)**	**No Opinion**	**(1), no. (%)**	**Disagree**	**(1), no. (%)**	**Strongly Disagree**	**(1), no. (%)**
Perceived Severity										
Worry about acquiring LTBI	48 (20.0)		84 (35.0)		43 (17.9)		49 (20.4)		16 (6.7)	
Worry about acquiring TB disease	30 (12.5)		62 (25.8)		54 (22.5)		68 (28.3)		26 (10.8)	
Worry about acquiring LTBI with MDR-TB strains	16 (6.7)		70 (29.7)		64 (26.7)		63 (26.3)		27 (11.3)	
Latent TB infection is very serious	30 (12.5)		87 (36.25)		39 (16.3)		70 (29.2)		14 (5.8)	
Perceived Benefits										
IC measures prevent nosocomial TB transmission	86 (35.8)		102 (42.5)		29 (12.1)		21 (8.75)		2 (0.8)	
UV is an effective IC	48 (20)		119 (49.6)		54 (22.5)		12 (5)		7 (2.9)	

measure					
Respirator protects HCW from TB exposure	116 (48.3)	94 (39.2)	22 (9.2)	5 (2.1)	3 (1.3)
Respirator protects HCW from MDR-TB exposure	89 (37.1)	109 (45.4)	36 (15)	5 (2.1)	1 (0.4)
It is important for Georgian HCWs to be tested for latent TB infection	100 (41.7)	106 (44.2)	23 (9.6)	9 (3.8)	2 (0.8)
It is important to test contacts of patients with TB (family, friends) for latent TB infection.	130 (54.2)	86 (35.8)	15 (6.3)	5 (2.1)	4 (1.7)
It is important to test children who have been exposed to TB for latent TB infection.	147 (61.3)	74 (30.8)	16 (6.7)	0 (0.0)	3 (1.3)
It is important to test individuals with	103 (42.9)	92 (38.3)	38 (15.8)	5 (2.1)	2 (0.8)

compromised immune systems for latent TB infection.					
Perceived Barriers					
UV lights can harm HCWs	31 (12.9)	70 (29.2)	57 (23.8)	73 (30.5)	9 (3.8)
If I tested positive for LTBI, I should not be treated because I will be exposed again in the future	33 (13.8)	70 (29.2)	50 (20.8)	69 (28.8)	18 (7.5)
If I tested positive for LTBI, I should not be treated because probably I have drug-resistant TB strains	23 (9.6)	43 (17.9)	58 (24.2)	93 (38.8)	23 (9.6)
Risks of treating LTBI outweigh benefits to treating LTBI	35 (14.6)	70 (29.2)	84 (35.0)	48 20.0)	3 (1.3)

NOTE. HCW, health care worker; LTBI, latent tuberculosis infection; TB, tuberculosis; MDR-TB, multidrug-resistant tuberculosis; IC, IC; UV, ultraviolet.

TB IC Related Behavior or Willingness to Exhibit TB IC-related Behavior

A total of 78% of HCWs from the NTP and only 36% of HCWs from the PHCs reported frequent use of respirators when they were around patients who were at risk for or who had active TB. TB IC–related behavior and willingness to implement TB IC–related behavioral change are outlined in Table 9.

Table 9. Tuberculosis IC Related Behavior or Willingness to Exhibit Tuberculosis IC Related Behavior (N=240)	
Characteristic	**No. (%)**
Respirator Use: How often do you wear a respirator when around patients who are at risk for or who have active TB?	
Frequent	144 (60.0)
Sometimes	49 (20.4)
Never	29 (12.1)
Missing	18 (7.5)
UV light Use: I do not want to work in an area where UV lights are used.	
Agree	90(37.5)
No Opinion	53(22.1)
Disagree	97(40.4)
LTBI Screening: Would you be willing to be tested each year for latent TB infection?	
Yes	125 (52.1)

No	59 (24.6)
Undecided	45 (18.8)
Missing	11(4.6)
LTBI treatment: If tested positive for latent TB infection, I should be treated.	
Agree	116 (48.3)
No Opinion	40 (16.7)
Disagree	84 (35.0)

NOTE. TB, tuberculosis; IC, IC; UV, ultraviolet; LTBI, latent tuberculosis infection.

Predictors of HCW Knowledge about TB

In our multivariate analysis, physicians were more likely to know symptoms suggestive of TB disease (aOR, 1.7; 95% CI, 1.0–2.9), TB diagnostic methods (aOR, 1.9; 95% CI, 1.1–3.1), high-risk groups for TB disease (aOR, 2.3; 95% CI, 1.3–4.0), and LTBI treatment rationale (aOR, 1.5; 95% CI, 1.0–2.5) than nurses (Table 4). HCWs who worked primarily with TB patients were more likely to know about the risk of LTBI progression to TB disease (aOR, 3.2; 95% CI, 1.6–6.4), highrisk groups for TB disease (aOR, 2.2; 95% CI, 1.0–4.8), LTBI treatment rationale (aOR, 2.3; 95% CI, 1.2–4.5), and LTBI treatment regimen (aOR, 4.2; 95% CI, 1.6–11.1) than those who did not work with TB patients (Table 10).

Table 10. Multivariate analysis for predictors of HCWs Tuberculosis Knowledge (N=240)

	Knowledge Outcomes [a] and Predictors						
Characteristic	LTBI Characteristics, aOR (95% CI)	Risk of LTBI Progress to TB, aOR (95% CI)	High-Risk groups for TB, aOR (95% CI)	TB Symptoms, aOR (95% CI)	TB Diagnosis, aOR (95% CI)	LTBI Treatment Rational, aOR (95% CI)	LTBI Treatment Regimen, aOR (95% CI)
Male vs.	1.4	**9.3** [c]	1.7	0.6	1.6	1.3	3.0
Female	(0.4, 5.5)	**(1.9, 44.9)**	(0.5, 6.0)	(0.2, 2.5)	(0.5, 5.4)	(0.4, 4.40)	(0.4, 25,8)
Age, y (60 < vs.	1.3	0.9	1.2	1.5	1.2	**1.7** [c]	1.1
52 – 60 vs.	(0.8, 2.0)	(0.6, 1.3)	(0.8, 1.8)	(0.9, 2.5)	(0.8, 1.8)	**(1.1, 2.6)**	(0.6, 1.9)

45 – 51 vs. 36 – 44 vs. ≤ 35)							
Occupation (Physician vs. Nurse vs. Other) [d]	1.6 (1.0, 2.6)	1.4 (0.8, 2.3)	**2.3** [c] **(1.3, 4.0)**	**1.7** [c] **(1.0, 2.9)**	**1.9** [c] **(1.1, 3.1)**	**1.5** [c] **(1.0, 2.5)**	0.6 (0.3, 1.1)
Works with TB patients vs. does not work with TB patients	1.6 (0.8, 3.2)	**3.2** [c] **(1.6, 6.4)**	**2.2** [c] **(1.0 4.8)**	1.6 (0.8, 3.3)	1.4 (0.7, 2.8)	**2.3** [c] **(1.2, 4.5)**	**4.2** [c] **(1.6, 11.1)**
Length of Employment, y (35 ≤ vs. 21 - 34 vs. 6 - 20 vs. ≤ 5) [d]	0.7 (0.4, 1.2)	0.7 (0.4, 1.3)	0.9 (0.5, 1.9)	0.6 (0.3, 1.3)	0.8 (0.5, 1.5)	0.5 (0.2, 0.9)	0.7 (0.3, 1.7)

NOTE. TB knowledge variables were coded as correct versus incorrect answers.

aOR, adjusted odds ratio; CI, confidence interval; HCW, health care worker; TB, tuberculosis; LTBI, latent tuberculosis infection; [a] Binary logistic regression was used; [c] Statistically significant effect.

[d] Ordinal variables.

Association between HCW TB Knowledge and Beliefs

HCWs who knew the risk of progression from LTBI to TB disease (P < .03) and the high-risk groups for TB disease (P< .01) were more likely to worry about acquiring LTBI with drug-resistant strains than HCWs who did not have this knowledge. HCWs who knew LTBI treatment rationale (P< .01) and TB diagnostics (P< .05) were more likely to think that screening of TB contacts for LTBI is important than those HCWs who did not demonstrate this knowledge. HCWs who knew LTBI characteristics (P< .04), LTBI treatment rationale (P <.01), and TB diagnostics (P< .01) more likely felt that immunocompromised individuals should be screened for LTBI than those who did not have this knowledge. Only those HCWs who knew LTBI characteristics (P< .01) perceived LTBI as a serious infection. As expected, HCWs, who worked primarily with TB patients considered themselves more susceptible to LTBI than HCWs, who did not (P <.01).

Predictors of TB IC–Related Behaviors

HCWs who indicated that they worried about becoming infected with drug-resistant TB (aOR, 1.7; 95% CI, 1.29–2.24), HCWs who thought it was important to screen TB contacts (aOR, 3.1; 95% CI, 1.25–7.77), and HCWs who were physicians (aOR, 1.6; 95% CI, 1.04–

2.42) were more likely to be willing to undergo annual screening for LTBI (Table 8). HCWs were more likely to refuse treatment for LTBI if they worked in TB facilities (inpatient TB facility: aOR 0.3; 95% CI, 0.12–0.68; outpatient TB facility: aOR, 0.2; 95% CI, 0.10–0.35), and they perceived a high personal risk of TB reinfection (aOR, 0.5; 95% CI, 0.36–0.64). Those who thought that LTBI was a potentially serious health condition were more willing to be treated for LTBI (aOR, 2.0; 95% CI, 1.48–2.60) (Table 8). Availability of respirators in HCFs was the only significant predictor of routine use of respirators (aOR, 5.1; 95% CI, 3.50–7.30). In multivariate analysis, employment in a TB outpatient facility (aOR, 3.1; 95% CI, 1.37–6.96), perceived susceptibility to LTBI in the future (aOR, 1.4; 95% CI, 1.02–2.03), and the perception that UV germicidal radiation was unlikely to harm HCWs (aOR, 0.4; 95% CI, 0.24–0.50) were identified as independent predictors of willingness to use UV lights in HCFs (Table 11).

