

**A STUDY TO EVALUATE IMMUNODIAGNOSTIC TESTS  
FOR  
TUBERCULOSIS INFECTION AND  
DETERMINANTS OF TB INFECTION IN A POPULATION OF  
HEALTH CARE WORKERS IN THE WESTERN CAPE OF  
SOUTH AFRICA**

By

Shahieda Adams

Thesis presented for the degree of

Doctor of Philosophy

in the School of Public Health and Family Medicine

University of Cape Town

**August 2014**

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Supervisors: Professor Keertan Dheda and

Co-supervisor: Professor Rodney Ehrlich

This thesis is presented in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in the School of Public Health and Family Medicine, Faculty of Health Sciences, University of Cape Town. The work on which this thesis is based is original research and has not, in whole or in part, been submitted for another degree at this or any other university. The contents of this thesis are entirely the work of the candidate.

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**Shahieda Adams**

August 2014

## ABSTRACT

## ABSTRACT

**Background:** Health care workers are at increased risk of acquiring latent tuberculosis infection (LTBI). The emergence of interferon-gamma release assays (IGRAs) for the diagnosis of LTBI, presents an opportunity for improved estimation of TB infection prevalence and incidence. Their utility in settings with high background prevalence TB and HIV infection is unknown. Major aims of the study were to:

- Evaluate the prevalence and factors associated with TB infection using both tuberculin skin test (TST) and IGRA assays in a sample of health care workers.
- Evaluate change in interval test response over one year to determine annual risk of infection and determinants associated with test conversion.

**Methods:** Participants completed a questionnaire on occupational and environmental characteristics including a TB symptom screen and underwent chest radiograph and rapid HIV test. Three tests for latent TB infection were administered: TST, QuantiFERON-TB Gold In-Tube (QFT-GIT) and a T-SPOT.TB test. All tests were repeated one year later.

**Results:** The prevalence of TB infection at baseline was 84%, 65% and 60% as measured by TST, QFT-GIT and T-SPOT.TB. There was only fair agreement between TST and IGRAs. HIV positive status was significantly associated with having a TST negative / T-SPOT.TB positive discordant test response (OR=4.72). TST had superior sensitivity than IGRAs for the diagnosis of LTBI.

In primary level staff a positive TST outcome, was negatively associated with HIV positive status (OR=0.41). Long employment duration was positively associated with TST (OR=4.17) and QFT-GIT (OR= 2.42) positivity.

Involvement in sputum collection (OR=3.25) and home-based care of TB patients (OR= 4.14) was associated with a positive IGRA test.

The conversion rate for TST and IGRAs was 38% and 22%, respectively. Reversion rates ranged from 1% - 16 % and was lowest for TST. Factors associated with conversion (for IGRAs) included employment sector, counselling of TB patients and a baseline positive TST.

**Conclusion:** The annual rate of TB infection was very high pointing to occupational exposure as a contributory factor. TST had superior sensitivity than IGRAs for LTBI diagnosis but poor uptake on serial testing. IGRAs had excellent uptake but its clinical utility was negatively influenced by high rates of reversion.

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## PREFACE

Results from the analysis of the study findings as well as the background review of the literature have been included in manuscripts for publication as well in presentations at conferences. These are listed below:

### PUBLICATIONS

1. Liesl Grobler, Shaheen Mehtar, Keertan Dheda, Shahieda Adams, Sanni Babatunde, Martie van der Walt and Muhammad Osman  
EVIDence to Inform South African TB policies (EVISAT) project  
**A systematic review of the epidemiology of tuberculosis in health care workers in South Africa** (*in submission 2014*)
2. Shahieda Adams, Rodney Ehrlich , Roslynn Baatjies, Richard N van Zyl-Smit, Qonita Said-Hartley, Rodney Dawson and Keertan Dheda  
**Incidence of Occupational Latent TB Infection in South African Healthcare Workers** (*submitted European Respiratory Journal, 2014*)

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## **CHAPTER 1**

### **INTRODUCTION**

## **CHAPTER 1**

### **1.1 INTRODUCTION**

#### **Tuberculosis as a public health problem in South Africa and globally**

Tuberculosis (TB) is an important public health problem globally with 8.8 million (range, 8.5–9.2 million) incident cases of TB and 1.1 million (range, 0.9–1.2 million) TB related deaths reported among HIV-negative people. HIV-associated TB contributes an additional 0.35 million (range 0.32–0.39 million) deaths. (World Health Organization, 2011:13) TB is the second leading cause of death from an infectious disease worldwide (after HIV, which caused an estimated 1.8 million deaths in 2008). Whilst the Millennium Development Goal of halting and reversing the TB epidemic may already have been reached as manifested in a slowly declining incidence rate it is unlikely that the more important long term elimination target set for 2050 will be met (Lonroth et al., 2010; Dye & Williams, 2010). Reasons for the continued global burden of tuberculosis may in part be attributed to a rise in the world population. Contributing factors to the epidemic may be broadly categorized into a failure to reduce transmission or an increase in host susceptibility to infection and disease. Low- and middle-income countries shoulder most of the global tuberculosis burden as a result of the synergy between these two factors which are highly prevalent in such countries.

In South Africa despite a relatively well-funded and functioning National Control Programme, TB has remained a pressing public health problem. The disease burden posed by TB in South Africa is considerable and the epidemic continues unabated, much of it fuelled by the high HIV prevalence in the region. South Africa is currently ranked third among high burden countries with an estimated 0.40 million–0.59 million incident cases in 2010, outranked by only India and China (World Health Organization, 2011). Whilst all of the high burden countries have demonstrated declines or stabilization

in their incident rates, South Africa has shown an increasing trend in incident rate with the latest annual incidence estimated to be 1170 per 100 000 persons. The importance of HIV infection as a risk factor is underscored by the latest estimate of a 60% HIV prevalence among incident TB cases in South Africa, relative to a general population HIV prevalence of 9.6 (Statistics South Africa, 2013).

### **Tuberculosis as an occupational risk of health workers**

It is well established that health care workers (HCWs) globally have an increased risk of tuberculosis infection and disease due to occupational exposure (Baussano et al., 2011; Joshi et al., 2006). In recognition of this risk, the World Health Organization (WHO) has advocated a more proactive approach than currently is the case towards protecting the health of HCWs. This includes improving infection control in health care facilities and the implementation of isoniazid prophylaxis for those highest risk of progression to TB disease (WHO, UNAIDS & ILO, 2010; World Health Organization, 2010; World Health Organization, 2009).

In many low and intermediate TB incidence settings, screening programmes for latent TB infection in HCWs are routinely implemented (Menzies, Joshi & Pai, 2007). This is coupled with targeted prophylactic treatment of those with latent TB infection (LTBI) to prevent progression to TB disease. This is not the case in many high TB incidence settings, including South Africa, where the focus until recently has been on the diagnosis and treatment of TB disease (Kasprowicz et al., 2011). Whilst the main reason for this discrepancy is the extent of the TB burden in such settings, another reason is the absence of highly sensitive and specific tests for the diagnosis of LTBI. The tuberculin skin test (TST) until recently has been the only test utilised for the diagnosis of LTBI. It has poor specificity for LTBI diagnosis as test outcome may be affected by immunosuppression, confounding due to prior *Bacille Calmette Guerin* (BCG) vaccination or exposure to environmental

mycobacteria and boosting when used in serial testing. These factors adversely influence the specificity of TST test response (Lalvani & Pareek, 2010). Furthermore the lack of a gold standard for LTBI diagnosis has resulted in the use of active TB as a surrogate measure to evaluate test sensitivity and specificity. This adversely influences TST test performance as cellular immunity is likely to be compromised in the setting of severe infection or immunosuppression.

This past decade has seen the introduction of interferon-gamma release assays (IGRAs), novel diagnostic assays for the diagnosis of LTBI, which have shown promise on account of superior specificity and equivalent sensitivity to that of TST. These features would allow for more targeted implementation of isoniazid prophylactic treatment (IPT) (Pai, Zwerling & Menzies, 2008; Menzies, Pai & Comstock, 2007). Several studies have confirmed that IGRAs have superior sensitivity for LTBI diagnosis in immunocompromised individuals, and improved sensitivity in the setting of active TB, when used as a proxy measure for latent TB infection. IGRAs are also not subject to boosting when used on their own (i.e. without preceding administration of TST) and are thought to be better correlated with recent TB exposure. This has resulted in them being proposed as ideal tests for serial screening of LTBI in HCWs and in outbreak investigations.

Based on the emerging evidence, IGRAs have been incorporated into screening protocols for HCWs in countries such as the USA and the UK (Mazurek et al., 2005; National Collaborating Centre for Chronic Conditions (UK) & Centre for Clinical Practice at NICE (UK), 2011). Other countries, such as Canada, have adopted a more cautious approach as more recent evidence emerging from systematic reviews and mature screening programmes has raised questions about the utility and performance of IGRAs for serial screening of HCWs in low and intermediate TB incidence settings (Dorman et al., 2014; Slater et al., 2013; Zwerling et al., 2013). These doubts are in the main about the interpretation of conversion and reversion

patterns, the use of standard cut-offs to denote test positivity and the variable associations with LTBI prevalence and incidence as well as with TB exposure. This lack of consistency in IGRA test results has resulted in calls for a better understanding of IGRA test response and the use of additional risk factor analysis in clinical decision making to inform the targeted implementation of both LTBI screening and IPT in low TB incidence settings (Pai, 2012).

The evidence base for the performance and utility of IGRAs has in the main come from low and intermediate TB incidence setting. Several authors have pointed to the limited research on the utility of IGRAs for the diagnosis of LTBI in HCWs in high TB incidence settings (Zwerling et al., 2013; Zwerling et al., 2012; Joshi et al., 2006; Pai et al., 2005). This is precisely the setting where HCWs could benefit most from targeted IPT and a resultant reduction in risk of contracting TB disease. A few studies conducted among select groups of HCWs in South Africa have illustrated an increased risk of TB disease and LTBI (Claassens et al., 2010; Claassens et al., 2013; Kranzer et al., 2010; Naidoo & Jinabhai, 2006; O'Donnell et al., 2010; van Rie et al., 2013). This is to be expected as HCWs in South Africa are exposed to high background population TB incidence, have an HIV prevalence which mirrors that of the population, and have additional occupational exposure with relatively poor implementation of infection control practices where they work, adding to their TB risk (Adams et al., 2012; Farley et al., 2012; Naidoo, Seevnarain & Nordstrom, 2012; Zungu & Malotle, 2011). This makes them an ideal population for targeted implementation of IPT.

The evidence base from high TB incidence countries for the use of IGRA assays in screening of HCWs is, however, sparse. A recent systematic review includes results from only five studies which have evaluated LTBI in HCWs from high TB incidence settings (Zwerling et al., 2012). Only two studies involved a head-to-head comparison between TST and IGRAs at baseline, whilst two studies had a prospective design and evaluated IGRA

test conversion over time. Pai et al (2005) in a study of HCWs in India found a stronger association between occupational exposure and IGRA test positivity than for TST, but this difference in association was not statistically significant. Neither longitudinal study reported data that showed IGRA conversion to be more strongly associated with TB exposure than TST conversion.

The one-year prospective study on which this thesis is based, was therefore undertaken to evaluate the use of IGRAs for LTBI screening in HCWs in a high TB incidence settings, against a background of high HIV prevalence.

## **1.2 STUDY AIM**

The aim of this study was to evaluate the use of interferon gamma release assays for the diagnosis of latent tuberculosis infection in a population of health care workers engaged in providing health care services to communities where high rates of tuberculosis (TB) and Human immunodeficiency virus (HIV) infection prevail.

## **1.3 OBJECTIVES OF THE STUDY**

1. Determine the prevalence of latent TB infection using tuberculin skin test (TST) and interferon gamma release assays (IGRAs) and evaluate the occupational and host factors associated with the occurrence of LTBI, as measured by the different tests.
2. Determine the baseline prevalence of TB disease in this population
3. Determine the level of agreement between the different tests and the factors associated with concordant and discordant test outcomes at baseline.

4. Perform a direct comparison between test performance of the different tests, using latent class analysis, in measuring sensitivity and specificity in this population.
5. Estimate the annual incidence of infection (based on test conversion) through the employment of a serial testing strategy and explore the occupational and host factors associated with risk of conversion.

#### **1.4 STRUCTURE OF THE THESIS**

**Chapter 2** is a comprehensive literature review of the problem of tuberculosis infection and disease in health care workers with a focus on determinants associated with such infection in this occupational group. Included in the review is a focus on studies which use both tuberculin skin tests and interferon gamma release assays as outcome measures of LTBI and which measure agreement between these two testing modalities. Research findings from studies which report results from serial screening are also included to evaluate changes in test response over time and the factors associated with test conversion and reversion. The aim of this chapter is to provide a context for the study and the questions asked.

**Chapter 3** provides a detailed description of the methods employed in the execution of the study. This includes a detailed description of the study setting and the population to develop a basis for comparison with results from similar studies conducted among different HCW populations.

**Chapter 4** presents the results of the baseline phase of the study. Findings with regard to the prevalence and determinants of LTBI using different test modalities are presented. This is only the second South African study which has evaluated the prevalence of LTBI (using both TST and IGRAs) among HCWs and the first to evaluate the relationship with LTBI occurrence and occupational factors in a HCW population.

**Chapter 5** evaluates agreement between TST and IGRAs for the diagnosis of LTBI. It includes an exploration of factors associated with concordant versus discordant test results using a multinomial regression model. A direct comparison between test performance is also done using latent class statistical modelling techniques.

**Chapter 6** evaluates change in LTBI test response over a one year interval. Estimates of annual incidence of infection (based on test conversion) are reported. Factors associated with risk of conversion are explored with a view to identifying consistent predictors of conversion across all test modalities. Potentially modifiable occupational risk factors influencing conversion are also evaluated.

**Chapter 7** concludes with a discussion of the implications of the main findings and limitations of the study. The implications for research and public health policy and occupational health programmes are outlined and recommendations are made in this regard.