Table 11. Multivariate Analysis for Predictors of Tuberculosis IC-Related Behaviors (N=240)									
	IC–Related Behavioral Outcomes and Predictors								
Characteristic	Respirator Use,	aOR (95% CI)[a]	UV Light Use	in HCF,	aOR (95% CI)[b] LTBI	Screening,	aOR (95% CI)[a]	LTBI	Treatment, aOR (95% CI)[a]
Modifying Factors									
TB inpatient vs. non-TB HCF	1.6	(0.48, 5.29)	1.3	(0.43, 3.61)	1.7	(0.72, 4.09)		**0.3**[c]	**(0.12, 0.68)**
TB outpatient vs. non-TB HCF	1.0	(0.42, 2.18)	**3.1**[c]	**(1.37, 6.96)**	0.6	(0.30, 1.17)		**0.2**[c]	**(0.10, 0.35)**
Occupation [d]					**1.6**[c]	**(1.04, 2.42)**		0.7	(0.42, 1.06)
Respirator availability [d]	**5.1**[c]	**(3.50, 7.30)**							
Perceived Threat									
Will test positive for LTBI in the future			**1.4**[c]	**(1.02, 2.03)**					
Worry about acquiring LTBI with MDR-TB strains	1.4	(0.97, 1.97)			**1.7**[c]	**(1.29, 2.24)**			
LTBI is very serious								**2.0**[c]	**(1.48, 2.60)**

Perceived Benefits			
UV light is an effective TB IC measure	1.6 (0.69, 3.46)		
It is important to test TB contacts for LTBI		**3.1 [c] (1.25, 7.77)**	
Perceived barriers			
UV lights can harm HCWs	**0.4 [c] (0.24, 0.50)**		
If I tested positive for LTBI, I should not be treated because I will be exposed again in the future			**0.5 [c] (0.36, 0.64)**

NOTE. Occupation was coded as "physician" or "nurse" or "other." Respirator availability was coded as "always," "most of the time," "sometimes," "rare," or "never." Respiratory use was coded as "frequent," "sometimes," or "never." UV light use in HCF, LTBI screening, and LTBI treatment were coded as "yes," "undecided," or "no." aOR, adjusted odds ratio; CI, confidence interval; UV, ultraviolet; HCF, health care facility; TB, tuberculosis; LTBI, latent tuberculosis infection; MDR-TB, multidrug-resistant tuberculosis; IC, Infection Control; HCW, health care worker; [an] Ordinal logistic regression was used; [b] Polytomous logistic regression was used; [c] Statistically significant effect; [d] Ordinal variable.

Discussion

We found a high prevalence of LTBI among Georgian HCWs, which was significantly higher among HCWs at TB facilities (55% QFT-GIT positive and 68% TST positive) compared to HCWs at non-TB HCFs (32% QFT-GIT positive and 54% TST positive). Furthermore, high rates of LTBI diagnostic test conversions were found among Georgian HCWs. The conversion rate (a negative test followed by a subsequent positive test) when using the TST was 31.2/100 person-years. The conversion rate by QFT-GIT was 23.0/100 person-years. This suggests high rates of occupational exposure to and infection with *Mycobacterium tuberculosis* among Georgian HCWs and highlights the need for implementation of effective TB infection control measures. In the survey about TB and TB IC related behaviors conducted among HCWs from the NTP and PHCs in Georgia, physicians compared to nurses were found to have greater knowledge related to TB and TB IC measures. Also, HCWs who worked primarily with TB patients were more educated about TB and related IC activities compared to HCWs, who did not see TB patients regularly. HCWs knowledgeable about TB and TB IC measures were more likely to perceive their susceptibility to TB infection, the severity of TB disease, and TB IC intervention benefits and barriers. Moreover, HCWs who perceived their susceptibility to TB infection and net benefit of TB IC measures were more likely to comply with IC interventions.

The high prevalence of LTBI among Georgian is consistent with reports from India [108], Russia [109], and Vietnam [110] which have not accomplished implementation of infection control measures and have reported prevalence of positive test results between 40-66% for TST and QFT-GIT among HCWs.

There are limited data on LTBI test conversion rated among HCWs in TB endemic countries (low and middle-income countries)[111]. The most striking findings of our study were high rates of LTBI test conversion among Georgian HCWs representing probable recent infection with *M. tuberculosis*. Much higher rates of conversion were seen among Georgian HCWs in our study of compared to other studies among HCWs in India that reported TST conversion rates of 2.7/100 person-years and QFT-GIT conversion rates of 7.7/100 person-years [112]. A study of Malaysian HCWs found QFT-GIT conversion rates of 9.9/100 person-years[113]. One reason for higher rates of conversion in our study Of note, these other studies were of HCWs from hospitals not specializing in TB care, whereas in our study, 61% of HCWs worked in facilities specializing in TB care. Our findings highlight a high rate of ongoing transmission of TB in Georgian HCFs especially TB HCFs, and the urgent need to implement effective TB IC measures.

There are limited data on IGRA performance in the serial testing of HCWs using in LMIC TB endemic countries. Our study of serial TST and IGRA testing in LMIC with high incidence of TB evaluates the relationship between epidemiologic factors (age, degree of occupational TB exposure, and TB exposure outside health care settings) and risk of LTBI prevalence and the LTBI diagnostic tests conversion.

Interestingly, in our study frequent occupation TB exposure was associated only with QFT-GIT positive results at baseline LTBI screening. Increasing age was associated with both positive TST and positive QFT-GIT at baseline. Other studies have also found a positive association between occupational TB exposure and IGRA positivity rates [114]. Of three cross-sectional studies of IGRAs and TST conducted in high-incidence settings[108-110], only one study from India evaluated the association between occupational risk factors for both TST and IGRA [108]. This study found a stronger, but non-significant, association between occupational risk factors and IGRA positivity than for TST positivity[108]. In the systematic review by Zwerling et al., among 22 cross-sectional studies of HCWs in low and moderate incidence TB countries, TST, QFT-GIT, and TSPOT.TB correlated well with established indicators of occupational risk of TB exposure, although no test was more consistently associated with these indicators of exposure [114].

Both in univariate and multivariate analysis, we did not find an association between occupational TB exposure and TST conversions. QFT-GIT conversion was positively associated with increasing age per year only, both in univariate and multivariate analysis. Interestingly, we also found that HCWs with BCG vaccination scar were less likely to have QFT-GIT conversion in multivariate analysis. A study from Japan of serial testing of HCWs with QFT-GIT found that HCWs, who worked in a TB ward were 20 times more likely to experience QFT-GIT conversion than those who did not work in a TB ward[91]. While we did not observe a significant association with TB exposure frequency and TST or QFT-GIT test conversion, we did find that 16/19 (84%) TST conversions and 19/24 (79%) QFT-GIT

conversions occurred among HCWs working at a TB facility. Our small sample size for serial testing: only 46 HCWs were TST negative on baseline testing and only 81 were negative on baseline QFT-GIT testing, limited our ability to detect significance. However, we found higher proportion of HCWs with consistently positive QFT-GIT results on both rounds of LTBI testing among HCWs with frequent (≥ twice per month) TB exposure at work compared to HCWs with rare (< twice per month) occupation TB exposure (48% vs. 38%, $p<0.001$).

Absence of a true gold standard test for LTBI presents a major challenge for assessing the performance of the LTBI diagnostic tests[85]. Therefore, we investigated epidemiologic factors and the agreement between the two LTBI diagnostic tests at baseline and repeated testing. We found higher proportion of concordant results of the two diagnostic tests for LTBI among HCWs without BCG vaccination scar vs. HCWs with the scar both at baseline (66% vs. 81%, $p<0.02$, n=308) and repeated testing (79% vs. 70%, $p<0.27$, n=163). It is important to note that the difference was significant only at baseline testing. Moreover, multivariate analysis showed that the HCWs with discordant LTBI test results TST positive / QFT-GIT negative group, were less likely to report frequent (≥ twice per month) occupational TB exposure and were more likely to have BCG vaccination scar found by inspection compared to the HCWs with concordant LTBI test results at baseline LTBI screening. Important to note that only increasing age was in positive association with discordant LTBI diagnostic test results at repeated testing.

Based on our study findings we can argue that TST and QFT-GIT performance differs across baseline and repeated testing with respect to the well-established indicators of TB exposure in the community and at work. Also, the performance of the two tests differs among HCWs with BCG vaccination scar compared to HCWs without the scar. The absence of cross-reactivity with BCG is an advantage of IGRAs over TST[111]. BCG vaccination was strongly associated with the pattern of TST-positive/ QFT-GIT negative test discordance, as has been reported by others[20, 115-117]. Our results support a role for IGRAs in accurately determining TB infection status at baseline screening of HCWs in high TB incidence country with high BCG vaccination coverage.

Georgia has been designated by the WHO as a high burden MDR-TB country[111]. It is particularly important to assess nosocomial TB Transmission to HCWs and to strengthen TB IC in HCFs in the setting of a highly endemic M/XDR-TB country, as there are no evidence-based guidelines for treatment of LTBI due to M/XDR-TB contact [118]. Moreover, prior to 2012 patients with infectious TB were diagnosed and treated in specialized inpatient and outpatient TB facilities of the NTP, although persons with undiagnosed TB or suspected cases of TB might have been seen at non-TB facilities and referred to a specialized TB facility later[1]. Currently, TB care is provided by diverse non-NTP public and private care providers[3]. This transition introduces a significant risk of nosocomial TB transmission in "non-TB" HCFs if effective TB infection control measures are not implemented. We found the same prevalence of LTBI among Georgian HCWs from non-TB HCFs (32%) as it is estimated in the general population of TB endemic countries[41]. This finding further highlights the importance

of preventing nosocomial TB transmission in non-TB HCFs in Georgia. TB IC should become part of the national infection control strategy[12].

Evidence supports that knowledge is a facilitator of compliance with interventions[9, 119, 120]. Nurses who work mainly with TB patients should be targeted for the training given their lack of knowledge on this topic. Furthermore, Georgian HCWs who work in non-TB HCFs need training about TB and TB IC, as persons with undiagnosed TB or suspected cases of TB may be seen at these facilities. This is especially true since TB services are currently being integrated with PHCs as part of the ongoing health system reforms in Georgia.

The survey data were analyzed based on the HBM. The model suggests that individuals conduct an internal assessment of the net benefits of changing their behavior and decide whether or not to act. The model identifies four aspects of this assessment: perceived susceptibility to ill-health (risk perception), perceived severity of ill-health, perceived benefits of behavior change, and perceived barriers to taking action.[95] Consistent with the HBM, UV light use is well-accepted by HCWs who believe that they are at risk of TB infection, but HCWs who think that UV lights can be harmful leads to their reluctance to use UV lights in HCFs. Perceived LTBI threat predicted HCWs' readiness to receive LTBI treatment while concern for re-infection with TB after LTBI treatment predicted HCWs refusal to be treated for LTBI. Given the reported high rates of occupational acquisition of TB infection, it is not surprising that Georgian HCWs believe that they remain at risk of TB even after treatment for LTBI. We also found that respirators are not always available for all HCWs, especially in non-TB HCFs. These findings emphasize the need to strengthen IC

measures in Georgian HCFs and provide important baseline information for the Georgian Ministry of Health, Labor, and Social Affairs that is currently implementing IC interventions in HCFs.

In summary, our study is the first survey of HCWs' knowledge, beliefs, and behaviors about TB IC and LTBI screening and treatment in Georgia. We were able to identify specific knowledge gaps and beliefs to be addressed during implementation of TB IC measures in Georgian HCFs. Researchers and HCF administrators should pursue the application of behavioral science methods to strengthen TB IC measures implementation process.[39] Based on our survey findings, a targeted campaign is needed to raise HCWs' awareness about TB and about the benefits of TB IC measures to prevent the nosocomial transmission of TB and the particular threats of drug-resistant TB in the country Georgia.

Limitations

Both, the longitudinal study of the rates and risk factors for LTBI among Georgian HCWs and the anonymous survey about the determinants of TB IC-related behaviors among Georgina HCWs, had several limitations. In the study of the rates and risk factors for LTBI among HCWs in Georgia only 45 participants were included from outside of the capital city, Tbilisi. Although TB services' infrastructure, level of TB IC measures implementation, and HCWs compliance to the existing TB IC measures are about the same across the Georgian HCFs, one third of the notified TB patients in Georgia undergo TB diagnosis and initial phase

of TB treatment in HCFs in Tbilisi. Therefore, the LTBI prevalence and incidence rates among Georgian HCWs might be slightly overestimated in this study. Furthermore, because of the high prevalence of LTBI, the number of uninfected HCWs who were at risk for LTBI test conversions was modest (81 were at risk for QFT-GIT conversion and 46 for TST conversion). HCWs are not routinely tested for LTBI in Georgia (and the vast majority of LMICs) so there may have been selection or volunteer bias on HCWs, who chose to participate in our study. Only 53% (163/308) of the HCWs enrolled in the study had repeated LTBI tests performed, which could have introduced bias with respect to conversion rates and risk factors. Finally, repeat testing occurred at different time intervals but this was clearly documented, so we were able to calculate conversion rates over time.

One limitation of the anonymous survey is that TB IC related behaviors were self-reported rather than observed. For instance, respirator use was measured by HCWs' responses to anonymous questions, rather than by observations of this behavior by the study team. Another limitation of our study is that convenience sampling was used, and 19% of those who were approached did not agree to complete the survey. Most of the non-responders (53 out of 58 HCWs) were nurses from the NTP, potentially introducing selection bias. Physician to nurse ratio in TB services and PHCs is about one to one in Georgia. Therefore, we expected the nearly equal proportion of nurses and physicians in our study population. Physicians comprised 48% of our population, and nurses comprised 40%, so our study slightly overrepresented physicians compared to nurses. A major strength of the anonymous survey is that it included various types of HCWs from across the whole country.

Conclusions

1. We found a high prevalence of LTBI among Georgian HCWs.
    i. LTBI prevalence was significantly higher among HCWs at TB facilities compared to HCWs at non-TB HCFs.

2. We found high rates of LTBI diagnostic test conversions among Georgian HCWs.
    i. The majority (80%) of TST and QFT-GIT conversions occurred among HCWs working at TB facilities

3. The performance of TST and QFT-GIT varied with respect to indicators of TB exposure both at baseline and at repeated testing.

    i. Indicators of occupational TB exposure - frequent contact with TB patients at work, was positively associated with only QFT-GIT positive results at baseline;
    ii. Increasing age was associated with both positive TST and positive QFT-GIT at baseline;
    iii. We did not find association between occupational TB exposure and TST or QFT-GIT conversions;

        Note: Our small sample size for serial testing: only 46 HCWs were TST negative on baseline testing and only 81 were negative on baseline QFT-GIT testing, limited our ability to detect significance.

iv. Increasing age was positively associated with QFT-GIT conversion, and HCWs with BCG vaccination scar were less likely to experience QFT-GIT conversion;

v. We found higher proportion of HCWs with consistently positive QFT-GIT results on both rounds of LTBI testing among HCWs with frequent (≥ twice per month) TB exposure at work compared to HCWs with rare (< twice per month) occupation TB exposure.

Note: As opposed to the small sample size for the detection of risk factors associated with QFT-GIT and TST conversions (concluson 3-iii), propotions of consitantly positive QFT-GIT test across the occupation TB exposure friequency were compared among 163 HCWs, who underwent serial testing for LTBI. Possibly, the larger samle size alowed us to detect statistically significant association between conistantly posittive QFT-GIT and the occupation TB exposure.

4. TST and QFT-GIT performance differs among HCWs with BCG vaccination scar compared to HCWs without the scar at baseline screening of HCWs

i. We found higher proportion of concordant results of the two diagnostic tests for LTBI among HCWs without BCG vaccination scar vs. HCWs with the scar both at baseline;

ii. HCWs with discordant LTBI test results TST positive / QFT-GIT negative group, were less likely to report frequent (≥ twice per month) occupational TB exposure and were more likely to have BCG vaccination scar found by inspection compared to the HCWs with concordant LTBI test results at baseline LTBI screening. Only increasing age was in positive association with discordant LTBI diagnostic test results at repeated testing.

5. We found that moderate knowledge of TB and TB IC among Georgian HCWs
    i. Physicians compared to nurses were found to have greater knowledge related to TB and TB IC measures.
    ii. HCWs, who worked primarily with TB patients, were more educated about TB and related IC activities compared to HCWs, who did not see TB patients regularly.

6. Consistent with the Health Belief Model,
    i. HCWs knowledgeable about TB and TB IC measures were more likely to perceive their susceptibility to TB infection, the severity of TB disease, and TB IC intervention benefits and barriers.
    ii. HCWs, who perceived their susceptibility to TB infection and net benefit of TB IC measures, were more likely to comply with IC interventions.

- UV light use is well-accepted by HCWs, who believe that they are at risk of TB infection, but HCWs who think that UV lights can be harmful leads to their reluctance to use UV lights in HCFs.
- Perceived LTBI threat predicted HCWs' readiness to receive LTBI treatment while concern for re-infection with TB after LTBI treatment predicted HCWs refusal to be treated for LTBI.

7. We found that respirators were not always available for all HCWs, especially in non-TB HCFs

8. Our study findings suggest a high rate of ongoing transmission of TB in Georgian HCFs especially TB HCFs and the urgent need to implement effective TB IC measures.

    Note: Prior to 2012 patients with infectious TB were diagnosed and treated in specialized inpatient and outpatient TB facilities of the NTP, although persons with undiagnosed TB or suspected cases of TB might have been seen at non-TB facilities and referred to a specialized TB facility later[1]. Currently, TB care is provided by diverse non-NTP public and private care providers[3]. This transition introduces a high risk of nosocomial TB transmission in non-TB HCFs too. Findings of our study about the same prevalence of LTBI among Georgian HCWs from non-TB HCFs (32%) as it is estimated in general

population of TB endemic countries[41] further highlights importance of preventing nosocomial TB transmission in non-TB HCFs in Georgia

Practical Recommendations

1. Based on our study findings that there are high rates of LTBI prevalence and incidence among Georgina HCWs, TB IC measures should urgently be implemented in Georgian HCFs.

2. Considering ongoing transition of TB services from the NTP specialized TB facilities to non-NTP public and private TB facilities, TB IC strategy should become integral part of the National IC strategy in Georgia[12].

3. The set of TB infection control measures should be monitored and evaluated[12]

    i. Introduce screening of HCWs at baseline and five years after TB IC measures implementation to assess change in nosocomial TB transmission rates[12]

    ii. Use QFT-GIT for screening of HCWs to monitor TB IC measures implementation in Georgian HCFs.

    Note: Although in resource-limited, highly endemic TB countries, resources would likely be better spent on strengthening TB IC measures than on the extra cost of IGRA screening, our study findings showed that none of the well-established indicators of TB occupational exposure was associated with TST positive test results either at baseline or repeated testing. Furthermore, we

found that rare occupational TB exposure and presence of BCG vaccination scar was strongly associated with TST positive/QFT-GIT negative test results at baseline. Our results support a role for IGRAs in accurately determining TB infection status at baseline screening of HCWs in high TB incidence country with high BCG vaccination coverage.

4. Operational research should be enabled and conducted[12]
    i. Further evidence from IGRA serial testing studies, including long-term follow up data of "converters" is needed, to be able to determine what changes in IGRA test values constitute the development of LTBI infection

5. Researchers and HCF administrators should pursue the application of behavioral science methods to strengthen TB IC measures implementation process[39]

6. Based on our survey findings, a targeted campaign should be introduced to raise HCWs' awareness about TB and about the benefits of TB IC measures to prevent the nosocomial transmission of TB and the particular threats of drug-resistant TB in the country Georgia.
    i. Nurses who work mainly with TB patients should be targeted for the training given their lack of knowledge on this topic
    ii. Georgian HCWs, who work in non-TB HCFs, need training about TB and TB IC, as persons with undiagnosed TB or suspected cases of TB may be

seen at these facilities. This is especially true since TB services are currently being integrated with PHCs as part of the ongoing health system reforms in Georgia

7. Include module on TB transmission and TB IC in the state Continues Medical Educating program
8. Introduce LTBI preventive therapy among HCWs only after documented decline in nosocomial TB transmission and decrease of TB prevalence < 100/100,000 population per year[47]

Note: As per the latest WHO guidelines on the management of latent tuberculosis infection[47] systematic testing and treatment of LTBI should be considered for HCWs from high-income or upper middle-income countries with an estimated TB incidence rate of less than 100 per 100 000 population. The Panel judged that these countries are most likely to benefit from systematic testing and treatment of LTBI for HCWs due to their current TB epidemiology and resource availability. Resource-limited countries and other middle-income countries that do not belong to the above category should implement treatment of LTBI among people living with HIV and child contacts below 5 years of age.