## **1.5 DEFINITIONS**

### **Health care worker**

In the South African context and for the purpose of this study, a health care worker was defined as any person directly engaged in providing a service at, or on behalf of the health care facility. This included those in direct employment by the health department and those employed by non-governmental organizations such as community based health care workers who provide support for health programmes e.g. TB adherence supporters. In addition, those employees who provide support services such as administrative, security, or cleaning services were also classified as health care workers and were considered eligible to participate in the study. This is in line with the approach adopted by the World Health Organization that proposes the inclusion of all those involved in the provision of health services, as well as management and support workers in such a definition. It furthermore includes subsectors of workers engaged in community-based and home-based care (WHO, UNAIDS & ILO, 2010; Oxlade et al., 2009).

### **Low, medium and high tuberculosis- incidence country**

The World Health Organization characterizes countries based on their annual TB incidence rate. Low TB incidence countries have a <50/100 000 annual incidence of TB; intermediate-incidence 50-99/100 000 and high incidence  $\geq 100/100\ 000$ .

### **Low, middle and high- income countries**

This refers to a definition employed by the World Bank to characterize a country by its economic status (The World Bank Group, 2014). For the current 2015 fiscal year, low-income economies are defined as those with a gross national income (GNI) per capita, calculated using the *World Bank Atlas* method, of \$1,045 or less in 2013; middle-income economies are those with a GNI per capita of more than \$1,045 but less than \$12,746; high-income economies are those with a GNI per capita of \$12,746 or more.

### **Latent TB infection**

LTBI is defined by the presence of an M. tuberculosis specific immune response in the absence of clinical and radiological disease (Chee et al., 2013).

### **Tuberculosis disease**

For the purpose of this study TB disease was defined as bacteriologically confirmed tuberculosis on either sputum smear microscopy or culture.

### **TST conversion**

This was defined as a negative baseline TST (induration of <10mm), and follow-up positive TST (induration  $\geq$  10mm), with an increase of at least 10mm, as per American Thoracic Society (ATS) and Centres for Disease Control (CDC) standards(American Thoracic Society, 2000).

### **TST reversion**

A positive TST at baseline ( $\geq$ 10 mm) which becomes negative upon retesting (< 10 mm induration) 1 year later.

### **QuantiFERON Gold-in-tube (QFT-GIT) conversion**

A QFT-GIT conversion was represented by a baseline interferon-gamma (IFN- $\gamma$ )<0.35 IU/ml and follow-up IFN- $\gamma$ >0.35 IU/ml.

### **TSPOT.TB conversion**

A conversion was represented by a negative test (<6 spots increment in Panels A and B) at baseline changing to a positive test (>6 spots increment in either Panel A or B) on follow-up testing.

### **IGRA reversion**

A change from a positive IGRA at baseline to a negative IGRA at follow-up.

**Employment sector**

This refers to the employer group who is responsible for delivery of health care and support services and provides employment to health care workers. Employees were categorized as belonging to four employment sectors viz. the Provincial health department, the local authority health department, non-governmental organizations or other (usually support services such as security which is outsourced)

## 1.6 REFERENCES

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## **CHAPTER 2**

### **LITERATURE REVIEW**

## **CHAPTER 2**

### **2.1 BACKGROUND AND REVIEW OF THE LITERATURE ON TUBERCULOSIS IN HEALTH CARE WORKERS IN HIGH BURDEN COUNTRIES**

In reviewing the literature on TB infection and disease risk in HCWs, a distinction has been made between estimates from low incidence TB settings (< 50 / 100 000 cases per year), intermediate setting (50 - 100 / 100 000 cases per year) and high TB incidence settings ( $\geq$  100 / 100 000 cases per year)(Zwerling et al., 2012; Baussano et al., 2011). Estimates from high TB burden countries usually refer to the 22 countries identified by the World Health Organization as contributing the greatest caseloads to the global TB burden, accounting for 81% of cases in 2010(World Health Organization, 2011). Others have evaluated HCW TB risk by the socio-economic status of their countries (using criteria defined by the World Bank), referring to low and middle income countries (LMICs) and high-income countries (HICs)(Menzies, Joshi & Pai, 2007; Joshi et al., 2006). These authors report that 90% of the global TB burden affects populations residing in LMICs and that HCW in high income countries generally have a very low occupational risk of TB. There is some overlap of these classifications as the high burden countries and LMICs include countries with either high or intermediate TB incidence rates. In reporting on these estimates in this thesis, as far as possible a distinction has been made between low and high TB incidence settings, as TB caseload is likely to differ considerably from setting to setting influencing the TB infection risk faced by HCWs.

For the purpose of this review, a broad definition of health care worker is employed to include all those engaged in the support and delivery of health services at a selected health facility. This is in line with the approach adopted by the World Health Organization that proposes the inclusion of all those involved in the provision of health services, as well as management and

support workers, in such a definition. It furthermore includes subsectors of workers engaged in community-based and home-based care (WHO, UNAIDS & ILO, 2010; Oxlade et al., 2009).

Whilst the primary focus of the thesis is on latent TB infection (LTBI), it is important to distinguish between LTBI and TB disease. In clinical practice LTBI is defined by the presence of an immunological sensitization to mycobacterial antigens in the absence of clinical signs and symptoms of active disease (Barry et al., 2009). As a consequence of this definition TB is commonly viewed as having a binary distribution – latent versus active disease. TB disease or active TB would require a course of chemotherapy with standard treatment regimens in accordance with a country-specific national TB control programme. Individuals with LTBI who are considered at high risk of progression to active TB would be offered isoniazid prophylaxis. This simplistic view of TB, whilst useful in the clinical setting, does not accurately reflect the diverse range of clinical phenotypes which represents the spectrum of LTBI. These may include individuals who have completely “cleared the infection to those who are actively replicating TB organisms” without clinical manifestation of disease. Active TB has also been shown to have “diverse pathological manifestations in both animal and human studies ranging from sterile tissues to liquefied cavities with large numbers of replicating organisms” (Barry et al., 2009: 846). To arrive at a more complete understanding of TB infection Barry et al (2009) propose that LTBI and active TB be viewed as part of a continuous spectrum of TB infection. Increasingly research is focused on trying to ascertain at which point of the spectrum the risk of progression from latent to active TB is highest. Preventative therapy should then be targeting this high risk group as part of TB control efforts.

Health care workers (HCWs), as a high risk occupational group, find themselves at the forefront of the dual epidemics of TB and HIV. It is well recognized that they have an increased risk of contracting tuberculosis on account of their occupational exposure to tubercle bacilli (Baussano et al.,

2011; Menzies, Joshi & Pai, 2007; Joshi et al., 2006). Furthermore this risk has been demonstrated to be greater among health care workers in LMICs which account for 90% of the global TB burden. Due to limited resources and the magnitude of the TB caseload in such countries, resources tend to be focused on community case-finding and implementation of the directly observed treatment supervision (DOTS) strategy. There is therefore little focus on prevention of TB in HCWs or the implementation of interventions aimed at reducing TB transmission in health care facilities (Farley et al., 2012; Naidoo, Seevnarain & Nordstrom, 2012; Kasprowicz et al., 2011; Harries, Maher & Nunn, 1997).

HCWs in high TB-incidence countries are at risk of contracting TB in their dual role as carers to those who have contracted TB and by virtue of belonging to communities where TB is highly prevalent. Whilst they are considered a high-risk occupational grouping for contracting TB, there is limited research that attempts to describe and quantify their risk in this regard. There is, however, a growing recognition globally that the increasing impact of TB and HIV on healthcare workers in such settings could have a profoundly negative influence on the success of important public health programmes and the capacity of health systems to respond effectively to the TB and HIV epidemics. To address this problem a Policy Guideline to promote access to care for HCWs with a specific focus on TB and HIV has recently been published (WHO, UNAIDS & ILO, 2010). The Joint WHO-ILO-UNAIDS Policy Guidelines on Improving Health Workers' access to HIV and TB prevention, Treatment, Care and Support Services outlines fourteen recommendations which require action with regard to policy, workplace practices, budget and monitoring and evaluation. It promotes the strengthening of occupational health policy to better protect health care workers and advocates regular screening, improved infection control, access to prophylactic and effective treatment for those at risk of or infected with TB and HIV.

Whilst the lack of progress in implementing TB prophylactic treatment in high incidence countries is primarily due to a lack of resources, there are also limited data to provide reliable estimates of TB infection risk in HCWs in such settings. Furthermore the available tools have suboptimal sensitivity in accurately diagnosing those with recent TB infection, in discriminating between latent and active TB and in identifying those at highest risk of progression from latent to active TB disease (Kasproicz et al., 2011). The deficiencies are further complicated by the lack of a gold standard for the diagnosis of LTBI, making it difficult to interpret results from screening programmes of high risk groups such as health care workers.

## **2.2 THE EPIDEMIOLOGY OF LATENT TB INFECTION AND TB DISEASE IN HEALTH CARE WORKERS IN HIGH BURDEN SETTINGS**

### **Prevalence and incidence of LTBI**

#### *Low TB incidence settings*

In low TB incidence countries such as the United States the risk of TB infection and disease in HCWs declined in tandem with a declining incidence of TB in the general population (Harries, Maher & Nunn, 1997). Following several outbreaks of MDR TB among HCWs in the US between 1985 and 1993, renewed focus was placed on the implementation of preventative strategies and infection control measures to reduce nosocomial transmission of TB (Jensen et al., 2005).

A review of TB infection prevalence in HCWs from high-income countries, which are generally associated with low TB incidence settings, demonstrated a median LTBI prevalence of 24% (4 – 46%) in HCWs. This is almost a third

of the estimate for HCWs in LMICs (Menzies, Joshi & Pai, 2007). Similarly the LTBI incidence in HCWs in low TB incidence countries at 2.9% (IQR 1.8 %- .8.2%), equates to less than half the incidence in high-TB incidence countries (Baussano et al., 2011). In low TB incidence settings the implementation of targeted surveillance and prevention of tuberculosis transmission among HCWs have been shown to decrease the overall risk of tuberculosis infection among HCWs (Baussano et al., 2007).

### *Intermediate and high TB incidence settings*

A systematic review of 51 studies by Joshi et al (2006: 2381-2382) estimated the burden of tuberculosis among HCWs working in LMICs. The prevalence of LTBI among HCWs was found to range from 33 % to 79%, with more than half of HCWs infected with TB in many settings. In this review LTBI *incidence* was measured in six studies only, which showed an annual risk of infection ranging from 3.9% to 14.3%, with the risk attributable to occupational exposure ranging from 2.6% to 11.3%. This suggests that most cases of TB infection in HCWs are attributable to occupational exposure. A more recent review by Baussano et al (2011) estimated the median incidence of LTBI in HCWs in high incidence countries to be 7.2% (IQR 4.1% - 14.3%), but makes no distinction between occupational and non-occupational infection.

Several other studies have evaluated LTBI prevalence among HCWs in intermediate and high incidence TB countries such as India, Russia, China, Thailand, Vietnam and Brazil (Christopher et al., 2010; He et al., 2010; Lien et al., 2009; Drobniowski et al., 2007; Pai et al., 2005; Roth et al., 2005; Yanai et al., 2003; Do et al., 1999). These studies have found the prevalence of LTBI as measured by TST or Interferon-gamma release assays (IGRAs) to vary between 40-70% and to be higher than that found in the general population. In Africa very few studies have been conducted to evaluate LTBI prevalence and risk factors in HCWs. This is due to a limited focus on LTBI diagnosis and treatment, given the magnitude of the TB burden in many

African countries and the limited resources available for testing. A study of HCWs in Cote d' Ivoire reported a LTBI prevalence of 79% , whilst one in Ugandan HCWs found the LTBI prevalence to be 57%(Kayanja et al., 2005; Kassim et al., 2000).

In South Africa the treatment of LTBI in the general population is limited to those considered to be at high risk of progression to active disease, such as HIV-infected individuals, children under the age of five years and individuals with silicosis (de Jager et al., 2014; National Department of Health South Africa, 2009). Until very recently there has been no clear guideline that specifically addresses LTBI in HCWs (Tshitangano, 2013).

Studies of HCWs from high TB incidence countries display considerable heterogeneity due to differences in methodological quality, study design, sampling strategy and populations sampled(Baussano, 2011: 493).This heterogeneity is reflected in the suboptimal precision of estimates generated to reflect TB infection risk in HCWs. In the absence of effective implementation of large scale surveillance and TB screening programs of HCWs in high burden countries, arriving at an accurate estimate of TB infection risk of HCWs, remains elusive.

### **Risk factors associated with latent TB infection in health care workers**

Few studies have tried to identify the host or occupational and environmental risk factors associated with increased susceptibility to TB infection in HCWs.

#### *Low TB incidence settings*

In HCWs from high income countries the prevalence of LTBI as demonstrated by a positive TST is consistently associated with non-

occupational factors such as older age, foreign birth, and previous BCG vaccination (Menzies, Joshi & Pai, 2007). Occupational factors that show variable association with LTBI are years of work in health care, working in internal or respiratory medicine and increased exposure to TB patients. Most studies evaluating LTBI incidence have been conducted in the US, with incidence rates substantially higher than that of the general population at 1% (0.2% – 12%). LTBI incidence in such settings is positively associated with increased risk of exposure (high numbers of TB patients) and an absence of infection control measures. The imprecision of LTBI incidence estimates may in part be due to the inclusion of study settings with variable TB infection rates and differences in testing protocols and definitions of conversion employed for LTBI.

#### *Intermediate and high TB incidence settings*

In LMICs the prevalence of LTBI has been found to be positively associated with increasing age and duration of employment in a health care facility. Similar associations have been demonstrated in studies from intermediate and high incidence countries such as India, Russia, China, Thailand, Vietnam and Brazil (Christopher et al., 2010; He et al., 2010; Lien et al., 2009; Drobniewski et al., 2007; Pai et al., 2005; Roth et al., 2005; Yanai et al., 2003; Do et al., 1999). In addition, the prevalence of LTBI has been found to be consistently associated with occupational TB exposure, occupational category and variably associated with BCG vaccination and male sex. With regard to occupational category, several studies have reported a higher prevalence of LTBI in nurses than in other HCWs (Joshi et al., 2006). Additional independent occupational predictors of LTBI are working in medical wards, participation in procedures such as sputum collection and performance of autopsies, and a history of contact with TB patients. A study of Ugandan HCWs found LTBI prevalence to be positively associated with employment location and older age (Kayanja et al., 2005). A recent study among South African HCWs and medical students in Johannesburg found

significant differences in LTBI prevalence between the two groups, with HCWs displaying a two to fourfold increase in LTBI prevalence compared to medical students ( 56.7% v. 26.6% TST positive; 69.2% v.15.2% IGRA positive) (van Rie et al., 2013). This suggests that occupational exposure to TB and/or duration of employment may be important risk factors for LTBI.

### **Prevalence and incidence of TB Disease**

TB disease is characterized by signs and symptoms of infection and / or microbiological or radiological evidence of disease. TB disease is considered to represent part of the TB infection spectrum which extends from sterilizing immunity through to fulminant active disease(Barry et al., 2009). In the absence of a gold standard to evaluate diagnostic test performance for LTBI, TB disease has been used as a proxy gold standard for LTBI, in order to evaluate test sensitivity. The assumption is that LTBI must be present concurrently or have preceded the development of TB disease.“Geographically LTBI prevalence mirrors that of TB disease with the highest prevalence of infection found in high TB disease burden regions such as Sub-Saharan Africa and the Indian subcontinent”(Kasprovicz, 2011:S1169).

#### *Low TB disease incidence settings*

Studies of TB disease risk in HCWs in high income countries have produced highly variable estimates, with most studies relying on TB case registries to describe TB associated morbidity and mortality.“The best-designed studies have demonstrated a two to threefold increased risk of TB disease among HCWs when matched for employment and socio-economic status with the general population” (Menzies, 2007: 600). This is more pronounced in younger HCWs. The median incidence of TB disease among HCWs was estimated to be 67/100 000 and 91/ 100 000 in low and intermediate TB incidence countries respectively. Comparison to TB incidence rates in the

general population have yielded an incidence rate ratio (IRR) of 2.0 and 1.4 respectively (Baussano et al., 2011).

### *Intermediate and high TB disease incidence settings*

Tuberculosis disease incidence among HCWs from LMICs has been found to be generally higher than in the general population. The estimated incidence due to nosocomial exposure ranges from 25 – 5361/100 000 per year. Baussano et al (2011) in reviewing TB disease among HCWs in high incidence countries estimated the median incidence of TB disease to be 1180/100 000 (IQR 91 – 3.222) which is fivefold greater than the median TB incidence rate in the population [IRR 5.4; IQR 1.7 -9.1]. The percentage of cases attributable to exposure in health care settings was estimated to be 81%.

Several studies have been conducted to evaluate TB disease in HCWs in Africa but have not reported on the potential role of occupational factors in contributing to their TB disease risk. In Malawi HCWs were found to be at greatly increased risk of all types of TB disease (IRR 11.9; 95% CI 9.5 -17.7) in a study conducted at 40 hospitals. The annual incidence of treated TB cases among this sample of HCWs was 3.6% (Harries et al., 1999). TB was also cited as an important cause of death among HCWs in urban areas in Malawi, accounting for 47% of deaths in one study(Harries et al., 2002). In Nigeria a study of hospital-acquired TB showed clinical staff to be at highest risk among HCWs of contracting occupational TB, with HIV co-infection being present in 47% of cases. Female prevalence was double that of males(Salami & Oluboyo, 2008). Similarly a Mozambican study highlighted a reported TB and HIV prevalence among staff of 21% and 28%, respectively (Casas et al., 2011).

Several studies have been conducted in different groups of South African HCWs to determine the prevalence and /or incidence of TB disease and evaluate factors associated with increased risk of contracting tuberculosis (Table 2.1). A study based on a record review of staff health records between 1986 and 1997 from four dedicated TB centres in Mpumalanga, South Africa, concluded that HCWs were not at increased risk of TB when compared to the general population (Balt, Durrheim & Weyer, 1998: 363). Wilkinson and Gilks (1998: 501) similarly demonstrated a lower incidence of TB among staff at a South African district hospital when compared to the general population during the same period. However this study demonstrated an increasing incidence of TB among health care workers which was attributed to the rising HIV epidemic during this period.

More recent studies, however, have shown the opposite association. In a retrospective cohort study which involved staff from eight regional hospitals in Kwazulu Natal, Naidoo and Jinabhai (2006:679) demonstrated a consistently higher incidence of TB in health care workers than the general population, with a median incidence of 1133/ 100 000. A substantial proportion of the cases (23.5%) presented with extra-pulmonary TB and 3% were diagnosed with multi-drug resistant TB. A recent screening programme of health care workers employed as adherence counselors and support staff in TB programmes located in the Western Cape demonstrated a TB and HIV prevalence of 5% and 20% respectively (Kranzer et al., 2010:224). This represents a five-fold increase in TB prevalence when compared to the general population, suggesting that these lay health care workers are at greatly increased risk of contracting TB. Similarly, the Desmond Tutu TB Centre has demonstrated a twofold increase in TB incidence in a small cohort of health care research workers compared to the general population (Claassens et al., 2010:1578). This suggests that HCWs are at increased risk of TB due to occupational exposure.

The emergence of extensively drug-resistant (XDR) tuberculosis has aggravated the occupational risk faced by HCWs in South Africa (Jarand et al., 2010; O'Donnell et al., 2010). HCWs in KZN were shown to have a fivefold increased risk of being hospitalized for multidrug resistant (MDR) TB (5.46, 95%CI 4.75 – 6.28) and a near sevenfold increased risk of being hospitalized for XDR TB (6.69, 95% CI 4.38 – 10.20) relative to the general population. They were also less likely to report previous TB treatment than the general population and had a similar HIV positive prevalence. This evidence strongly points to occupational exposure as a contributing risk factor to drug resistant TB in HCWs.

A recent large study evaluating infection control measures at primary care facilities in five provinces of South Africa found the standardized incidence ratio for smear positive TB disease in HCWs to be double that of the general population (Claassens et al., 2013). HCWs have been shown to have HIV infection rates which approximates that of the general population ranging from 11 – 20% among HCWs in SA (Table 2.2) (Kranzer et al., 2010; Connelly et al., 2007; Shisana et al., 2004). Given these high rates of HIV infection coupled with occupational exposure to tuberculous bacilli and high background prevalence of TB disease in the community, there are likely to be many HIV-infected HCWs in South Africa with recent LTBI and therefore at considerable risk of progressing from LTBI to active TB disease.

### **Risk factors associated with the development of TB disease**

Several risk factors have been identified as being associated with the progression from LTBI to TB disease. These are well-established risk factors such as HIV co-infection, malnutrition and young age. Diabetes mellitus, heavy alcohol intake or problem drinking and smoking, iatrogenic immunosuppression and indoor air pollution are considered emerging risk factors (Narasimhan et al., 2013; Kasproicz et al., 2011). For these reasons, in TB control programmes, LTBI testing has been limited to those considered

at highest risk of developing TB disease. In South Africa, such interventions are primarily aimed at children under the age of five years, high-risk TB contacts, HIV-co-infected individuals and silicotic individuals.

There has been less focus on identifying occupational and environmental risk factors associated with the development of TB disease. Whilst it is known that certain occupations, such as HCWs and mineworkers, face an increased risk of developing TB disease, few studies have investigated the relationship between occupational and environmental exposures and TB risk in high burden settings. In the South African mining sector, resources are being devoted to research and interventions to address the epidemic of silicosis and tuberculosis, especially since the emergence of HIV, which has greatly increased TB rates among mineworkers (Dharmadhikari et al., 2013). Increasingly too, efforts are being focused on the diagnosis and treatment of LTBI among mineworkers in an attempt to improve TB control in this high risk population and reduce TB disease incidence (Churchyard et al., 2014; Lewis et al., 2013; Fielding et al., 2011). This has culminated in the recent development of a clinical guideline on isoniazid preventive therapy for patients with silicosis in South Africa (de Jager et al., 2014).

There is similarly an increasing awareness of the need for improved protection of HCWs, but there has been limited and fragmented implementation of programmes to adequately protect HCWs in South Africa (Naidoo et al., 2013; Adams et al., 2012; Naidoo, Seevnrain & Nordstrom, 2012; Zungu & Malotle, 2011). Whilst the high rate of TB among SA HCWs is indicative of a substantial occupational transmission risk, the effectiveness of tools to evaluate infection control practices at primary care clinics in SA is not proven. This suggests that there is a need for such evaluation tools to be tested and validated for local high burden settings (Claassens et al., 2013). TB prevention programmes for HCWs will need to be informed by accurate estimates of TB infection and disease prevalence and incidence and a complete understanding of occupational and environmental risk factors

associated with increased transmission of tuberculous bacilli in healthcare settings.

### *Low TB incidence settings*

In addition to well-known host factors of which HIV co-infection is the most important, several studies from low TB burden settings have highlighted occupational risk factors as contributing to TB disease incidence in HCWs. Specific occupations, shorter duration of employment and occupational location characterized by increased exposure to TB patients have been associated with greatly increased risk of TB disease in HCWs in several studies (Skodric-Trifunovic et al., 2009; Jo et al., 2008; Sotgiu et al., 2008; Hosoglu et al., 2005; Cuhadaroglu et al., 2002; Babus, 1997). Occupational titles were not uniform across all studies, and differed by study setting, making direct comparisons difficult. Most studies, however, showed nurses and staff with direct exposure to TB patients such as pulmonologists to have an increased risk of LTBI, compared to other HCWs.

Some studies from low burden settings have shown TB incidence rates to be similar to that of the general population. The authors of a review on tuberculosis risk in HCWs argue that comparison to the general population may underestimate the contribution of occupational exposure (Menzies, Joshi & Pai, 2007). This on account of HCWs higher socio-economic status, being younger in age and healthier than the general population (on account of the healthy worker effect), which should result in them having a lower background risk of TB disease. The excess risk may therefore plausibly be attributed to occupational exposure. Baussano et al (2011) estimates that 49% of TB cases of TB in HCWs in low incidence countries are attributable to exposure in health care settings.

### *Intermediate and high incidence TB disease settings*

TB disease in HCWs has been positively associated with low HCW: TB patient ratio as well as with certain locations and occupational categories within health facilities (Joshi et al., 2006). Associations with occupational category have been shown in countries such as China where medical staff had a higher TB prevalence than administrative staff (6.7/1000 v. 2.5/1000) (He et al., 2010). Russian HCWs in Samara Oblast similarly were found to have a tenfold increase incidence of TB disease when compared to the general population, with staff in the TB services at greatest risk (Dimitrova et al., 2005). HCWs at a university hospital in Brazil showed high rates of extra-pulmonary TB (48%) (do Prado et al., 2008). In Thailand the TB disease incidence rate of HCWs within a large hospital setting was positively associated with work site (emergency room) and occupation (nursing) (Jiamjarasrangi, Hirunsuthikul & Kamolratanakul, 2005). In a study of hospital staff in Kenya strong associations were demonstrated between TB disease in HCWs and occupational exposure even after controlling for living conditions (Galgalo et al., 2008). In Indian HCWs low body-mass index (BMI), frequent contact with patients and employment location in the medical wards or microbiology laboratories were found to be independently associated with increased risk of acquiring TB (Mathew et al., 2013).

Studies from South Africa have evaluated risk factors for TB disease in HCWs, focusing mainly on host and not occupational determinants. One study highlighted the increasing incidence of TB disease in tandem with the growing prevalence of HIV infection (Wilkinson & Gilks, 1998). Naidoo and Jinabhai (2006) similarly showed an increasing incidence of TB among HCWs with younger age-group and paramedical staff having the highest TB incidence rates. In KZN. HCWs with drug-resistant TB (MDR-TB or XDR-TB) were mainly female (78%), of younger age (median age, 35 years), with a high percentage of HIV infection among those tested (67%) (O'Donnell et al., 2010). In another study evaluating the impact of infection control measures

at primary care facilities, TB disease risk was similar for HCWs in primary care and those in hospital-based settings (Claassens et al., 2013).

Studies among HCWs in South Africa, as elsewhere, have been conducted with fairly small sample sizes on select groups of HCWs and have been conducted in specific localities. These deficiencies limit their generalizability, i.e. they do not provide a representative estimate with good precision of TB risk of HCWs in South Africa. This limitation is further compounded by the varying prevalence of TB within different regions of the country. However, all the studies with the exception of those performed by Balt and Wilkinson show that HCWs in South Africa, as elsewhere, face a greater risk of TB infection and disease than the general population. The factors associated with such risk are poorly understood and the role of occupational and environmental factors in driving TB infection and disease rates in HCWs has not been elucidated. Identification of those factors is necessary for the implementation of targeted interventions in the future that is aimed at decreasing HCWs risk to TB infection and disease in high burden settings.

**Table 2.1 Incidence estimates from studies conducted among South African health care workers to evaluate their risk of tuberculosis compared to that of the general population**

Study author (year)	year	N	Estimates of TB incidence	Risk ratio (95% CI)
Balt (1998)	1986 - 1997	726*	275 / 100 000	1.0
Wilkinson (1998)	1991 - 1996	388	558 / 100 000	0.4
Naidoo (2006)	1999	3711	1024 / 100 000	2.7
	2000	3819	1206 / 100 000	3.8
	2001	4807	834 / 100 000	1.9
	2002	6887	945 / 100 000	1.7
	2003	8445	1149 / 100 000	1.5
	2004	4226	1641 / 100 000	-
				1133 / 100 000**
Claassens (2008)	2005 - 2007	180	4477 / 100 000	2.3
Claassens (2013)	2006	1439	834 / 100 000	2.4 (1.2 – 2.4)
	2007	1649	1 092 / 100 000	3.0 (1.8 – 4.7)
	2008	1917	887 / 100 000	2.3 (1.3 – 3.7)

N= number of health care workers observed during study period. 95% CI=95% confidence interval and is only provided if quoted in the study. Risk ratio is calculated by dividing the incidence of TB in HCWs by the incidence in a community-based reference population.

\* Measured as nursing years; \*\* Median incidence over the time period of the study

**Table 2.2 HIV prevalence among South African health care workers**

<b>Study author (year)</b>	<b>n</b>	<b>HIV prevalence (95% CI)</b>
Shisana (2004)	595	15.7% (12.2 -19.9)
Connely (2007)	2032	11.5%
Kranzer (2010)	215	20%
Van Rie (2014)	199	11%

n=sample size.95% CI=95% confidence interval and is only provided if quoted in the study.