Bibliography

1. Mirtskhulava V, Kempker R, Shields KL, et al. Prevalence and risk factors for latent tuberculosis infection among health care workers in Georgia. Int J Tuberc Lung Dis 2008;12(5):513-9.
2. Lomtadze N, Aspindzelashvili R, Janjgava M, et al. Prevalence and risk factors for multidrug-resistant tuberculosis in the Republic of Georgia: a population-based study. Int J Tuberc Lung Dis 2009;13(1):68-73.
3. World Health Organization. Global tuberculosis report 2014. Geneva: World Health Organization; 2014.
4. Emergence of Mycobacterium tuberculosis with extensive resistance to second-line drugs--worldwide, 2000-2004. MMWR Morb Mortal Wkly Rep 2006;55(11):301-5.
5. Extensively drug-resistant tuberculosis (XDR-TB): recommendations for prevention and control. Wkly Epidemiol Rec 2006;81(45):430-2.
6. Fennelly KP, Iseman MD. Health care workers and tuberculosis: the battle of a century. Int J Tuberc Lung Dis 1999;3(5):363-4.
7. Granich R, Binkin NJ, Jarvis WR, et al. Guidelines for the prevention of tuberculosis in health care facilities in resource-limited settings. Geneva: World Health Organization; 1999.
8. Harries AD, Maher D, Nunn P. Practical and affordable measures for the protection of health care workers from tuberculosis in low-income countries. Bull World Health Organ 1997;75(5):477-89.
9. Jensen PA, Lambert LA, Iademarco MF, Ridzon R, Cdc. Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care settings, 2005. MMWR Recomm Rep 2005;54(RR-17):1-141.
10. Joshi R, Reingold AL, Menzies D, Pai M. Tuberculosis among health-care workers in low- and middle-income countries: a systematic review. PLoS Med 2006;3(12):e494.
11. Menzies D, Joshi R, Pai M. Risk of tuberculosis infection and disease associated with work in health care settings. Int J Tuberc Lung Dis 2007;11(6):593-605.
12. World Health Organization. WHO policy on TB infection control in health-care facilities, congregate settings and households. Geneva: World Health Organization; 2009.
13. Mirtskhulava V, Whitaker JA, Kipiani M, et al. Determinants of tuberculosis infection control-related behaviors among healthcare workers in the country of georgia. Infect Control Hosp Epidemiol 2015;36(5):522-8.
14. Jones-Lopez EC, Ellner JJ. Tuberculosis infection among HCWs. Int J Tuberc Lung Dis 2005;9(6):591.
15. Sepkowitz KA. Tuberculosis and the health care worker: a historical perspective. Ann Intern Med 1994;120(1):71-9.
16. Bock NN, Jensen PA, Miller B, Nardell E. Tuberculosis infection control in resource-limited settings in the era of expanding HIV care and treatment. J Infect Dis 2007;196 Suppl 1:S108-13.
17. Zahnow K, Matts JP, Hillman D, et al. Rates of tuberculosis infection in healthcare workers providing services to HIV-infected populations. Terry Beirn Community Programs for Clinical Research on AIDS. Infect Control Hosp Epidemiol 1998;19(11):829-35.
18. Manusov EG, Bradshaw RD, Fogarty JP. Tuberculosis screening in medical students. Fam Med 1996;28(9):645-9.
19. Bailey TC, Fraser VJ, Spitznagel EL, Dunagan WC. Risk factors for a positive tuberculin skin test among employees of an urban, midwestern teaching hospital. Ann Intern Med 1995;122(8):580-5.
20. Dorman SE, Belknap R, Graviss EA, et al. Interferon-gamma release assays and tuberculin skin testing for diagnosis of latent tuberculosis infection in healthcare workers in the United States. Am J Respir Crit Care Med 2014;189(1):77-87.
21. Dooley SW, Villarino ME, Lawrence M, et al. Nosocomial transmission of tuberculosis in a hospital unit for HIV-infected patients. JAMA 1992;267(19):2632-4.

22. Fraser VJ, Kilo CM, Bailey TC, Medoff G, Dunagan WC. Screening of physicians for tuberculosis. Infect Control Hosp Epidemiol 1994;15(2):95-100.
23. Menzies D, Fanning A, Yuan L, FitzGerald JM. Tuberculosis in health care workers: a multicentre Canadian prevalence survey: preliminary results. Canadian Collaborative Group in Nosocomial Transmission of Tuberculosis. Int J Tuberc Lung Dis 1998;2(9 Suppl 1):S98-102.
24. Plitt SS, Soskolne CL, Fanning EA, Newman SC. Prevalence and determinants of tuberculin reactivity among physicians in Edmonton, Canada: 1996-1997. Int J Epidemiol 2001;30(5):1022-8.
25. Stuart RL, Bennett NJ, Forbes AB, Grayson ML. Assessing the risk of tuberculosis infection among healthcare workers: the Melbourne Mantoux Study. Melbourne Mantoux Study Group. Med J Aust 2001;174(11):569-73.
26. Schwartzman K, Loo V, Pasztor J, Menzies D. Tuberculosis infection among health care workers in Montreal. Am J Respir Crit Care Med 1996;154(4 Pt 1):1006-12.
27. Liss GM, Khan R, Koven E, Simor AE. Tuberculosis infection among staff at a Canadian community hospital. Infect Control Hosp Epidemiol 1996;17(1):29-35.
28. Boudreau AY, Baron SL, Steenland NK, et al. Occupational risk of Mycobacterium tuberculosis infection in hospital workers. Am J Ind Med 1997;32(5):528-34.
29. Menzies D, Fanning A, Yuan L, FitzGerald JM. Hospital ventilation and risk for tuberculous infection in canadian health care workers. Canadian Collaborative Group in Nosocomial Transmission of TB. Ann Intern Med 2000;133(10):779-89.
30. Zarzuela Ramirez M, Cordoba Dona JA, Perea Milla E, Benitez E, Escolar Pujolar A, Lopez Fernandez FJ. [Analysis criteria's influence on incidence and factors associated tuberculin conversion in health care workers]. Med Clin (Barc) 2000;114(13):493-5.
31. Chen B, Wang X, Zhong J, et al. Tuberculosis among healthcare workers in southeastern China: A retrospective study of 7-year surveillance data. Int J Environ Res Public Health 2014;11(11):12042-52.
32. Tudor C, Van der Walt M, Margot B, et al. Tuberculosis among health care workers in KwaZulu-Natal, South Africa: a retrospective cohort analysis. BMC Public Health 2014;14:891.
33. Blumberg HM, Watkins DL, Berschling JD, et al. Preventing the nosocomial transmission of tuberculosis. Ann Intern Med 1995;122(9):658-63.
34. Members of the Ad Hoc Committee for the Guidelines for Preventing the Transmission fo Tuberculosis in Canadian Health Care F, Other Institutional S. Guidelines for preventing the transmission of tuberculosis in Canadian Health Care Facilities and other institutional settings. Can Commun Dis Rep 1996;22 Suppl 1:i-iv, 1-50, i-iv, 1-55.
35. Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet 2006;368(9547):1575-80.
36. Basu S, Andrews JR, Poolman EM, et al. Prevention of nosocomial transmission of extensively drug-resistant tuberculosis in rural South African district hospitals: an epidemiological modelling study. Lancet 2007;370(9597):1500-7.
37. Nodieva A, Jansone I, Broka L, Pole I, Skenders G, Baumanis V. Recent nosocomial transmission and genotypes of multidrug-resistant Mycobacterium tuberculosis. Int J Tuberc Lung Dis 2010;14(4):427-33.
38. Guidelines for preventing the transmission of tuberculosis in Canadian Health Care Facilities and other institutional settings. Can Commun Dis Rep 1996;22 Suppl 1:i-iv, 1-50, i-iv, 1-55.
39. Kanjee Z, Catterick K, Moll AP, Amico KR, Friedland GH. Tuberculosis infection control in rural South Africa: survey of knowledge, attitude and practice in hospital staff. J Hosp Infect 2011;79(4):333-8.
40. Harries AD, Hargreaves NJ, Gausi F, Kwanjana JH, Salaniponi FM. Preventing tuberculosis among health workers in Malawi. Bull World Health Organ 2002;80(7):526-31.
41. Uplekar M, Weil D, Lonnroth K, et al. WHO's new End TB Strategy. Lancet 2015.