### **2.3 AGREEMENT BETWEEN IGRA ASSAYS AND TST AND A COMPARISON OF TEST PERFORMANCE USING LATENT CLASS ANALYSIS**

It is estimated that one third of the world's population is latently infected with TB. (Dye et al., 1999) This large reservoir of infected individuals are at increased risk of developing active TB. Estimates based on the natural history of TB suggest that 5% of infected individuals will progress to active TB within the first 2 years following initial infection. Immune competent infected individuals have a lifetime risk of 5 - 10% for developing TB (Lalvani & Pareek, 2010). The diagnosis and treatment of LTBI are increasingly being recognized as an important strategy to aid tuberculosis control worldwide (Chee et al., 2013).

Groups at high risk of progression have been identified as; immunocompromised individuals, young children, those with recent TB infection and high risk occupational groups such as health care workers and mineworkers. Whilst programmes of targeted LTBI diagnosis and treatment are routinely implemented in low TB incidence countries, this has been far more difficult to implement in high TB incidence countries (Kasproicz et al., 2011). Recent strategies proposed by WHO especially relevant to South Africa, which accounts for a quarter of the global burden of HIV-associated TB, include intensified case-finding (ICF), improved TB Infection control, provision of antiretroviral therapy (ART) and isoniazid preventative therapy (IPT) (World Health Organization, 2008). Whilst large-scale ART programmes have been rolled out throughout Africa, there has been less focus on infection control and IPT. This is in part due to the limitations of existing tools to diagnose LTBI and identify those individuals at most at risk of progression to active disease (Kasproicz et al., 2011).

## **Diagnosis of latent TB infection**

Globally the diagnosis of LTBI has primarily been made using the tuberculin skin test (TST). The past decade has seen the development of novel diagnostic tests for LTBI, in the form of interferon-gamma release assays (IGRAs). A large body of evidence has accumulated as researchers have focused on their utility in diagnostic programmes. These include serial screening of high risk populations such as health care workers in order to implement targeted IPT. However, very little research has been conducted among HCWs to evaluate test performance and utility in high burden settings (Zwerling et al., 2012).

### **Tuberculin skin test**

#### *Test properties and immunology*

The TST has been around for more than a century and until fairly recently the diagnosis of LTBI globally has been made using only this test (Chee et al., 2013; Lalvani & Pareek, 2010). A positive skin test in the absence of symptoms or signs of clinical disease is considered diagnostic of LTBI. The TST detects a delayed hypersensitivity to purified protein derivative (PPD) in the form of a skin reaction. PPD constitutes a mix of over 200 *M. Tuberculosis* proteins. This renders it susceptible to cross-reactivity with bacille Calmette- Guerin (BCG) and several species of environmental mycobacteria, diminishing its specificity. It also has suboptimal sensitivity in anergic individuals such as those who are HIV-infected or those on immunosuppressive medication, leading to a high rate of false negatives. The TST measures a cellular immune response which manifests as a skin induration 48-72 hours after intradermal inoculation with PPD. It requires trained personnel to administer and read the TST and two visits within the required time to obtain the result. These logistic requirements in part have

contributed to low uptake of TST in resource poor countries despite the affordability of the test.

### *Test performance*

The lack of a gold standard for LTBI has also complicated the interpretation of TST test performance. In evaluating sensitivity of TST, active TB has been used as a proxy measure for LTBI, as it is assumed that LTBI must be present for active TB to develop. This is less than ideal as it is known that with overwhelming infection with TB bacilli and HIV co-infection, cellular immunity may be impaired leading to a falsely negative TST. Furthermore TST is not able to discriminate between LTBI and active TB.

A further reason for low programmatic uptake is that some authorities have considered TST to have suboptimal test performance. TST sensitivity has been estimated to range from 70%-80% in community based studies, which excluded high risk occupational groups, using active TB as a surrogate for LTBI(Menzies, Joshi & Pai, 2007). The specificity of TST has been evaluated in populations at very low risk for LTBI and has ranged from 56% in BCG vaccinated to 98% in non- BCG vaccinated individuals.

TST may also be subject to boosting when used in serial screening, making it difficult to discriminate between true conversion or new infection and immune priming as a consequence of antecedent TST administration. Serial administration of TST has also resulted in larger positive TST skin reactions on follow-up testing(Menzies, 1999;Mandalakas et al., 2008; French et al., 2006). Boosting typically manifests as increased tuberculin reactions upon retesting in the absence of new infection. It is believed to result from recall of waned cell-mediated immunity. The boosting effect is maximal if the interval between the first and second test is between 1 and 5 weeks, but has been shown to be present one or more years after a first negative tuberculin test.

In low burden countries, the prevalence of boosting is common in foreign-born, elderly individuals and BCG-vaccinated populations. TST positivity is also considered to be a reflection of cumulative exposure and does not always correlate well with recent exposure especially in BCG vaccinated populations. This feature renders it less useful as a tool for contact tracing and outbreak investigations because of the risk of false positives (Menzies, Pai & Comstock, 2007).

### *Test utility*

Despite these drawbacks TST does have some predictive value as recent LTBI, reflected in TST conversion, has been associated with a 5% risk of progression to active TB in the first 2 years following infection. In addition those with LTBI have a lifetime risk of 5% -10 % of developing active TB. (Blower et al., 1995; Lalvani & Pareek, 2010). The risk is greatly increased in individuals who are HIV positive and disease progression rates are estimated at 3.5–16.2 % per person-year of observation in HIV positive contacts of index cases in LMICs (World Health Organization, 2012). TST positivity is also associated with IPT benefit as HIV-infected individuals with a positive TST have been shown to have a greater reduction after IPT in subsequent risk of developing TB disease than those with negative TST (Akolo et al., 2010).

## **Interferon- gamma release assays**

### *Test properties and immunology*

Two new T-cell-based assays have been developed for diagnosing LTBI and have been commercially available in recent years (Menzies, Pai & Comstock, 2007: 340). The one test is an enzyme-linked immunosorbent assay (ELISA), the QuantiFERON-TB Gold-in -tube (QFT-GIT) (Cellestis Ltd, Carnegie,

Australia) which measures the production of antigen-specific interferon-gamma (IFN-  $\gamma$ ) by circulating T-cells in whole blood. The other is the T-SPOT.TB (Oxford Immunotec, Oxford, UK) which uses an Elispot technique to measure peripheral blood mononuclear cells (PBMCs) that produce IFN- $\gamma$ . Both assays are characterized by the use of more specific *M. Tuberculosis* antigens – the early secretory protein-6 (ESAT-6) and the culture filtrate protein-10 (CFP-10) which are encoded by genes found in the region of difference (RD1) on the *M.tuberculosis* genome. These genes are not present in the genome of *M.bovis* BCG or certain non-tuberculous mycobacteria such as *M.avium*. resulting in improved specificity of the tests and equivalent sensitivity when compared to TST. IGRAs do however come at a higher cost than TST, a requirement for good laboratory infrastructure and are invasive (requires the taking of blood samples), all of which may serve as barriers to their practical uptake in low-resource settings.

### *Test performance*

Results from meta analyses show IGRAs to have very good specificity, unaffected by BCG vaccination, for LTBI diagnosis whilst T-SPOT.TB has superior sensitivity over TST and QFT-GIT for TB disease (Pai, Zwerling & Menzies, 2008; Diel, Loddenkemper & Nienhaus, 2010). The pooled sensitivity of T-SPOT.TB has been reported as 90% (86-93%) which is superior to that of QFT-GIT at 70% (64 – 93%), using active TB as a surrogate marker for LTBI. Estimates of pooled specificity have been reported as 96% (94 – 98%) for the QFT-GIT and 93% (86 – 100%) for the TSPOT.TB (including its pre-commercial ELispot version). The meta analyses have in the main been based on studies conducted among BCG vaccinated individuals at low risk for LTBI, in low TB incidence countries.

## *Test Utility*

IGRAs have certain advantages over the TST in that they are in vitro tests. This feature reduces the risk of potential adverse effects and eliminates the effect of boosting. These advantages together with superior test qualities make them ideal for serial screening of high risk populations such as health care workers. They have thus been recommended for incorporation into LTBI screening guidelines in both the United States (USA) and the United Kingdom (UK) (Mazurek et al., 2010; National Institute for Health and Clinical Excellence (NICE), 2006; Mazurek et al., 2005). The Centers for Disease Control (CDC) have recommended that QFT may replace TST in the diagnosis of LTBI in certain instances, whilst the UK's National Institute for Clinical Excellence (NICE) has recommended the use of IGRAs as an adjunct to TST for the diagnosis of LTBI.

Preliminary studies evaluating the use of IGRAs in screening programmes in low TB incidence settings have suggested that such a strategy is cost-effective. This on account of superior sensitivity and specificity which may substantially decrease the number of individuals needing further investigation and IPT following screening for LTBI (Nienhaus et al., 2011). Whilst the screening costs associated with IGRA testing may be considerable initially, this is offset by the reduction in post-screening costs through better targeted implementation of IPT.

In the absence of a gold standard for LTBI, comparison of diagnostic performance between TST and IGRAs has relied on the use of surrogate markers for LTBI. These have included the presence of active TB, degree of exposure to infectious cases and to assess specificity, the presence of IGRA negativity in low risk populations (healthy BCG vaccinated individuals with no risk factors for TB disease) (Lalvani & Pareek, 2010). Using degree of exposure to TB as a surrogate for LTBI, both IGRA assays have been shown to agree at least as well with TB exposure as the TST. This has rendered

IGRAs useful for outbreak and contact tracing investigations in low burden settings.

Preliminary research has also suggested that IGRAs may be less affected by energy and therefore more suitable for the screening of immunocompromised individuals, especially those who are HIV-infected and at high risk of progression to TB disease (Lalvani & Pareek, 2010). A recent review of the utility of IGRAs for the diagnosis of LTBI in HIV-infected showed T-SPOT.TB to be less affected by immunosuppression than QFT and TST. However, IGRAs were not consistently more sensitive than TST in studies that were based on direct comparison between IGRAs and TST in the same sample (Cattamanichi et al., 2011).

There is also no consistent evidence that either IGRA is more sensitive than TST for the diagnosis of active tuberculosis. Currently neither the TST nor IGRAs are considered to have value for active tuberculosis diagnosis in adults, especially in the presence of HIV co-infection (Metcalfe et al., 2011; Ling et al., 2011).

#### *Utility in high TB incidence settings*

Whilst very few studies have been performed utilizing IGRAs for LTBI diagnosis in HCWs in high TB burden settings, preliminary evidence from both community and occupational studies have suggested that IGRA performance is different in such settings and that IGRAs may have suboptimal sensitivity for the detection of LTBI (Zwerling et al., 2012; Dheda et al., 2009).

IGRAs have some predictive value for progression to TB disease which have varied according to risk groups (Chee et al., 2013). Reported estimates of progression from latent to active TB disease have ranged from 8% in HIV-infected individuals and silicotics to 12.9% in close contacts of infectious cases (Chee et al., 2013). Whilst very few longitudinal studies have been conducted in high burden settings, a systematic review and meta analyses (inclusive of high burden settings and HCW studies) that evaluated the predictive value of IGRAs for development of TB disease across varied populations did not show IGRAs to have stronger predictive value for TB disease than TST (Rangaka et al., 2012). In contrast, a recent study has shown QFT conversion to be associated with an approximately 8-fold higher risk of progression to tuberculosis disease (than non-converters) in a cohort of adolescents in a high TB incidence setting in South Africa (Machingaidze et al., 2012).

## **Comparison of TST and IGRAs**

### **Concordance studies among health care workers**

Many studies have been conducted in HCWs, predominantly in low TB incidence countries where LTBI testing of high risk populations such as HCWs form an integral part of the TB Control Programme. Head to head comparisons between TST and IGRAs have been done evaluating LTBI prevalence, test performance, association with TB exposure and active TB disease. Cost benefit analyses have also been carried out, leading to some countries adopting IGRAs as part of their diagnostic testing for LTBI. In contrast the evidence base from high incidence countries is very sparse, despite the fact that HCWs from such countries could benefit greatly from a diagnostic test that has greater sensitivity and specificity than TST and is relatively unaffected by immunosuppression and BCG vaccination.

### *Low TB incidence settings*

Studies among HCWs in low burden countries have generally revealed poor agreement between IGRA assays and TST (Swindells et al., 2009). A more recent review by Zwerling et al (2012: 64) have found that out of 25 studies that compared IGRA with TST, all but one showed a lower prevalence of LTBI as measured by a positive IGRA (QFT or T-SPOT.TB) than that by TST. In 17 of these 24 studies a statistically significant difference was found in comparing test positivity. A higher prevalence of BCG vaccination in the study population was not necessarily associated with a higher prevalence of positive TST or a larger difference between the TST and IGRA test positivity. Concordance was generally weak between TST and IGRAs in these studies, with kappa ( $\kappa$ ) values ranging widely from 0.05 (in a study using a 12mm TST cut-off) to 0.56 (in a study using a 15 mm TST cut-off) among BCG-vaccinated individuals. Three studies showed that agreement could be improved by employing a more stringent TST cut-off (i.e. 15 mm rather than 10 mm). The predominant type of discordance in all studies that reported on discordance was that of TST+/IGRA- subjects. The prevalence of positive IGRA was lower than that of positive TST. This difference in prevalence was significant in studies from low and intermediate TB incidence settings.

### *Intermediate and High TB incidence settings*

Only four studies have been performed that evaluated TST and QFT-GIT in a head-to-head comparison among HCWs in high TB incidence settings (Table 2.3)(van Rie et al., 2013; He et al., 2012; Lien et al., 2009; Pai et al., 2005). These have showed fair to substantial agreement and an inconsistent association of BCG vaccination with such agreement. Three of the studies measured agreement between QFT and TST at varying cut-points for TST. Pai et al (2005) showed good agreement at a TST cut-point of 10mm but

poorer agreement when comparing QFT to a lower or higher than 10mm cut-point for TST. A study of Chinese HCWs showed worsening of agreement with increase in TST cut-point with best agreement occurring at a TST level of  $\geq 5$  mm. Among Indian HCWs, factors associated with discordance (TST +/- IGRA-) were increasing age, increasing years in health care and occupational category (attending physicians/ faculty vs. medical students), whilst BCG vaccination had no association with concordance. None of the factors remained significant following multivariate analysis (Pai et al., 2005).

The above finding is in contrast to that in Vietnamese HCWs among whom Lien et al (2009) showed poorer agreement in BCG vaccinated than in non-BCG vaccinated individuals ( $k$  0.29 vs. 0.55). Age, years in health care, type of hospital and BCG vaccination were associated with TST +/- IGRA - discordance, with BCG vaccination remaining a significant predictor of discordance following multivariate analysis(Lien et al., 2009). In a South African study more HCWs tested positive on IGRA than TST (69.2% vs. 56.7) whilst medical students were more likely to test positive on TST than IGRA (26.6% vs. 15.2%)(van Rie et al., 2013).Factors associated with discordance were not reported in this study.

### **Latent Class analysis**

In the absence of a gold standard for LTBI, studies have relied on clinical scenarios where the presence of a specific immune response to *M. Tuberculosis* is likely to serve as surrogate measures for true latent infection. These have included having active TB disease, a history of active TB or being a close contact of an infectious index case (Chee et al., 2013). Although these surrogate measure are not optimal as Tcell responses may frequently be absent, IGRAs have been shown to be well correlated with measures of exposure, with equivalent and in some cases superior sensitivity to TST. For specificity, analyses have focused on the performance of TST and IGRAs to detect an immune response in low risk settings and has

shown IGRAs to have superior specificity especially in BCG vaccinated populations. The use of tests with dichotomous outcomes in the setting where no gold standard is available does, however, affect the accuracy of prevalence estimates generated by such tests as well as measures of test performance such as sensitivity and specificity.

Latent class analysis is a statistical method that has been used to estimate prevalence and diagnostic test sensitivity in the absence of results from a perfect diagnostic test or gold standard. It therefore has particular application in the context where multiple tests may be used to determine disease status in the absence of a true gold standard as in LTBI (Dendukuri, Hadgu & Wang, 2009; Pai et al., 2008). LCA modeling also allows for the consideration of conditional dependency as tests may not be independent of each other and may exhibit a degree of correlation, as between IGRA assays, which may affect estimates. To estimate the sensitivity and specificity of different diagnostic tests, latent class analysis is based on the premise that the results of various imperfect tests for the same condition are influenced by a common underlying latent variable, which represents the true disease status (Christopher et al., 2011; Christopher et al., 2010; Dendukuri, Hadgu & Wang, 2009; Pai et al., 2008). It therefore allows for a more direct comparison of test sensitivity and specificity in a given population. The availability of model-based techniques offers a more realistic approach to prevalence estimation that accounts for the imperfect nature of the test, and allows simultaneous analysis of multiple imperfect dichotomous tests. Pai et al (2008) evaluated LTBI prevalence in Indian HCWs using both mixture and LCA models. They have shown that the application of LCA using both TST and QFT for the diagnosis of LTBI, provided more information on the sensitivity, specificity and predictive value of such tests than when using only one test. The authors suggest that future studies should consider using LCA and at least three diagnostic tests to improve prevalence estimation of LTBI.

## **2.4 SERIAL SCREENING FOR LATENT TB INFECTION IN HEALTH CARE WORKERS**

Serial screening of HCWs has been implemented in low TB incidence countries as part of prevention efforts to allow for targeted treatment of LTBI and prevention of progression to active TB in HCWs. The superior specificity of IGRAs together with the absence of a boosting effect make them ideal tests to be employed in serial testing programmes for HCWs. Some countries have incorporated IGRAs into surveillance programmes that employ serial testing for LTBI.

Estimates of the median annual incidence of LTBI based on TST conversion in HCWs in countries with low, intermediate and high TB incidence have been reported as 3.8%, 6.9% and 8.4% respectively (Baussano et al., 2011). When compared with the general population this translates into incident rate ratios (IRRs) of 2.4, 2.4 and 3.7 respectively and is indicative of the higher risk of LTBI infection among HCWs, which rises in tandem with background TB prevalence.

The evidence base for utilization of IGRAs in serial testing is growing, but studies have yielded varying results. A recent review presents results of eight studies from low and intermediate TB incidence countries that have reported IGRA conversion rates among HCWs (Zwerling et al., 2012). Estimates of conversion have ranged from 1.8% to 14.4% with most studies showing higher rates of IGRA conversion than TST conversion. IGRA conversion was not shown to be consistent with TB exposure in all studies. One study showed an association between male gender and older age with IGRA conversion.

On the other hand, reversion rates from both low and intermediate TB incidence countries reported in three studies have ranged from 40% to 53%, with baseline values closest to the cut-point more likely to revert to a negative IGRA test at follow-up. These findings were replicated in subsequent studies of German and Portuguese HCWs with the highest conversion and reversion rates produced when using conventional dichotomous definitions of positive and negative (Ringshausen et al., 2010; Torres Costa et al., 2011; Torres Costa et al., 2009). More recent data from HCW screening programmes have highlighted areas of uncertainty relating to the interpretation of sequential IGRA assays results and the influence on LTBI treatment programmes (Slater et al., 2013; Zwerling et al., 2013a; Fong et al., 2012; Loddenkemper, Diel & Nienhaus, 2012; Zwerling et al., 2012; Dorman et al., 2014). These latest data suggest that sequential IGRA results display considerably variability, resulting in high rates of conversion and reversion, which to some extent is influenced by type of test used and cut-off definition employed to denote a positive test. This raises concerns about whether the current cut-points are sensitive enough to accurately identify new infection and provide an accurate picture of LTBI rates in high risk populations.

Only two studies from high incidence settings have evaluated IGRA conversion in HCWs in high TB incidence settings (Zwerling et al., 2012). The rates of conversion have ranged from 11.6% to 21% and have varied depending on which cut-point was used. Reversion rates were even higher and have been reported as ranging from 18% to 40%. Neither study reported data to show that IGRA conversion was more strongly associated with TB exposure than TST conversion. Given the limited research and the evidence emerging from low incidence settings, the World Health Organization (WHO) has in the interim issued a report [by the Strategic and Technical Advisory Group for Tuberculosis (STAG-TB)] that discourages the use of IGRAs for the screening of LTBI among high risk groups such as HCWs in LMICs (World Health Organization, 2010).

## 2.5 RESEARCH GAPS AND AREAS OF UNCERTAINTY

Despite the increased utilization of IGRAs for baseline and serial screening of HCWs in high income countries and low TB incidence settings, many areas of uncertainty remain (Loddenkemper, Diel, & Nienhaus, 2012). These have in the main been related to sensitivity of IGRAs in certain high risk groups, limited prognostic power for identification of those at highest risk of progression to active TB, limited utility in active TB and unexplained high rates of reversion and conversion (Whitworth et al., 2013).

Whilst IGRAs are considered to have equivalent sensitivity to TST, the sensitivity of the assays appears to be reduced in key subgroups such as children and HIV-infected individuals, leading to an increased rate of false negatives (Moyo et al., 2011; Cattamanchi et al., 2011; Metcalfe et al., 2011). This has implications for the screening of HCWs with high background prevalence of LTBI and HIV working in high TB prevalence settings as such individuals are at high risk of progression from LTBI to active disease. *Studies conducted among HCWs in high TB incidence settings have not reported on HIV status or evaluated the potential influence of HIV status on prevalence estimates.*

In low incidence settings IGRAs generate lower prevalence estimates for LTBI than TST and are better correlated with TB exposure, resulting in lower numbers needed to treat (NNT) to prevent progression to active TB. But the prognostic power of IGRAs is still not optimal as IGRAs are not able to distinguish those with recent LTBI and therefore at highest risk of progression, from those with more remote exposure to TB. Among HCWs in high TB incidence settings, TB transmission is likely to be ongoing given the high background rate of TB and the poor implementation of control measures in health care facilities. *It is unclear how IGRA performance is affected in such settings, and longitudinal studies are needed to evaluate LTBI incidence and factors associated with acquisition of recent infection.*

The use of IGRAs in serial screening have also raised concerns about the interpretation of sequential test results arising from such screening and whether observed changes are due to innate variability of these assays or represent true conversion and therefore new infection(Zwerling et al., 2013a; Fong et al., 2012; Zwerling et al., 2012). Based on findings of some of these more recent large studies which are based on more mature serial screening programmes, IGRAs appear to have considerable variability which negatively affects their short-term reproducibility (Slater et al., 2013; Joshi, Monson & Woods, 2012; Nienhaus et al., 2013). The exact cause of the high rates of conversion and reversion has not been identified. These latest findings have resulted in a more cautious approach to the use of IGRAs in serial screening of high risk groups in low-incidence settings such as the USA and Canada(Canadian Tuberculosis Committee, 2010; Mazurek et al., 2010).

The evidence base from high TB incidence settings for the use of IGRAs in high risk populations such as HCWs remains sparse. Only three studies have been conducted among HCWs evaluating a head to head comparison of both TST and IGRAs or the utility of IGRAs in serial testing of HCWs(Zwerling et al., 2013b; Zwerling et al., 2012).Evidence from high incidence settings have shown that HCWs are at increased risk of LTBI using the TST. It is not known whether IGRAs are really more sensitive than TST at detecting LTBI in HCWs living in countries characterized by high background TB and HIV prevalence. *The role and contribution of occupational TB exposure, as opposed to community exposure, to LTBI prevalence and incidence have also not been elucidated.* This is despite it being known that infection control measures and practices are poorly implemented in health care facilities in countries such as South Africa, and despite the high TB burden and the emergence of extremely drug-resistant TB(Farley et al., 2012; Naidoo, Seevnarain & Nordstrom, 2012).Furthermore, to arrive at a true comparison of TST and IGRAs with respect to specificity, factors associated with discordance, specifically TST positive IGRA negative discordance, require closer examination.

Studies that have evaluated serial testing using IGRAs in high incidence settings, have shown considerable variability in IGRA test results. This is reflected in high rates of conversion and reversion, with no study showing IGRA conversion to be better correlated with TB exposure than TST conversion. This raises questions about the test stability in such settings and the interpretation of sequential IGRA tests. Concurrent administration of TST with IGRA at baseline may also result in boosting of subsequent IGRA test response, further confounding serial test interpretation. There is emerging agreement on the need for the revision of IGRA cut-points to account for such variability and to accurately denote true conversion. The use of alternative definitions of conversion has been proposed (Zwerling et al., 2012). Such definitions include “an absolute increase from the baseline result (as with TST), a proportional increase from baseline or a grey zone” to account for innate test variability (Zwerling, 2012: 68).

Despite numerous systematic reviews which have evaluated IGRA performance, data from high TB incidence countries which have been included in such reviews have been limited in informing clear recommendations (Cattamanchi et al., 2011; Metcalfe et al., 2011). Specifically, the utility of IGRAs in HCW screening in such settings is uncertain (Zwerling et al., 2012). To answer some of these questions studies of HCWs from high TB incidence settings using a longitudinal study design are needed. Such studies should evaluate prevalence estimates, compare test agreement and performance between IGRAs and TST, as well as provide estimates of LTBI incidence and factors associated with recent infection. Quantification of test conversion and reversions should also be evaluated to inform the need for improved estimations of LTBI incidence and revised criteria for what truly constitutes new infection. This might well be different for HCWs in high TB incidence settings from those working in low TB incidence settings. This thesis aims to answer some of these questions.

**Table 2.3 LTBI prevalence and test agreement among health care workers in countries with intermediate or high TB incidence using TST and IGRA assays**

Study author (year)	N	LTBI Prevalence		Test agreement	Kappa (95% CI)
Country		TST*	IGRA*		
Pai (2005) India	719	41%	40%	81%	0.61 (0.56 – 0.67)
Mirtskhulava (2007) Georgia	265	77%	60%	74%	0.43 (0.33 – 0.55)
Lien (2009) Vietnam	255	66%	47%	73%	0.44 (0.43 - .45)
He (2012) China	999	54%	68%	67%	0.19 (0.13 – 0.25)
Zhang (2013) ** China	96	55%	29%	59%	0.18 (0.01 – 0.52)
Van Rie (2014) South Africa	199	45%	48%	68%	0.37

CI: confidence interval. \* Cut- points used were 10mm for TST, cut-point  $\geq 0.35$  IU/ml for QFT-GIT and  $\geq 6$  spot forming units for TSPOT.TB.

\*\*TST compared to QFT-GIT in all studies except Zhang (2013) where the comparison is with TSPOT.TB.

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## **CHAPTER 3**

### **METHODS**

## **CHAPTER 3**

### **3.1 STUDY SETTING AND PARTICIPANTS**

#### **3.1.1 Study design and setting**

This was a longitudinal study conducted among health care workers employed at seven health care facilities in the greater Cape Town area, South Africa. The selection of facilities was made following consultations with health service managers and the researchers. Factors taken into consideration were that such facilities should serve communities with a high TB and HIV prevalence, that there should be an interest in and need for such research and that research staff would receive support and co-operation from management and staff at the facilities. Facilities chosen as study sites were three clinics and two community health centres classified as primary level care facilities, and two TB hospitals, classified as secondary level facilities.

The five primary care facilities identified as study sites were the Delft community health centre, Nolungile Community health centre, Nyanga clinic, Kuyasa clinic, and Site B clinic. Nolungile, Kuyasa and Site B were located in Khayelitsha, an area with one of the highest TB and HIV prevalence in the Western Cape (Garone et al. 2001). The two secondary level facilities were the Brooklyn Chest and D.P. Marais TB hospitals which function as referral centres for clinically complicated or drug resistant TB cases referred from primary level facilities.

There were marked differences in the patient profile and scope of services delivered at these two types of facilities. The primary health care facilities in the sample were community based health centres which offered a range of health services including diagnostic and treatment services for tuberculosis

sufferers and primary health care services which were both preventive and curative in nature. These facilities relied on staff supplied by non-governmental organizations for the successful implementation of important public health programmes such as the national tuberculosis programme and utilized large numbers of community health care workers to support such programmes. Facilities were based within the communities allowing for quick access to primary health care and a high load of undiagnosed patients entering through the doors, this in many cases representing their first contact with a health service.

In contrast the two TB hospitals included in the study were both secondary level facilities aimed primarily at providing long-term or in-hospital curative care for patients with complicated or drug-resistant tuberculosis. They functioned as referral centres and were located at some distance away from the communities that they served. Patients were admitted as in-patients after being referred from primary care centres. The facilities therefore offered a level of specialized care not available at the primary level. Patients served were those with confirmed TB, with possible complications and or drug resistant TB and who would benefit from specialist in-hospital and long-term care.