42. Wenger PN, Otten J, Breeden A, Orfas D, Beck-Sague CM, Jarvis WR. Control of nosocomial transmission of multidrug-resistant Mycobacterium tuberculosis among healthcare workers and HIV-infected patients. Lancet 1995;345(8944):235-40.
43. Larsen NM, Biddle CL, Sotir MJ, White N, Parrott P, Blumberg HM. Risk of tuberculin skin test conversion among health care workers: occupational versus community exposure and infection. Clin Infect Dis 2002;35(7):796-801.
44. Moro ML, Errante I, Infuso A, et al. Effectiveness of infection control measures in controlling a nosocomial outbreak of multidrug-resistant tuberculosis among HIV patients in Italy. Int J Tuberc Lung Dis 2000;4(1):61-8.
45. Panlilio AL, Burwen DR, Curtis AB, et al. Tuberculin skin testing surveillance of health care personnel. Clin Infect Dis 2002;35(3):219-27.
46. Medicine Io. Ending neglect: the elimination of tuberculosis in the United States. Washington, DC: National Academy Press; 2000.
47. WHO. Guidelines on the management of latent tuberculosis infection: World Health Organization.
48. Horsburgh CR, Jr., Rubin EJ. Clinical practice. Latent tuberculosis infection in the United States. N Engl J Med 2011;364(15):1441-8.
49. Sia IG, Wieland ML. Current concepts in the management of tuberculosis. Mayo Clin Proc 2011;86(4):348-61.
50. Dutta NK, Karakousis PC. Latent tuberculosis infection: myths, models, and molecular mechanisms. Microbiol Mol Biol Rev 2014;78(3):343-71.
51. Lillebaek T, Dirksen A, Baess I, Strunge B, Thomsen VO, Andersen AB. Molecular evidence of endogenous reactivation of Mycobacterium tuberculosis after 33 years of latent infection. J Infect Dis 2002;185(3):401-4.
52. Jasmer RM, Nahid P, Hopewell PC. Clinical practice. Latent tuberculosis infection. N Engl J Med 2002;347(23):1860-6.
53. Schluger NW. Advances in the diagnosis of latent tuberculosis infection. Semin Respir Crit Care Med 2013;34(1):60-6.
54. Pai M. Spectrum of latent tuberculosis - existing tests cannot resolve the underlying phenotypes. Nat Rev Microbiol 2010;8(3):242; author reply 242.
55. Vernon A. Treatment of latent tuberculosis infection. Semin Respir Crit Care Med 2013;34(1):67-86.
56. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: controlling tuberculosis in the United States. Am J Respir Crit Care Med 2005;172(9):1169-227.
57. Herrera V, Perry S, Parsonnet J, Banaei N. Clinical application and limitations of interferon-gamma release assays for the diagnosis of latent tuberculosis infection. Clin Infect Dis 2011;52(8):1031-7.
58. Koch R. An Address on Bacteriological Research. Br Med J 1890;2(1546):380-3.
59. Tissot F, Zanetti G, Francioli P, Zellweger JP, Zysset F. Influence of bacille Calmette-Guerin vaccination on size of tuberculin skin test reaction: to what size? Clin Infect Dis 2005;40(2):211-7.
60. Menzies D. Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. Am J Respir Crit Care Med 1999;159(1):15-21.
61. Valenti WM, Andrews BA, Presley BA, Reifler CB. Absence of the booster phenomenon in serial tuberculin skin testing. Am Rev Respir Dis 1982;125(3):323-5.
62. Bass JA, Jr., Serio RA. The use of repeat skin tests to eliminate the booster phenomenon in serial tuberculin testing. Am Rev Respir Dis 1981;123(4 Pt 1):394-6.
63. Miller EJ. Booster phenomenon in serial tuberculin testing. Am Rev Respir Dis 1979;120(3):705.
64. Thompson NJ, Glassroth JL, Snider DE, Jr., Farer LS. The booster phenomenon in serial tuberculin testing. Am Rev Respir Dis 1979;119(4):587-97.

65. Menzies R, Vissandjee B, Amyot D. Factors associated with tuberculin reactivity among the foreign-born in Montreal. Am Rev Respir Dis 1992;146(3):752-6.
66. Lind A, Larsson LO, Bentzon MW, et al. Sensitivity to sensitins and tuberculin in Swedish children. I. A study of schoolchildren in an urban area. Tubercle 1991;72(1):29-36.
67. Gulnar SB, Bulut BU. Influence of BCG vaccination on tuberculin reactivity in healthy Turkish school children. Acta Paediatr 1997;86(5):549.
68. Wood-Baker R, Walker D, Dargaville P. The effect of Bacille Calmette-Guerin (BCG) vaccination on intradermal tuberculin reactivity in Tasmania. Aust N Z J Med 1997;27(1):82-3.
69. Kuyucu N, Kuyucu S, Bakirtas A, Karacan C. BCG revaccination and tuberculin reactivity. Indian J Pediatr 2001;68(1):21-5.
70. Moreno S, Blazquez R, Novoa A, et al. The effect of BCG vaccination on tuberculin reactivity and the booster effect among hospital employees. Arch Intern Med 2001;161(14):1760-5.
71. Wang L, Turner MO, Elwood RK, Schulzer M, FitzGerald JM. A meta-analysis of the effect of Bacille Calmette Guerin vaccination on tuberculin skin test measurements. Thorax 2002;57(9):804-9.
72. Bierrenbach AL, Cunha SS, Barreto ML, et al. Tuberculin reactivity in a population of schoolchildren with high BCG vaccination coverage. Rev Panam Salud Publica 2003;13(5):285-93.
73. Hizel K, Maral I, Karakus R, Aktas F. The influence of BCG immunisation on tuberculin reactivity and booster effect in adults in a country with a high prevalence of tuberculosis. Clin Microbiol Infect 2004;10(11):980-3.
74. Yeh YP, Luh DL, Chang SH, Suo J, Chang HJ, Chen TH. Tuberculin reactivity in adults after 50 years of universal bacille Calmette-Guerin vaccination in Taiwan. Trans R Soc Trop Med Hyg 2005;99(7):509-16.
75. Mazurek GH, Jereb J, Vernon A, LoBue P, Goldberg S, Castro K. Updated guidelines for using Interferon Gamma Release Assays to detect Mycobacterium tuberculosis infection - United States, 2010. MMWR Recomm Rep 2010;59(RR-5):1-25.
76. QuantiFERON TB Gold (In-Tube Method) [Package Insert]. Valencia, CA: Cellestis Inc. 2006.
77. Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A. Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. MMWR Recomm Rep 2005;54(RR-15):49-55.
78. Position statement on interferon-gamma release immunoassays in the detection of latent tuberculosis infection, October 2007. Commun Dis Intell Q Rep 2007;31(4):404-5.
79. Updated recommendations on interferon gamma release assays for latent tuberculosis infection. An Advisory Committee Statement (ACS). Can Commun Dis Rep 2008;34(ACS-6):1-13.
80. World Health Organization. Use of tuberculosis interferon-gamma release assays (IGRAs) in low- and middle-income countries : policy statement. Geneva: World Health Organization; 2011.
81. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. Ann Intern Med 2007;146(5):340-54.
82. Behr MA, Wilson MA, Gill WP, et al. Comparative genomics of BCG vaccines by whole-genome DNA microarray. Science 1999;284(5419):1520-3.
83. Pai M, Riley LW, Colford JM, Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. Lancet Infect Dis 2004;4(12):761-76.
84. Diel R, Loddenkemper R, Meywald-Walter K, Gottschalk R, Nienhaus A. Comparative performance of tuberculin skin test, QuantiFERON-TB-Gold In Tube assay, and T-Spot.TB test in contact investigations for tuberculosis. Chest 2009;135(4):1010-8.
85. Ewer K, Deeks J, Alvarez L, et al. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of Mycobacterium tuberculosis infection in a school tuberculosis outbreak. Lancet 2003;361(9364):1168-73.
86. Gandra S, Scott WS, Somaraju V, Wang H, Wilton S, Feigenbaum M. Questionable effectiveness of the QuantiFERON-TB Gold Test (Cellestis) as a screening tool in healthcare workers. Infect Control Hosp Epidemiol 2010;31(12):1279-85.

87. Ringshausen FC, Nienhaus A, Schablon A, Schlosser S, Schultze-Werninghaus G, Rohde G. Predictors of persistently positive Mycobacterium-tuberculosis-specific interferon-gamma responses in the serial testing of health care workers. BMC Infect Dis 2010;10:220.
88. Ringshausen FC, Nienhaus A, Torres Costa J, et al. Within-subject variability of Mycobacterium tuberculosis-specific gamma interferon responses in German health care workers. Clin Vaccine Immunol 2011;18(7):1176-82.
89. Slater ML, Welland G, Pai M, Parsonnet J, Banaei N. Challenges with QuantiFERON-TB Gold assay for large-scale, routine screening of U.S. healthcare workers. Am J Respir Crit Care Med 2013;188(8):1005-10.
90. Torres Costa J, Silva R, Sa R, Cardoso MJ, Nienhaus A. Serial testing with the interferon-gamma release assay in Portuguese healthcare workers. Int Arch Occup Environ Health 2011;84(4):461-9.
91. Yoshiyama T, Harada N, Higuchi K, Nakajima Y, Ogata H. Estimation of incidence of tuberculosis infection in health-care workers using repeated interferon-gamma assays. Epidemiol Infect 2009;137(12):1691-8.
92. Carpenter CJ. A meta-analysis of the effectiveness of health belief model variables in predicting behavior. Health Commun 2010;25(8):661-9.
93. Chang LC, Hung LL, Chou YW, Ling LM. Applying the health belief model to analyze intention to participate in preventive pulmonary tuberculosis chest X-ray examinations among indigenous nursing students. J Nurs Res 2007;15(1):78-87.
94. Harrison JA, Mullen PD, Green LW. A meta-analysis of studies of the Health Belief Model with adults. Health Educ Res 1992;7(1):107-16.
95. Green EC, Murphy E. Health Belief Model. In: The Wiley Blackwell Encyclopedia of Health, Illness, Behavior, and Society: John Wiley & Sons, Ltd; 2014.
96. Ferrara G, Losi M, Meacci M, et al. Routine hospital use of a new commercial whole blood interferon-gamma assay for the diagnosis of tuberculosis infection. Am J Respir Crit Care Med 2005;172(5):631-5.
97. Targeted tuberculin testing and treatment of latent tuberculosis infection. American Thoracic Society. MMWR Recomm Rep 2000;49(RR-6):1-51.
98. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. Am J Respir Crit Care Med 2000;161(4 Pt 1):1376-95.
99. Blumberg HM, Kempker RR. Interferon-gamma release assays for the evaluation of tuberculosis infection. JAMA 2014;312(14):1460-1.
100. Flaskerud JH. Is the Likert scale format culturally biased? Nurs Res 1988;37(3):185-6.
101. Komorita SS. Attitude Content, Intensity, and the Neutral Point on a Likert Scale. J Soc Psychol 1963;61:327-34.
102. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform 2009;42(2):377-81.
103. Kraemer HC. Measurement of reliability for categorical data in medical research. Stat Methods Med Res 1992;1(2):183-99.
104. Xiao Chen PE, Michael Mitchell, Christine Wells. Regression with SPSS. In: UCLA Institute for Digital Research and Education. SPSS Web Books.
105. Kleinbaum DG, Klein M, Pryor ER. Logistic regression : a self-learning text. 3rd ed. New York: Springer; 2010.
106. Binomial logistic regression in SPSS. In: Statistical Guides and SPSS Tutorials by Laerd Statistics.
107. McCrum-Gardner E. Which is the correct statistical test to use? Br J Oral Maxillofac Surg 2008;46(1):38-41.