A study protocol was developed and approval obtained from the University of Cape Town's Faculty of Health Sciences Research Ethics Committee for conducting this study in November 2008. The baseline phase of the study commenced in May 2009 and was concluded in June 2010. Recruitment at the seven facilities took place sequentially over a period of one year using a trained nurse interviewer to perform the data collection, administer tuberculin skin test (TST) and draw bloods for the processing of study assays. Data collection for the follow-up phase of the study immediately followed the baseline phase and took place from July 2010 to June 2011, allowing for a one year interval between test responses as per the study protocol. The

study was commenced at the primary level facilities and concluded at the secondary level facilities for both phases.

Prior to starting with recruitment, presentations were done to staff outlining the objectives of the study, clarifying questions from staff and inviting them to participate in the study. Leaflets were distributed among staff outlining the objectives and benefits of the study and posters were put up in the facility, providing information on the study.

### **3.1.2 Participants**

For the purposes of the study a broad definition of health care worker was employed to include all those engaged in the support and delivery of health services at a selected health facility. This is in line with the approach adopted by the World Health Organization that proposes the inclusion of all those involved in the provision of health services, as well as management and support workers, in such a definition. It furthermore includes subsectors of workers engaged in community-based and home-based care (WHO, UNAIDS & ILO, 2010; Oxlade et al., 2009). Participation in the study was left up to individual choice following group presentations and information sessions and occurred during work-time only.

The study sample therefore included both support staff (administrative, security staff and community health care workers) and clinical staff (interns, researchers, nurses and doctors). Presentations were made to staff at all facilities informing them about the study and inviting their participation. Exclusion criteria were stipulated as pregnancy, allergy to tuberculin and age younger than eighteen years. A formal sample size calculation was made based on the expected estimates of LTBI in the population. It was estimated that a sample size of 300-400 HCWs would produce fairly precise estimates, i.e. within 5% of the expected estimate for LTBI positivity detected by either

TST or IGRA. Sample sizes were calculated using Stata and was based on a range of LTBI prevalence (30% - 60%) as the exact population prevalence was unknown. It was assumed that TST positivity would be approximately 60% and IFN- $\gamma$  positivity approximately 40% in the study sample. This was in keeping with data from a recent review of LTBI prevalence in HCWs in low- and middle-income countries (LMICs) which reported an LTBI prevalence of 33-79% (median prevalence 54%) detected by TST (Joshi et al., 2006).

**Table 3.1 Possible prevalence of LTBI, desired precision and sample size estimation**

<b>Prevalence of LTBI</b>	<b>Desired precision</b>	<b>Sample size (n)</b>
<b>TST or IGRA positivity</b>		
60%	54% – 66%	300
60%	55% – 65%	400
50%	44% – 55%	300
50%	45% – 55%	400
40%	35% – 45%	400
40%	34% – 46%	300
30%	25% – 36%	300
30%	26% – 35%	400

## **3.2 BASELINE DATA COLLECTION INSTRUMENTS AND INVESTIGATIONS**

### **3.2.1 Questionnaire**

Each participant was required to answer a standard questionnaire eliciting data on age, sex, home language, current medical conditions, HIV testing, place of employment, duration of employment, job category, smoking,

exposure to secondary smoke, alcohol use and HIV status(appendix 1). A section of the questionnaire focused on specific occupational tasks (e.g. interviewing, examining, or performing sputum collection from TB patients), awareness of the presence of environmental control measures (facility implementation of ventilation measures, provision of N95 masks)and the practice of administrative controls (infection control policy, early triage, separation of TB patients) to minimize TB risk in the facilities of employment. Questions on health workers' perception of their risk of infection were also included.

Previous studies in clinical settings have described the use of symptom scores as a diagnostic tool to distinguish subjects with and without TB (den Boon et al., 2006). However, these are not always applicable to survey settings as the prevalence of TB and symptoms is higher in clinical settings. This questionnaire followed a method used previously in a survey setting to characterize TB symptoms. Questions focused on the presence and duration of the cardinal TB symptoms of cough, haemoptysis, weight loss, night sweats, chest pain and fever. Questions on factors which could potentially confound LTBI test response such as BCG vaccination and a history of previous TB were included. Parental employment status during childhood was used as a proxy variable for socio-economic status and a detailed section on smoking history and alcohol consumption was included.

To evaluate the risk of exposure to tuberculosis infection, an exposure index was developed using exposure indicators demonstrated in one study to be a reasonable accurate reflection of such exposure and potentially associated with tuberculous infection and disease.(Menzies, Joshi &Pai, 2007; Joshi et al., 2006).Exposure was evaluated both in the community and occupational context. Community contact questions that were included as part of the exposure index related to the infectivity of the index / contact case (ranging from unknown sputum result to sputum positive contact),proximity of living arrangements to the contact (e.g. ranging from no contact to sleeping in same room) and duration of contact (ranging from nil to  $\geq 12$  hours). For occupational exposure, the index incorporated the type of service provision

to patients as a proxy measure for potential contact with infectious cases (ranging from non-clinical to clinical services provision or a combination thereof), proximity to TB patients in the facility (no direct exposure to direct exposure in the same room) and the average duration of contact per day with TB patients (ranging from 0 – 3 hours to  $\geq 12$  hours). Maximum scores of 11 and 8, were assigned to community and occupational exposure indices respectively. Participant scores were treated as a numeric variable to evaluate the relationship with LTBI test outcomes. Questions were also posed to determine whether participants had been provided with health and safety training on TB infection control.

The questionnaire was developed in English and translated into Afrikaans and Xhosa, two local languages spoken in the Western Cape. Back translation was done to assess the validity and reproducibility of the content. A trained interviewer administered the questionnaire in a standardized manner. As far as possible subjects were interviewed in their language of choice.

### **3.2.2 Chest radiograph**

All participants were required to undergo a postero-anterior digital chest radiograph to exclude active TB. The chest radiograph was read by an occupational health physician and pulmonologist, two experienced clinicians trained in the Chest Radiograph Review System (CRRS); a radiological review system specifically aimed at developing competency in the radiological diagnosis of TB (Pinto et al., 2013). The two readers classified the films as normal, inactive TB, active TB or other pathology (not TB related). The two readers did not read strictly according to the CRRS format, relying on prior training and clinical experience to classify radiographs. Where disagreement occurred between the two readers, an expert radiologist was requested to read the film to arrive at a final classification. This final classification was recorded as the chest radiograph result.

### **3.2.3 HIV test**

All participants were requested to have an HIV test which was a rapid enzyme-linked immunosorbent assay (ELISA) test which was performed in the field in accordance with the manufacturer's instructions. A positive result was followed up with a confirmatory test using a rapid ELISA test from a different manufacturer as per the study protocol and current department of health screening practice. The first-line test used in this study was the Abbot's Determine ® HIV-1/2 test (Direct relief international, 2014), whilst the second line test was the SD Biotec (Standard diagnostics incorporated, 2013). This procedure was in accordance with current testing practice employed in large HIV testing programmes of the City of Cape Town's health department as well as WHO guidelines (World Health Organization, 2004).

Participants were allowed to report their HIV test result if previously tested, without undergoing the test in this study. All participants were provided with pre-and post-test counselling using a strategy that incorporated the advice, consent, test and support (ACTS) approach. This methodology has been adopted by the health department's HIV counselling and testing (HCT) programmes. Both the nurse interviewer and principal investigator underwent training in ACTS prior to starting the data collection. Refusal to disclose a previous HIV result or undergo a test as part of this study did not compromise their eligibility for inclusion or continued participation in the study.

### **3.2.4 Tuberculin skin test**

All participants were required to undergo the tuberculin skin test. The one step TST protocol was employed to test health care workers at baseline. Two tuberculin units (0.1ml) of RT23 PPD (Statens Serum Institute, Copenhagen), were injected intra-dermally on the volar aspect of the forearm. The

induration was then measured by a trained reader after 48 – 72 hours using the ballpoint and ruler method (Moran-Mendoza et al., 2013). An induration of  $\geq 10$  mm was considered a positive test at baseline. In the case of an HIV positive individual an induration of  $\geq 5$  mm was considered a positive test at baseline.

### **3.2.5 Interferon-gamma release assays**

Venesection was performed by a trained nurse to obtain blood samples for the interferon-gamma assays (IGRAs). All bloods were taken at the same occasion when TST was done or within 3 days of TST administration, to eliminate the effect of boosting (van Zyl-Smit et al., 2009a; van Zyl-Smit et al., 2009b). Blood samples were transported to the laboratory on the same day and within a few hours of it being collected for processing. Two IGRA assays were used to evaluate LTBI test response viz. the QuantiFERON-TB Gold In-Tube (QFT-GIT) and the T-SPOT.TB.

#### *QuantiFERON-TB Gold In-Tube*

All health care workers were tested at baseline with the QFT-GIT test (Cellestis Ltd, Carnegie, Australia). Blood samples were drawn directly into three blood tubes supplied by the manufacturer that contained the following *M. tuberculosis* antigens: early secretory antigen-6 (ESAT-6), culture filtrate protein-10 (CFP-10) and TB7.7 combined, the nil control or the positive control mitogen (PHA) (Whitworth et al., 2013). Whole blood was incubated at 37°C overnight (16-24 hours). Incubation with antigens allowed for the activation of antigen-specific effector T-cells and subsequent interferon-gamma (IFN- $\gamma$ ) release into the extra-cellular fluid. Following incubation, the blood tubes were centrifuged to separate the plasma (supernatant) containing the secreted IFN- $\gamma$  and samples could then be stored at 4°C for up to 28 days (or at -20°C for longer periods) prior to assay output.

development. The final step involved the measurement of IFN-  $\gamma$  release using an enzyme-linked immunosorbent assay (ELISA). In the QFT-GIT ELISA, development of a yellow colour indicated that IFN-was released into the plasma TB-specific T-cells. It was expected for colour to develop in the positive control as well but not (or very minimally) in the negative control well. Colour in the TB antigen well was considered indicative of infection.

Optical density was measured using a micro plate reader and results were calculated using specialized analysis software supplied by the manufacturer. A series of IFN-standard concentrations were run alongside the samples in order to quantify IFN-levels. QFT- GIT was considered positive if the if the interferon-gamma response minus the nil antigen was  $\geq 0.35$  IU/ml.

#### *T-SPOT.TB*

A second IGRA assay the T-SPOT.TB assay (Oxford Immunotec, Abingdon, UK) was administered at baseline. The test was performed in accordance with the manufacturer's instructions and blood was drawn at the same time as for the QFT-GIT. Venous blood was drawn into cell preparation tube (CPT) vacutainers (Becton Dickinson, Oxford, UK) and was transported within 6hours to the laboratory. In brief peripheral blood mononuclear cells (PBMCs) were isolated from heparinized whole blood, before culturing PMBCs at 37°C for 18 ( $\pm$  2 hours) with Mtb antigens. (Whitworth et al., 2013)Incubation takes place in four wells of a 96 well plate, precoated with IFN- $\gamma$  –specific monoclonal antibodies. PBMCs at a concentration of 250,000 cells/well in AIM V® cell culture medium (Invirogen Corporation, Carlsbad, USA) are exposed to ESAT-6 and CFP-10 individually and to positive phytohemagglutinin (PHA) and negative (culture media controls).Overnight incubation of the cells takes place at a temperature of 37°C in 5% CO<sub>2</sub>. This results in activation and release of IFN- $\gamma$  from antigen specific effector T cells that are present in the culture. This IFN- $\gamma$  will be captured by monoclonal antibodies present on the membrane of the 96-well plate.

Immediately following culture, cells and antigens are washed off with phosphate buffered solution (PBS) and conjugate (an alkaline phosphatase-conjugated secondary IFN- $\gamma$  specific monoclonal antibody). Addition of an alkaline phosphatase chromogen substrate results in the formation of a visual spot where alkaline phosphatase is present. Each spot represents the release of IFN- $\gamma$  by a single T-cell responding to specific antigenic stimulation. The result for the TSPOT.TB assay is expressed in number of spot-forming cells (SFCs) per well. It is expected that SFCs be detected in the positive control and be absent from the negative well. SFCs detected in either or both of the antigen containing wells is indicative of tuberculosis infection. Enumeration of the SFCs in the TSPOT.TB may be done immediately or at another date. An ELIspot reader with appropriate software supplied by Oxford Immunotec is used to generate counts. However in low-resource settings, a microscope or magnifying glass may be used. In this study a cut-off of six or more spots was treated as a positive result. To minimize inter-operator and inter-laboratory variability, all assays were done by the same operator in the same laboratory.

Samples were transported on the same day to the laboratory usually within a few hours of the blood samples being drawn. Stimulation happened in the test tube which was coated with reagent and then incubated overnight. Tests were batched for analysis which happened at regular intervals every few days. An Elispot reader was used to analyse the result for T-SPOT.TB.

In this study, the use of cut-points for test positivity was based on the manufacturer's instructions and software provided for assay analysis at the time that the study was conducted. For T-SPOT.TB this represented a 6 spot increment. Later recommendations, based on emerging evidence from studies performed, advised that a 5-7 spot increments be treated as a grey zone, and that an 8 spot or greater increment be considered a positive test. Whilst the original criterion of a 6 spot increment was used in our study, the

revised cut point was taken into consideration in the multivariate analysis. Current evidence suggests that further revision of cut points is required which may be considerably higher than those currently recommended (Slater et al., 2013).

The table below outlines the differences in methodology and processing of the two IGRA assays and is adapted from an article by Whitworth et al(2013).For further information links are provided to the relevant websites for both QFT-GIT and TSPOT.TB in the reference list(Oxford Immunotec, 2013; Qiagen, 2011).