108. Pai M, Gokhale K, Joshi R, et al. Mycobacterium tuberculosis infection in health care workers in rural India: comparison of a whole-blood interferon gamma assay with tuberculin skin testing. JAMA 2005;293(22):2746-55.
109. Drobniewski F, Balabanova Y, Zakamova E, Nikolayevskyy V, Fedorin I. Rates of latent tuberculosis in health care staff in Russia. PLoS Med 2007;4(2):e55.
110. Lien LT, Hang NT, Kobayashi N, et al. Prevalence and risk factors for tuberculosis infection among hospital workers in Hanoi, Viet Nam. PLoS One 2009;4(8):e6798.
111. World Health Organization. Global tuberculosis report 2013 (in IRIS). Geneva: World Health Organization; 2013.
112. Pai M, Joshi R, Dogra S, et al. Serial testing of health care workers for tuberculosis using interferon-gamma assay. Am J Respir Crit Care Med 2006;174(3):349-55.
113. Rafiza S, Rampal KG. Serial testing of Malaysian health care workers with QuantiFERON(R)-TB Gold In-Tube. Int J Tuberc Lung Dis 2012;16(2):163-8.
114. Zwerling A, van den Hof S, Scholten J, Cobelens F, Menzies D, Pai M. Interferon-gamma release assays for tuberculosis screening of healthcare workers: a systematic review. Thorax 2012;67(1):62-70.
115. Mancuso JD, Mazurek GH, Tribble D, et al. Discordance among commercially available diagnostics for latent tuberculosis infection. Am J Respir Crit Care Med 2012;185(4):427-34.
116. Vinton P, Mihrshahi S, Johnson P, Jenkin GA, Jolley D, Biggs BA. Comparison of QuantiFERON-TB Gold In-Tube Test and tuberculin skin test for identification of latent Mycobacterium tuberculosis infection in healthcare staff and association between positive test results and known risk factors for infection. Infect Control Hosp Epidemiol 2009;30(3):215-21.
117. Zwerling A, Cojocariu M, McIntosh F, et al. TB screening in Canadian health care workers using interferon-gamma release assays. PLoS One 2012;7(8):e43014.
118. Leung CC, Rieder HL, Lange C, Yew WW. Treatment of latent infection with Mycobacterium tuberculosis: update 2010. Eur Respir J 2011;37(3):690-711.
119. Palanduz A, Gultekin D, Kayaalp N. Follow-up of compliance with tuberculosis treatment in children: monitoring by urine tests. Pediatr Pulmonol 2003;36(1):55-7.
120. White MC, Tulsky JP, Goldenson J, Portillo CJ, Kawamura M, Menendez E. Randomized controlled trial of interventions to improve follow-up for latent tuberculosis infection after release from jail. Arch Intern Med 2002;162(9):1044-50.

Appendix 1. Questionnaire

Date _____/_____/____

A. **Patient Identification Code** ☐☐☐ - ☐☐☐☐

Health Care Facility _____

☐ Medical School

☐ Non TB Health Facility

☐ TB Outpatient Clinic

☐ TB Inpatient Clinic

☐ Other

Location of the Facility _____

☐ Tbilisi

☐ Other

Name

Last_____ First_____

Contact Phone Number: _____

B. Demographics

Date of birth ____/___/_____ (dd-mm-year)

Gender

☐ Male

☐ Female

Country of birth

☐ Georgia

☐ Other (specify)

Year arrived in Georgia _____

Ethnicity

☐ Georgian

☐ Other (Specify) _____

Education

☐ Graduate

☐ Undergraduate

☐ High School

☐ Did not finish high school

Profession

☐ Physician

☐ Nurse

☐ Lab Worker

☐ Outreach worker

☐ Medical Student

☐ Other _____

C. Vaccination

Prior History of **BCG** Vaccination

☐ Yes (If yes, how many total vaccinations? ___)

☐ No

☐ Don't Know

BCG Scar

☐ Yes

☐ No

Baseline LTBI Testing

Medical History

Last **TST**

☐ Yes

(If yes, year of the last **TST**_____; result of the last **TST** ☐ Negative ☐ Positive)

☐ No

☐ Don't Know

Known **Household** Tuberculosis Contact

☐ Yes

(If yes, ☐ immediate; ☐ extended)

☐ No

☐ Don't Know

Known Tuberculosis Contact **outside Work and Household**

☐ Yes

(If yes, ☐ immediate; ☐ extended)

☐ No

☐ Don't Know

Prior History of **TB Disease**

☐ Yes (If yes, Year? _____)

☐ No

Did you begin TB treatment? ☐ Yes ☐ No ☐ Don't Know

Did you complete TB treatment? ☐ Yes ☐ No ☐ Don't Know

Were you declared cured of TB? ☐ Yes ☐ No ☐ Don't Know

Employment History

Job Title: _____ Years in Current Position: _____

Occupation

☐ Administrative and Technical Staff

☐ Physician

☐ Nurse

☐ Lab Worker

☐ Outreach worker

☐ Medical Student

☐ Other

How much exposure to TB do you have at work?

☐ Daily

☐ Frequent

☐ Rare

☐ Very Rare

Prior Positions: _____

Years in Prior Position: _____

Total number of years in Health-care (including medical training) _____

Appendix 2. Data Collection Form

Patient Identification Code ☐☐☐ - ☐☐☐☐

Name: First, Last _____

I round:

Tuberculin Skin Test (TST) / Normal Value: < 10 mm /

Date TST placed: ____/____/_____ **Location placed:** ☐ Left Forearm ☐ Right Forearm

Date TST read: ____/____/_____

Result: _____ mm of induration (If no induration, mark "0")

QuantiFERON-TB Gold in Tube Test /Normal Value: < 0.35 IU /ml /

Date Blood Drown: ____/____/_____

Result: _____IU/ml

II round:

Tuberculin Skin Test (TST) / Normal Value: < 10 mm /

Date TST placed: ____/____/_____ **Location placed:** ☐ Left Forearm ☐ Right Forearm

Date TST read: ____/____/_____

Result: _____ mm of induration (If no induration, mark "0")

QuantiFERON-TB Gold in Tube Test /Normal Value: < 0.35 IU /ml /

Date Blood Drown: ____/____/_____

Result: _____IU/ml

QuantiFERON-TB Gold in Tube Test (QFT-GIT) /Normal Value: < 0.35 IU /ml /

Date Blood Drown: ____/____/_____

Result: _____IU/ml

Additional Comments: ----------

Appendix 3. Healthcare Provider Survey about Latent Tuberculosis Infection

Thank you for taking the time to complete this survey about your experiences with Tuberculosis (TB). This is an anonymous survey. Neither your name (nor any other identifying information) will be collected or linked to your responses on this survey, so please respond to each question as accurately and honestly as possible.

First, please, answer the following questions about your exposure to TB

1. Which city do you work in? (Check only one answer)

_____ Tbilisi

_____ Abastumani

_____ Batumi

_____ Kutaisi

_____ Poti

_____ Zugadidi

_____ Other

2. Which of the following health facility do you work at? (Check only one answer)

_____ Medical School

_____ Non TB Health Facility

_____ TB Outpatient Clinic

_____ TB Inpatient Clinic

_____ Other

[PLEASE CONTINUE TO THE NEXT PAGE]

3. Do you work primarily with tuberculosis patients?

_____ Yes

_____ No

Please answer the following questions about TB transmission, infection and treatment.

4. TB organisms are most commonly transmitted from person-to-person in which of the following ways? (Check only one answer).

_____ Blood and bodily fluids

_____ Aerosol

_____ Food

_____ Shared objects

5. Which of the following groups are among those at an increased risk for developing active TB? (Check all that apply).

_____ Young children

_____ Healthcare workers

_____ HIV-infected individuals

_____ Individuals with heart disease

[PLEASE CONTINUE TO THE NEXT PAGE]

6. Common symptoms of active TB disease include all of the following EXCEPT: (Check all that apply).

_____ Cough

_____ Night Sweats

_____ Weight loss

_____ Diarrhea

_____ Vomiting

7. Individuals with latent TB infection have which of the following characteristics: (Check all that apply).

_____ They are asymptomatic

_____ They are at risk of progressing to active TB disease

_____ They are infectious and can spread tuberculosis to others

_____ They will likely have a skin test or blood test result indicating latent TB infection

8. Generally, what percentage of people who have latent TB infection and a normal immune system will go on to develop active TB at some point in their lives? (Check only one answer).

_____ <1%

_____ 5-10%

_____ 30-50%

_____ >80%

[PLEASE CONTINUE TO THE NEXT PAGE]

9. Which of the following tests is required to diagnose active pulmonary TB? (Check all that apply).

_____ TB skin test

_____ TB blood tests

_____ Chest X-ray

_____AFB smear and culture of sputum

10. The primary rationale for treating latent TB infection is to: (Check only one answer)

_____ Reduce the risk that a person with latent TB can infect others

_____ Reduce the risk that TB infection will progress to disease

_____ Reduce the risk that a person with latent TB will be infected again in the future

_____ Reduce the risk that a person will develop multidrug-resistant TB

11. Which of the following regimens is the *preferred* method for the treatment of latent TB infection? (Check only one answer).

_____ Isoniazid for 6-9 months

_____ Rifampin and Pyrazinamide for 2 months

_____ Rifampin for 4 months

_____ Ofloxacin for 3 months

[PLEASE CONTINUE TO THE NEXT PAGE]

12. The TB skin test can cause tuberculosis infection.

_____ True

_____ False

_____ I don't know

Next, we are interested in your thoughts about latent TB infection. For the following questions, please indicate the degree to which you agree or disagree with the below statements by circling your answer.

	Strongly Agree (1)	Agree (2)	No Opinion (3)	Disagree (4)	Strongly Disagree (5)
13. I worry about acquiring latent TB infection.	1	2	3	4	5
14. I worry about acquiring active TB infection.	1	2	3	4	5
15. I worry about acquiring latent TB infection with multi-drug resistant TB.	1	2	3	4	5
16. Latent TB infection is very serious.	1	2	3	4	5

[PLEASE CONTINUE TO THE NEXT PAGE]

Please indicate the degree to which you think the following situations may occur by circling your answer.

	No Chance (1)	Little Chance (2)	Some Chance (3)	Very Good Chance (4)
17. I would test positive for latent TB infection if I were tested today.	1	2	3	4
18. At some point in the future, I will test positive for latent TB infection.	1	2	3	4
19. At some point in the future, I will test positive for active TB.	1	2	3	4

[PLEASE CONTINUE TO THE NEXT PAGE]

Next, we are interested in your thoughts about preventing the transmission of TB infection. For the following questions, please indicate the degree to which you agree or disagree with the below statements by circling your answer.