**Table 3.2 Comparison of commercially available IGRAs for the diagnosis of TB**

	<b>T-SPOT.TB</b>	<b>QuantiFERON Gold in-tube</b>
Sample collection	1 x tube heparinized blood Minimum blood volume=5ml	3 x QFT blood tubes containing nil control. Mtb antigens (combined) or mitogen control  1 ml blood per tube  Total blood volume = 3 ml
Pre-incubation sample preparation	PBMC isolation and addition of PMBCs to T-SPOT.TB 96 well plate (250 000/well) containing negative control Panel A and B Mtb antigens (individually) or positive control  <b>Mtb antigens: ESAT-6, CFP-10</b>	Inversion of tubes to ensure mixing of antigens/ controls with blood  <b>Mtb antigens: ESAT-6, CFP-10, TB 7.7</b>
Incubation	16-20 hour incubation in TSPOT.TB in 96 well plate (37°, 5% CO <sub>2</sub> )	16 – 24 hour incubation in QFT blood tubes (37°C)
Post-incubation sample preparation	Removal of cells and antigens / controls by washing	Centrifugation of QFT blood tubes for separation of plasma
Assay preparation	ELIspot for enumeration of IFN- $\gamma$ producing cells  Result determination using ELIspot reader	ELISA for quantification of plasma IFN- $\gamma$ levels  Result determination using ELISA reader

Adapted from Whitworth et al, 2013.

### **3.2.6 Sputum microscopy and culture**

Participants who tested positive on symptom screen or had a chest x-ray suggestive of active TB were investigated further using sputum microscopy and culture, to exclude active TB. Two sputum samples were collected on separate days for TB investigation and culture was performed on both samples. Investigation of sputum was done at the National Health Laboratory Service based at Groote Schuur hospital. Microscopy was done using Fluorescent techniques and auramine staining. The results were reported within 48hours as number of acid fast bacilli (AFBs) seen on smear per high power field. All TB sputum samples were further investigated by means of culture using a liquid medium. The Mycobacterial Growth Inhibitor Tube (MGIT) 960 system was used which detects bacterial growth using a fluorescence- quenching based oxygen sensor within each tube. As mycobacteria multiply within the tube, oxygen is consumed and fluorescence is detected by the system. A positive culture may be due to growth of TB but also bacteria, fungi and non-tuberculous mycobacteria. Smear microscopy requires approximately 10 000 TB bacilli per ml of sputum to be detected/positive. Culture can be positive with only 10 - 100 TB bacilli per ml of sputum. For a tube that flagged positive (culture positive result), the growth in the tube was stained and examined microscopically to detect the presence of mycobacteria and tested for the presence of TB using molecular probe-based assays (PCR) to rapidly confirm the presence of MTB complex.

## **3.3 FOLLOW-UP DATA COLLECTION INSTRUMENTS AND INVESTIGATIONS**

### **3.3.1 Questionnaire**

At follow-up one year later a questionnaire was again administered inclusive of a symptom screen (appendix 2). Additional questions related to smoking

status, HIV status and employment characteristics were included. Questions were also included to assess compliance with clinical referral at baseline and whether participants had found it easy to access clinical care. In addition, responses were elicited on their preferred model for the delivery of occupational health services to HCWs.

### **3.3.2 Chest radiograph**

Participants were requested to undergo a routine chest radiograph at the same facility using the same digital technology. The same two readers who evaluated the films at baseline, reviewed the follow-up films. Where necessary the previous films were consulted to verify whether interval radiographic change had occurred. These were also made available to the chest radiologist to resolve disagreement between the two readers where this occurred.

### **3.3.3 HIV test**

As previously, all participants were requested to have an HIV rapid test (ELISA) which was performed in the field in accordance with the manufacturer's instructions. A positive result was followed up with a confirmatory test using a rapid ELISA test from a different manufacturer as per the study protocol and current department of health screening practice. Participants were allowed to report their HIV test result if previously tested, without undergoing the test in this study. All participants were provided with pre-and post-test counselling using a strategy that incorporated the ACTS approach. Refusal to disclose a previous HIV result or undergo a test as part of this study did not compromise their eligibility for inclusion or continued participation in the study.

### **3.3.4 Tuberculin skin test**

The tuberculin test was repeated using the one step TST protocol on all participants, irrespective of their baseline result. Two tuberculin units (0.1ml) of RT23 PPD (Statens Serum Institute, Copenhagen), was injected intradermally on the volar aspect of the forearm. The induration was then measured by a trained reader after 48 – 72 hours. An induration of  $\geq 10$ mm was considered a positive test at baseline. In the case of an HIV positive individual an induration of  $\geq 5$ mm was considered a positive test at baseline. Refusal to undergo a repeat TST test did not disqualify participants from continuing in the study.

### **3.3.5 Interferon-gamma release assays**

Both IGRAs were repeated using the same methodology described earlier. All participants were required to undergo repeat testing irrespective of their test result at baseline.

### **3.2.6 Sputum microscopy and culture**

Participants who tested positive on symptom screen or had a chest x-ray suggestive of active TB were investigated further using sputum microscopy and culture, to exclude active TB. Two sputum samples were collected on separate days for TB investigation and culture was performed on both samples.

### **3.4 STATISTICAL ANALYSIS**

#### **Prevalence of LTBI and associated factors**

Statistical analyses were performed using Stata version 11 (Stata Corp, College Station, Texas). The outcomes evaluated included test positivity for TST, QFT-GIT and TSPOT.TB. The prevalence of TB associated risk factors and test outcomes were calculated. Stratified analysis was carried out to differentiate between staff from primary and secondary facilities following preliminary analysis which showed important demographic differences between the two groups. Results were separately summarized.

Risk factors for test positivity were evaluated using odds ratios (ORs) with 95% confidence intervals (95% CIs) presented for all estimates. Univariate logistic regression analysis was performed to evaluate the relationship between risk factors for TB infection and all three test outcomes. Individual multivariate regression models were then run for all variables of interest in relation to test outcomes, adjusting for covariates such as age and gender. Adjusting the analysis for multiple testing was not done. Multivariate models were also applied to the strata investigating determinants separately for staff employed at primary and secondary level facilities. Confidence intervals for all point estimates were calculated based on the following formula: point estimate  $\pm$  1.96 x standard error of the mean (SEM)

Logistic regression was used to develop models that best predicted test outcomes for each of the three tests. Through a process of model building, a model was derived which best predicted a positive test outcome for each of TST and the two IGRA assays individually. The best predictive model was determined by examination of the log likelihoods, the log-likelihood ratio tests, associated p-values and the Akaike's information Criterion (AIC) generated by successive models, with the final model chosen as

representing that with the lowest AIC. The strength and precision of the associations were summarized using odds ratios, confidence intervals and p values.

### **Agreement analysis and latent class modelling**

At baseline, agreement analysis was conducted to evaluate concordance between test outcomes and factors associated with discordance were examined. The kappa statistic ( $\kappa$ ) was used to quantify agreement between tests and 95% confidence intervals were calculated for each kappa value. The kappa statistic measure of agreement is scaled to be 0 when the amount of agreement is equal to what would be expected to be observed by chance and 1 when there is perfect agreement. Kappa categories of agreement according to Landis and Koch are therefore: poor if below 0; slight if  $\leq 0.20$ ; fair if 0.21 – 0.40; moderate if equal to 0.41-0.60, substantial if 0.61 -0.80 and almost perfect if 0.81-1.00(Munoz & Bangdiwala, 1997). In view of the variability observed in studies with serial administration of IGRAs and the potential impact of boosting of IGRA test response by TST, agreement analyses using higher cut-offs for test positivity were also performed on baseline results. Agreement analyses were also carried out on the test outcomes generated from repeat testing of the cohort one year later. This agreement was also calculated on absolute values using standard cut-offs for test positivity and did not take into consideration the baseline test results.

Factors associated with discordance were explored using a multinomial logistic regression model that compared discordant groups with a comparison group displaying perfect agreement between test outcomes. Discordant groups for both IGRA assays were defined as having a positive TST/negative IGRA or a negative TST/ positive IGRA.

To estimate the sensitivity and specificity of different diagnostic tests, latent class analysis (LCA) was used. This is a statistical modelling technique that is based on the premise that the results of various imperfect tests for the same condition are influenced by a common underlying latent variable which represents the true disease status(Christopher et al., 2010; Dendukuri, Hadgu & Wang, 2009; Pai et al., 2008). A pre-condition for a meaningful LCA model (i.e. for the model to be identifiable) is that the number of diagnostic tests used on the study sample must provide at least as many degrees of freedom as the number of parameters to be estimated. If not then a Bayesian estimation approach can be used to place reasonable bounds on some parameters about which prior information is available. This would allow for the estimation of the remaining parameters conditional on this prior information.

For this study design that involved three dichotomous tests, the problem is identifiable if it is assumed that the results of individual diagnostic tests for a given disease status are independent (conditional independence) and that observed associations between tests are due solely to the latent variable. In this study this condition could not be met as there were similarities in the technological properties and immunological mechanism underlying the two IGRA assays, which could potentially result in correlation between test results (conditional dependence). An alternative model was therefore also considered that allowed for conditional dependence between QFT and TSPOT given subjects' truly positive, conditional dependence between QFT and TSPOT given subjects truly negative. The conditional dependence is assumed to be positive in both groups.

In the analysis, a non-informative prior is used for the prevalence which is unknown. Prior information on the accuracies of the three tests was elicited from Pai et al (2008) using data on the sensitivity and specificity of TST and QFT-GIT based on systematic reviews and meta-analyses. The prior 95% CI of each parameter is constructed in such a way that the prior 95% CI covers

all the 95% CIs from individual studies included in the meta-analysis. Therefore it is equivalent to the prediction interval resulting from the meta-analysis. Non-informative priors were used for the conditional dependence terms and the method introduced by Dendukuri and Joseph (2001) was used to construct the priors. The prior set is represented in Table 5.1.

In applying a latent class model, subjects with both determinate and indeterminate results were included, as participants with indeterminate results in one test may still have relevance in the analysis on account of determinate results in the remaining two tests. A fixed effects model allowing for conditional dependence between QFT-GIT and TSPOT.TB was used to fit the data (Dendukuri & Joseph, 2001). WinBUGS software was used to analyse the data. Twenty thousand samples were drawn from the posterior distribution after discarding a burn-in of 1000 iterations. Convergence of the Monte Carlo Markov chain was assessed using the Gelman-Rubin statistic which is named BGR within WinBUGS.

### **Test conversion and factors associated with incident TB infection**

During the follow-up phase of the study the main outcomes of interest were test positivity at follow-up and the annual rate of TST and IGRA conversion which was used to define annual incidence of infection. The prevalence of TB associated risk factors, occupational and environmental factors and test outcomes was calculated at baseline for this cohort. Differences between participants included in the follow up and those lost to follow-up were explored using Chi-squared statistics, to evaluate the likely operation of attrition bias. Further analysis focused on identifying predictors of TST and IGRA conversion. Univariate and multivariate logistic regression analysis to explore the strength of associations with conversion were performed. An annual incidence of infection on the different tests was calculated.

### **3.5 ETHICS**

The study was approved by the research Ethics Committee of the University of Cape Town's Health Science Faculty. All participants gave written informed consent for participation in the study at recruitment (appendix 3) prior to commencement of the survey and during participation in the follow-up phase of the study (appendix 4).

Prior to giving consent a verbal explanation was provided to participants of the study purpose and a description of the study requirements. A written copy of this document which formed part of the consent document (appendices 3 and 4) and which included the contact details of the researcher was provided to participants. Issues of confidentiality and autonomy were also addressed in this document.

An undertaking was given to communicate all results to participants in a confidential personal report at baseline (appendix 5) and at follow-up (appendix 6). The only results not reported to participants were those for the IGRA assays which were being administered as research assays, as their utility in high burden settings was unknown. All results were immediately available once laboratory tests were processed, except in the case of those needing further investigations for active TB.

In accordance with the prevailing standard of care and clinical practice, all HIV infected individuals with a positive TST and in whom active TB was excluded, were referred for Isoniazid (INH) prophylaxis, by means of a formal referral letter completed by the study's principal investigator at both baseline and follow-up (Appendix 7).

Those individuals diagnosed with active TB during the course of the survey were referred for TB treatment to a facility of their choice. They were also reported to the Compensation Commissioner, Department of Labour, as cases of occupational TB in accordance with the Compensation for Occupational Injuries and Diseases Act of 1993. Participants were consulted during all such process and due consideration for confidentiality was upheld.

HIV-infected individuals, who worked in high risk settings e.g. TB clinics, were also recommended for redeployment to a lower risk setting, with their consent. This was achieved by means of a formal discussion between the facility manager and the principal investigator.

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## **CHAPTER 4**

### **PREVALENCE AND DETERMINANTS OF LATENT TUBERCULOSIS INFECTION IN HEALTH CARE WORKERS**

## CHAPTER 4

### 4.1 INTRODUCTION AND OBJECTIVES

It is well established that health care workers are at increased risk of contracting TB infection and disease due to occupational exposure (Baussano et al., 2011; Menzies, Joshi & Pai, 2007). In high TB and HIV settings, such as South Africa, this risk has not been well studied. This is on account of scarce surveillance data and limited occupational health service provision to this high risk population (Adams et al., 2012; Zungu & Malotle, 2011). The implementation of infection control strategies have been shown to have a positive impact on TB risk in health care workers in low TB incidence settings (Centers for Disease Control and Prevention, 2005). A recent study, however, that was conducted in South African primary health care facilities has not shown a protective association between infection control practices and TB incidence in healthcare workers. The researchers conclude that this may call into question the suitability and validity of current tools used to measure infection control implementation in this study setting (Claassens et al., 2013). This suggests that validated tools are required which are adapted to local conditions to measure infection control measure implementation and that accurate data are needed to evaluate trends in TB infection and disease among health care workers.

The emergence of novel interferon  $\gamma$ -release assays (IGRAs) with claimed equivalent sensitivity to tuberculin skin test (TST) and improved specificity has raised the possibility of more accurate diagnosis of latent tuberculosis infection (LTBI) in high risk populations such as health care workers (Pai et al., 2008). Most of these studies have been performed in low TB incidence countries with limited BCG vaccination where targeted LTBI screening and treatment of high risk groups form an integral part of TB control programs. Consequently IGRA assays have been incorporated into surveillance programmes in countries such as the USA and UK (Mazurek et al., 2010;

National Collaborating Centre for Chronic Conditions (UK) & Centre for Clinical Practice at NICE (UK), 2011). In some cases it is advocated that IGRAs be used in conjunction with TST, whilst in others it is used in place of TST. Little is known about the utility of these assays in high-incidence settings such as Sub-Saharan Africa where health care workers work at the forefront of the HIV and TB epidemics, but within settings that often lack proper infection control measures. Several studies have shown that health care workers in South Africa have a relatively high HIV prevalence approximating that of surrounding communities (Kranzer et al., 2010; Naidoo & Jinabhai, 2006; Wilkinson & Gilks, 1998; Shisana et al., 2004; Connelly et al., 2007). Coupled with occupational exposure, HIV co-infection places those infected at high risk of progression from latent infection to active TB disease.