	Strongly Agree (1)	Agree (2)	No Opinion (3)	Disagree (4)	Strongly Disagree (5)
20. Implementation of effective TB infection control measures can prevent transmission of TB in hospitals.	1	2	3	4	5
21. UV light is an effective TB infection control measure.	1	2	3	4	5
22. UV lights can harm health care workers.	1	2	3	4	5
23. I do not want to work in an area where UV lights are used	1	2	3	4	5
24. Using respirators to prevent exposing healthcare workers to TB is important.	1	2	3	4	5
25. Using surgical masks by TB patients to prevent TB transmission is important	1	2	3	4	5

26. Using respirators to prevent the transmission of multi-drug resistant TB is important.	1	2	3	4	5
27. Using TB isolation rooms with those who have active TB in order to prevent transmission is important.	1	2	3	4	5
28. Instruction for those at high risk for or who have active TB in respiratory hygiene/cough etiquette is important.	1	2	3	4	5

[PLEASE CONTINUE TO THE NEXT PAGE]

Please answer the next additional questions about your experience with infection control practices for latent TB infection.

29. How frequently are respirators available to you? (Check only one answer).

_____ Always

_____ Most of the time

_____Sometimes

_____ Rarely

_____ Never

30. How often do you wear a respirator when around patients who are at risk for or who have active TB or TB disease? (Check only one answer).

_____ Always → Skip to question 32

_____ Most of the time → Go to the next question

_____Sometimes → Go to the next question

_____ Rarely → Go to the next question

_____ Never → Go to the next question

31. What is the *primary* reason why you do not wear a respirator all of the time? (Check only one answer).

_____ Respirators are not available to me when I need them.

_____ I have not been instructed to wear a respirator.

_____ I do not believe masks are effective at preventing TB transmission.

_____ Respirators are uncomfortable.

_____ Other (please specify: _____)

[PLEASE CONTINUE TO THE NEXT PAGE]

32. Are TB isolation rooms or wards used for patients in your facility? (Check only one answer).

_____ Yes

_____ No

_____ I don't know

Next, we are interested in your thoughts about testing for latent TB infection. For the following questions, please indicate the degree to which you agree or disagree with the below statements by circling your answer.

	Strongly Agree (1)	Agree (2)	No Opinion (3)	Disagree (4)	Strongly Disagree (5)
33. It is important for Georgian healthcare workers to be tested for latent TB infection.	1	2	3	4	5
34. It is important to test contacts of patients with active TB (family, friends) for latent TB infection.	1	2	3	4	5
35. It is important to test children who have been exposed to TB for latent TB infection.	1	2	3	4	5
36. It is important to test individuals with compromised immune systems for latent TB infection.	1	2	3	4	5

Please answer the next additional questions about testing for latent TB infection.

37. Would you be willing to be tested each year for latent TB infection? (Check only one answer).

_____ Yes → Go to the next question

_____ No → Skip to question 39

_____ Undecided → Go to the next question

38. Which of the following latent TB infection testing methods would you prefer? (Check only one answer).

_____ The skin test

_____ The blood test

_____ Either (I don't have a preference)

[PLEASE CONTINUE TO THE NEXT PAGE]

We are also interested in your thoughts about treating latent TB infection. For the following questions, please indicate the degree to which you agree or disagree with the below statements by circling your answer.

	Strongly Agree (1)	Agree (2)	No Opinion (3)	Disagree (4)	Strongly Disagree (5)
39. If I tested positive for latent TB infection, I should be treated.	1	2	3	4	5
40. If I tested positive for latent TB infection, I should not be treated because I will be exposed again in the future.	1	2	3	4	5
41. If I tested positive for latent TB infection, I should not be treated because the TB I have is probably resistant to the medications.	1	2	3	4	5
42. The benefits to treating latent TB infection outweigh the risks of treating latent TB infection.	1	2	3	4	5

Finally, we would like to know a little more about you. Please answer the below questions as honestly and accurately as possible.

[PLEASE CONTINUE TO THE NEXT PAGE]

43. How old are you (in years)?

I am _____ years old.

44. What is your biological sex? (Check one):

_____ Male

_____ Female

45. Approximately how many years have you worked in healthcare full-time?

_____ years

46. What is your primary job title? (Check only one answer):

_____ Administrative and Technical Staff

_____ Physician

_____ Nurse

_____ Laboratory Worker

_____ Outreach worker

_____ Medical Student

_____ Other

[PLEASE CONTINUE TO THE NEXT PAGE]

47. In what type of patient care are you involved? (Check one):

_____ I am not involved in patient care directly→ Skip to question 49

_____ Primarily inpatient

_____ Primarily outpatient

_____ Both inpatient and outpatient

48. With which patient population do you primarily work? (Check one):

_____ Adults

_____ Children

_____ Both adults and children

49. Have you had the BCG vaccine? (Check one):

_____ Yes

_____ No

_____ I don't remember

50. Have you had a TB skin test before? (Check one):

_____Yes → Go to the next question

_____ No → Skip to question # 54

_____ I don't remember → Skip to question # 54

[PLEASE CONTINUE TO THE NEXT PAGE]

51. Was the TB skin test positive? (Check one):

_____ Yes → Go to the next question

_____ No → Skip to #54

_____ I don't remember

52. Approximately how long ago was the most positive TB skin test?

_____ months, _____years ago

53. Have you been treated for latent TB infection (positive TB skin test) before? (Check one):

_____ Yes

_____ No

_____ I don't remember

54. Have you been diagnosed with active TB before? (Check one):

_____ No→ YOU HAVE COMPLETED THE SURVEY

_____ I don't remember→ YOU HAVE COMPLETED THE SURVEY

_____ Yes

55. Have you been treated for active TB before? (Check one):

_____ Yes

_____ No

_____ I don't remember

You have completed the survey. We appreciate you taking the time to share your thoughts

Appendix 4. Methodology for Drawing Blood

1. Clean the skin with alcohol swab and allow drying.
2. A total of 3 ml of blood will be drawn for the QuantiFERON-TB Gold in Tube (QFT-3G) test.
    a. The blood may be collected directly into the vacutainer tubes or collected with a syringe (without heparin) and transferred into the vacutainer tubes.
    b. Invert each tube several (at least 5) times immediately after collecting the blood to mix it with heparin.
    c. The set of QFT-3G tubes consists of a "Nil Control" gray-topped tube, a blue-topped tube that contains TB antigens, and an "Positive Control" orange-topped tube that contains Mitogen. One (1) ml of blood should be collected into each of the QFT-3G tubes (e.g. up to the black line on the side). **Do not overfill the tubes**. Leave the QFT-3G connected to the needle for 2 seconds after blood stops flowing.
3. Cover the puncture site with a Band-Aid and confirm that bleeding has stopped.
4. Shake the blood in the QFT-3G tubes for 5 seconds and place them upright in a tube-rack. The QFT-3G tubes must be mixed more vigorously to wash the antigens off the tube walls where they were sprayed during the manufacturing process. After being shaken, the QFT-3G tubes should NOT be mixed any further.
5. **Label the tubes with the Subject's number.**
6. Record collection time on the appropriate forms.

7. Transport the tubes of blood at room temperature to the lab for further processing. The blood should be taken to the lab as soon as possible and definitely within 12 hours. The QFT-3G tubes should be transported upright in a tube rack.

Appendix 5. QuantiFERON-TB Gold In-tube

Antigen Storage and Preparation for use

1. **QFT-3G tubes containing antigen:**

a) A set of QFT-3G tubes is used for each test to be performed. A set QFT-3G tubes consists of a "Nil Control" gray-topped tube, a blue-topped tube that contains TB antigens, and an "Positive Control" orange-topped tube that contains Mitogen.

b) For long-term storage, these tubes should be stored at 2 to 8°C (refrigerated). Prior to use the tubes can be kept at room temperature for periods up to 2 weeks.

Stimulation of Blood with Antigens for QFT-3G and Storage of Plasmas

I. Stimulation of blood for QFT-3G assay:

1. Place QFT-3G tubes upright in 37°C incubator (without additional mixing). Incubation of QFT-3G tubes should begin as soon as possible after blood collection, and must begin within 12 hrs of obtaining blood.

2. Record time QFT-3G incubation began on the appropriate form.

3. Incubate the QFT-3G tubes upright at 37°C for 20 to 40 hours. Record time incubation ended on the appropriate form.

4. After incubation is completed, centrifuge the tubes in a clinical centrifuge at 1,500 to 2,200g for 5 minutes at room temperature (22±5°C) to get the cells and debris out of the plasma and below the gel plug.

5. Transfer at least 300 µL of stimulated plasma from each QFT-3G tube to 1.2 ml micro-tubes appropriately labeled and positioned as described below (and illustrated in Table 1).

   A. Wrap a 3.25 X 3/8 inch label with the subject's ID number around 3 of the 1.2 ml micro-tubes.

   *B. Use a black indelible marker to mark the first tube, that will contain the "Nil" QFT-3G plasmas.

   C. Place tube is consecutively numbered boxes labeled with the study name, initial date used, and "QFT-3G Plasmas".

   D. Record the storage position of each plasma on the appropriate form.

Table 1: Storage layout for QFT-3G plasma samples (32 subjects / plate)

	1*	2	3	4*	5	6	7*	8	9	10*	11	12
A	N(1)	T(1)	M(1)	N(9)	T(9)	M(9)	N(17)	T(17)	M(17)	N(25)	T(25)	M(25)
B	N(2)	T(2)	M(2)	N(10)	T(10)	M(10)	N(18)	T(18)	M(18)	N(26)	T(26)	M(26)
C	N(3)	T(3)	M(3)	N(11)	T(11)	M(11)	N(19)	T(19)	M(19)	N(27)	T(27)	M(27)
D	N(4)	T(4)	M(4)	N(12)	T(12)	M(12)	N(2)	T(20)	M(20)	N(28)	T(28)	M(28)
E	N(5)	T(5)	M(5)	N(13)	T(13)	M(13)	N(2)	T(21)	M(21)	N(29)	T(29)	M(29)
F	N(6)	T(6)	M(6)	N(14)	T(14)	M(14)	N(2)	T(22)	M(22)	N(30)	T(30)	M(30)
G	N(7)	T(7)	M(7)	N(15)	T(15)	M(15)	N(2)	T(23)	M(23)	N(31)	T(31)	M(31)
H	N(8)	T(8)	M(8)	N(16)	T(16)	M(16)	N(2)	T(24)	M(24)	N(32)	T(32)	M(32)

N = NIL QFT-3G plasma, T= TB antigen stimulated QFT-3G plasma, and M=Mitogen stimulated QFT-3G stimulated plasma; Numbers in parentheses indicate samples from 32 different patients (1) through (32). Example: N(1) = Nil QFT-3G plasma from first subject's blood sample.

III. Storage of Plasmas for QFT-3G assay:

1. Tubes containing stimulated plasmas should be refrigerated or frozen within 12 hours of collection. Caps should be placed on tubes within 24 hours.

2. After capping tubes, place each box of microtubes in a separate Zip Lock bag and store for up to 2 weeks at 4° C. The plasmas should be stored at -70° C for longer periods. The freezer should **not** be frost-free.

Note: Performing ELISAs prior to refrigeration decrease clot formation in plasmas.

ELISA Methods for QFT-3G

Note: QFT-3G ELISA uses the "QuantiFERON-CMI" kit and is for "Nil", "TB antigen", and "Mitogen" stimulated plasmas collected from the QFT-3G tubes.

Please be familiar with the current "QuantiFERON-CMI" package insert but follow these instructions when performing the QFT-3G ELISAs for this study.

While QFT-3G ELISA uses different reagents which are specifically for the CMI assay, the procedure is similar to the QFT-1G assay. Differences in the procedure for performing the QFT-1G ELISA as compared to QFT-3G ELISA are indicated with an *.

1. If not previously done and available,

*a) reconstitute the CMI IFN-γ standard by adding 1.5mL of deionised or distilled water to the vial. Mix gently to minimize frothing but ensure complete resolubilization. Record the date the standard was reconstituted on the vial. Reconstituted stock CMI IFN-γ Standard may be kept for up to 3 months if stored at 2°C to 8°C.

b) reconstitute freeze dried Conjugate (to make 100X Concentrate) by adding 0.3mL of deionised or distilled water. Ensure complete resolubilization of the Conjugate by mix thoroughly but gently to minimize frothing. Return the Conjugate to 2 to 8 C as soon as possible. Reconstituted Conjugate should be used with 3 months.

c) dilute 1 part Wash Buffer 20X Concentrate with 19 parts deionised or distilled water and mix thoroughly. Each plate uses about 1 liter of diluted wash buffer. Diluted wash buffer should be used within 2 weeks of preparation.

2. Except for Conjugate (100X Concentrate), other reagents including standards, are brought to room temperature before use. Allow at least 60 min for equilibration.

*3. Prepare fresh dilutions for IFN-γ standards for each assay. Use the reconstituted stock CMI IFN-γ Standard to produce a dilution series of 3 IFN-γ concentrations as follows:

a) label 4 microtubes S1 to S4.

b) add 200 μL of Green Diluent to the 3 tubes labeled S2 to S4.

c) set S4 aside to avoid adding IFN-γ

d) add 200 μL of the reconstituted stock CMI IFN-γ Standard to the tubes labeled S1 and S2.

e) mix S2 thoroughly, change the tip and transfer 200 μL from S2 to S3.

f) mix s3 thoroughly. Do not add IFN-γ to S4.

4. Vortex plasma samples before performing ELISA.

5. Record requested information on ELISA worksheet (Appendix 3g including staff, reagent lot numbers, test samples, times, and temperatures.

6. Dilute the required amount of Conjugate 100X Concentrate in Green Diluent (as shown in Table 1). Add 50 μl of freshly prepared Conjugate to the required wells of an ELISA plate using a multichannel pipette. Use the diluted Conjugate within 30 minutes of preparation.

TABLE 1. CONJUGATE Preparation Table

# of Strips	Volume of 100 x Conjugate	Volume of Green Diluent
1	5μL	0.5 mL

2	10µL	1.0 mL
3	15µL	1.5 mL
4	20µL	2.0 mL
5	25µL	2.5 mL
6	30µL	3.0 mL
7	35µL	3.5 mL
8	40µL	4.0 mL
9	45µL	4.5 mL
10	50µL	5.0 mL
11	55µL	5.5 mL
12	60µL	6.0 mL

*7. Use a multichannel pipette to add 50 µl of Mitogen (M) and Nil (N) stimulated plasmas, and *M. tuberculosis*-specific antigen stimulated plasmas (e.g. SA1 through SA8, or SA1b to SA8b) from each patient, and diluted standards to the appropriate ELISA wells as shown in the worksheet template on page **Error! Bookmark not defined.**. Add QFT-3G Nil, QFT-3G TB, and QFT-3G Mitogen stimulated plasmas in place of SA6, SA7, and

SA8 if available. Standards should be added last and incubation time begun once they are added.

Note: **Pipetting should be done with extreme care to avoid pipette tips becoming blocked with cryoprecipitate that may be present in thawed plasmas**. Tip volumes should be checked before addition to ELISA wells. Plasma samples may be cleared of clotted material by centrifugation.

8. Place the lid on the ELISA plate and mix with a microplate shaker for 1 min with waveform set at 20 and amplitude set at 6 (not 9 as for blood).

*9. Incubate plates away from direct sunlight at room temperature (22°C +/- 5°C) for **2 hours** at room temperature (as distinct from 1 hour for the QuantiFERON-TB ELISA)

10. Wash the ELISA wells 6 times with wash buffer (concentrate diluted 1:20). Please refer to package insert for more detailed instructions for washing plates.

11. Tap ELISA plates face down on absorbent paper to remove residual wash buffer.

12. Dilute the required amount of Chromogen 100X Concentrate in Enzyme Substrate Buffer (as shown in Table 2) and add 100 µl of freshly prepared enzyme substrate solution to each well. Begin timing the incubation when substrate is added to first well.

TABLE 2. SUBSTRATE Preparation Table

# of Strips	Volume of Chromogen 100x Concentrate	Volume of Substrate Buffer
1	10 µL	1 mL
2	20 µL	2 mL
3	30 µL	3 mL
4	40 µL	4 mL
5	50 µL	5 mL
6	60 µL	6 mL
7	70 µL	7 mL
8	80 µL	8 mL
9	90 µL	9 mL
10	100 µL	10 mL
11	110 µL	11 mL
12	120 µL	12 mL

13. Place the lid on the ELISA plate and mix with a microplate shaker for 1 min with waveform set at 20 and amplitude set at 6.

14. Incubate away from direct sunlight at room temperature for precisely 30 min.

15. Add 50 µl of Enzyme Stopping Solution to each well. Enzyme Stopping Solution should be added to wells in the same order and at the same speed as the substrate in step 12.

16. Place the lid on the ELISA plate and mix gently on a microplate shaker or in the ELISA instrument.

17. Read the absorbance (optical density) of each well at 450nm (with a 620nm reference filter) using an ELISA plate reader within 5 min of adding the stopping solution.

18. Print absorbance readings from the ELISA plate reader and attach to ELISA worksheet.

*19. Use the **QuantiFERON-CMI Analysis Software** supplied by Cellestis to do the following:

    a. Determine the mean absorbance values for each of the standard IFN-γ samples and construct a linear standard curve with IU/ml IFN-γ versus absorbance.

    b. Assess the validity of the ELISA test.

    c. Convert the absorbance value of each test plasma into IFN-γ IU/ml using the standard curve.

20. Keep paper and electronic copies of the ELISA worksheet and the IFN-γ results.

21. Immediately report any episodes where 2 QFT-CMI ELISAs fail in a row, or if more than 1 in any series of 10 fails. Await technical input before running additional ELISAs.

QFT-3G ELISA Worksheet

IFN-☐ ELISA assay number: ☐

Date of assay: ☐☐ month ☐☐ day ☐☐ Year

Lab: _____ Operator(s): _____

QuantiFERON Kit (Batch number): _____

ELISA Plate Setup:

	1	2	3	4	5	6	7	8	9	10	11	12
A	N(1)	T(1)	M(1)	N(9)	T(9)	M(9)	N(17)	T(17)	M(17)	N(25)	T(25)	M(25)
B	N(2)	T(2)	M(2)	N(10)	T(10)	M(10)	N(18)	T(18)	M(18)	N(26)	T(26)	M(26)
C	N(3)	T(3)	M(3)	N(11)	T(11)	M(11)	N(19)	T(19)	M(19)	N(27)	T(27)	M(27)
D	N(4)	T(4)	M(4)	N(12)	T(12)	M(12)	N(2)	T(20)	M(20)	N(28)	T(28)	M(28)
E	N(5)	T(5)	M(5)	N(13)	T(13)	M(13)	N(2)	T(21)	M(21)	S1	S1	S1
F	N(6)	T(6)	M(6)	N(14)	T(14)	M(14)	N(2)	T(22)	M(22)	S2	S2	S2
G	N(7)	T(7)	M(7)	N(15)	T(15)	M(15)	N(2)	T(23)	M(23)	S3	S3	S3
H	N(8)	T(8)	M(8)	N(16)	T(16)	M(16)	N(2)	T(24)	M(24)	S4	S4	S4

N = NIL QFT-3G plasma, T= TB antigen stimulated QFT-3G plasma, and M=Mitogen stimulated QFT-3G stimulated plasma; Numbers in parentheses indicate samples from 28 different patients (1) through (28). Example: N(1) = Nil QFT-3G plasma from first subject's blood sample; and S1 through S4: High Standard to Zero Standard for IFN-γ.

Appendix 2 (continued)

Subject Numbers							
1		9		17		25	
2		10		18		26	
3		11		19		27	
4		12		20		28	
5		13		21		Standard Conc.	
6		14		22		S1	
7		15		23		S2	
8		16		24		S3	
						S4	

Sample Incubation	
Start Time	Stop Time
Start Temp	Stop Temp

Substrate Incubation	
Start Time	Stop Time

Printouts attached:

	YES	NO
Absorbance readings from plate reader:	☐	☐
Calculations IU/mL (Excel spreadsheet):	☐	☐

Electronic Results File: _____