Strategies aimed at decreasing TB risk among healthcare workers need to be informed by data that provide estimates of TB infection and disease among health care workers. Evaluation of occupational and environmental risk factors and their association with TB infection and disease may assist in the identification of potentially modifiable factors. This in turn needs to be addressed in further research or in policies on health and safety or infection control aimed at improving protection of health care workers.

In an attempt to generate some of these data and also to evaluate the utility of interferon  $\gamma$ -release assays (IGRAs) in a local health care worker population, a cross sectional study was conducted from May 2009 to June 2010 among staff at seven health care facilities in the greater Cape Town area, South Africa. These participants also served as the cohort for the follow-up phase of the study, which is the subject of the analyses in chapter 6.

The data collected allowed for the calculation of estimates for the main outcomes of interest as previously outlined in the thesis objectives:

1. To determine the prevalence of LTBI using both TST and IGRAs;
2. To evaluate occupational and environmental factors that may be associated with LTBI;
3. As a secondary objective, to evaluate the prevalence of active TB in this population.

## **4.2 METHODS**

### *4.2.1 Structured questionnaire administered by trained nurse interviewer*

A structured interview was administered to all participants by a nurse. The questionnaire elicited data on relevant demographic factors such as age, sex, home language, current medical conditions, HIV testing, place of employment, duration of employment, job category, smoking, exposure to secondary smoke, alcohol use and HIV status. It also focused on participants' awareness of potential occupational risk factors and environmental control measures in their places of employment. Questions related to TB specific factors such as BCG vaccination, previous TB screening and treatment as well as current TB symptoms were included. The questionnaire is reproduced in appendix 1.

### *4.2.2 Tuberculin skin test*

The one step TST protocol was employed to test health care workers at baseline. Two tuberculin units (0.1ml) of RT23 PPD (Staten Serum Institute, Copenhagen), was injected intra-dermally on the volar aspect of the forearm. The induration was then measured by a trained reader after 48 – 72 hours.

An induration of  $\geq 10$ mm was considered a positive test at baseline. In the case of an HIV positive individual an induration of  $\geq 5$ mm was considered a positive test at baseline.

#### *4.2.3 Interferon $\gamma$ -release assays*

All health care workers were tested at baseline with the QuantiFERON-TB Gold In-Tube (QFT-GIT) test (Cellestis Ltd., Carnegie, Victoria, Australia) in accordance with the manufacturer's instructions. THE QFT-GIT was considered positive if the interferon-gamma response minus the nil antigen was  $\geq 0.35$  IU/ml.

The T-SPOT.TB assay (Oxford Immunotec, Abingdon, UK) was performed in accordance with the manufacturer's instructions and blood was drawn at the same time as for the QFT-GIT. A cut-off of six or more spots was treated as a positive result.

#### *4.2.4 HIV test*

All participants were requested to have an HIV test which was a rapid enzyme-linked immunosorbent assay (ELISA) test which was performed in the field in accordance with the manufacturer's instructions. A positive result was followed up with a confirmatory test using a rapid ELISA test from a different manufacturer as per the study protocol. Participants were allowed to report their HIV test result if previously tested, without undergoing the test in this study.

#### *4.2.5 Digital Chest radiograph*

All participants were required to undergo a postero-anterior digital chest radiograph to exclude active TB. The chest radiograph was read by two experienced clinicians trained in the Chest Radiograph Review System (CRRS); a radiological review system specifically aimed at developing competency in the radiological diagnosis of TB (Pinto et al., 2013).

#### *4.2.6 Sputum microscopy and culture*

Participants who tested positive on symptom screen or had a chest x-ray suggestive of active TB were investigated further using sputum microscopy and culture, to exclude active TB. Two sputum samples were collected on separate days for TB investigation and culture was performed on both samples. If sputum could not be obtained, participants were provided with a referral letter to a TB clinic of their choice for further investigation and exclusion of active TB.

#### *4.2.7 Statistical methods*

Statistical analyses were performed using Stata version 11 (Stata Corp., College Station, Texas). The outcomes evaluated were test positivity for TST, QFT-GIT and T-SPOT.TB. The prevalence of potential TB associated risk factors and test outcomes were calculated. Stratified analysis was carried out to differentiate between staff from primary and secondary facilities and results were separately summarized.

Risk factors for test positivity were evaluated using odds ratios (ORs) with 95% confidence intervals (95% CIs). Univariate logistic regression analysis was performed to evaluate the relationship between potential risk factors for

TB infection and all three test outcomes. Individual multivariate regression models were then run for all variables of interest in relation to each test outcome, adjusting for covariates such as age and gender. Multivariate models were also applied in a stratified analysis, looking at determinants separately for staff employed at primary versus secondary level facilities.

Logistic regression was used to develop models that best predicted test outcomes for each of the three different tests. Through a process of model building, a model was derived which best predicted a positive test outcome for the different TST and the two IGRA assays, individually. The best predictor model was determined by examination of the log likelihoods, the log-likelihood ratio tests, associated p-values and the Akaike's information Criterion (AIC) generated by successive models with the final models chosen as representing that with the lowest AIC . The strength and precision of the associations were summarized using odds ratios, confidence intervals and p values.

## **4.3 RESULTS**

### *4.3.1 Participant description*

The total study population sample eligible for participation was 764 health care workers from seven facilities. The latter were selected by a method of purposive sampling. Researchers were guided by Department of Health staff in choosing facilities that served high TB and HIV prevalence communities. Based on records supplied by the human resource management of the seven facilities, a total of 764 health care workers were employed and therefore eligible for the study.

Since participation was voluntary, 510 health care workers were recruited to participate in the survey, representing a 67% participation rate. The results of only 505 participants were included in the analysis as five participants did not meet the inclusion criteria (one was not in the employ of any of the facilities; whilst four others were pregnant).

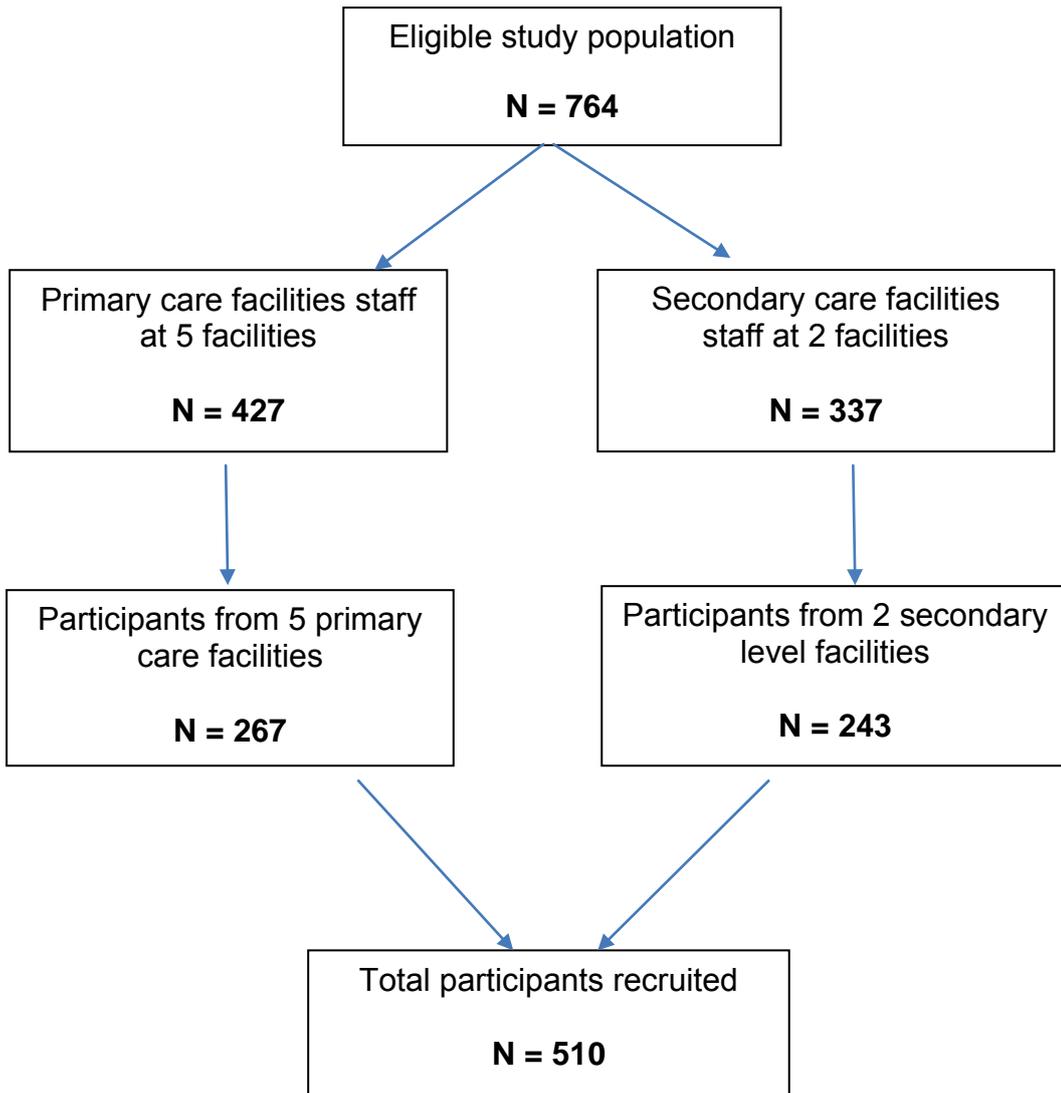
Participants were drawn from five primary level facilities, of which three were clinics, viz. Ubuntu, Kuyasa and Nyanga clinics, and two community health centres viz. the Delft and Nolungile Community Health Centres (CHCs). Clinics differ from the CHCs in the spectrum of services offered, but all facilities provide diagnostic and treatment services for potential TB patients. The facilities were located in areas that serve communities with a high prevalence of tuberculosis and HIV infection. Five facilities were located in Khayelitsha, which has a population of approximately 400 000 (based on 2011 census data) and a TB case notification rate of over 1600/100 000, 70% of which are co-infected HIV (City of Cape Town, 2011; Médecins Sans Frontières, 2011).

The other two facilities were secondary level hospitals dedicated to the care of TB patients with complicated, multi-drug-resistant or extremely drug resistant TB cases. The Brooklyn Chest and DP Marais Hospitals are in the main referral centres situated a distance away from the communities that they serve. Patients referred are known TB patients requiring a greater level of expertise and care to manage their disease.

With respect to employer category, participants were drawn from those employed by the Western Cape Provincial Health Department, the City of Cape Town Health Department or non-governmental organizations (NGOs) supporting health care programmes at a facility. Health care workers were defined broadly as any employee or trainee based at the facility and engaged in the support and delivery of health services to the community. This meant that clinical staff as well as administrative workers, community based health

workers, students in training and those tasked with the cleaning and security of facilities, were eligible for inclusion in the study.

**Figure 4.1. Recruitment and participation**



### **4.3.2 Demographic characteristics of participants**

Health care workers who participated in the study were predominantly female (74%), Xhosa-speaking (51%) (Table 4.1) and were evenly representative of primary and secondary level facilities. Participants were relatively young with marginally more health care workers aged below forty (52%) than above forty (48%). Participants were characterized by a high prevalence of current smokers (22%) and current alcohol use (32%).

A large proportion of the participants(37%) reported being on treatment for other medical conditions .The most common conditions were asthma and hypertension with 8% reportedlybeing diagnosed or on treatment for diabetes .

There was substantial awareness of the importance of HIV testing as 83% of participants indicated that they had previously been tested,with 97% of them willing to disclose their test result.Twelve percent reported having an earlier positive HIV test result and there was good uptake with 70% indicating their willingness to undergo a repeat test as part of the study protocol. The HIV prevalence, defined as reporting a positive test or being diagnosed positive in the study, was 11% in this population.

Analysis of parental employment during childhood which was used as a proxy measure for childhood socio-economic status showed that only 8% of participants had neither parents working with the remainder having one or both parents working. Whilst data on educational status specifically was not collected, participants were asked about whether they had a formal health qualification with 38% indicating that they possessed some form of training in healthcare. Overcrowding, a known risk factor for tuberculosis, was found to not be a problem as the median number of people sharing a bedroom was 2 [IQR: 1- 2].Nurses (27%) and support staff (27%) comprised the largest

occupational categories of participants, followed by lay health care workers (17%) and administration staff (14%).

In recognition of the differences in the scope and functions of the two types of facilities included in the study, a stratified analysis was carried out to explore demographic differences between participants from primary and secondary facilities. Significant differences were found in the gender and age distribution between the facilities with staff at primary level facilities comprising more females (79% vs. 68%  $P=0.005$ ) and younger employees (58% vs. 45%  $P=0.001$ ). Fewer staff at primary level facilities reported having a professional qualification (31% vs. 44%  $P=0.002$ ) and this was reflected in the marked differences in representation across different occupational categories with significantly fewer nursing staff in employment (21% vs. 34%;  $P=0.000$ ) but a far higher number of lay health care workers (31% vs. 2%;  $P=0.000$ ). Primary level staff also had significantly shorter duration of employment in a health care environment (median 9 versus 12 years;  $P=0.005$ ) when compared to secondary level staff. These differences are to be expected and are in large part driven by the level of service and care provided at the different facilities.

#### **4.3.3 TB associated variables**

Evaluation of TB associated variables demonstrated high coverage of BCG vaccination (91%) which was classified as either having a history of or evidence of a vaccination scar upon inspection, as evidence of BCG vaccination (Table 4.2). Forty percent of participants indicated that they had previously been screened for TB. The most common screening tools employed were either chest radiography or sputum microscopy. There was a high prevalence of individuals who had previously been treated for TB (13%) in this study population. Screening for current active TB demonstrated a high prevalence of chest x-ray abnormality (23%) (X-ray compatible with active or

inactive TB) and a positive TB symptom screen (26%) (defined as yes to the presence of any TB symptoms).

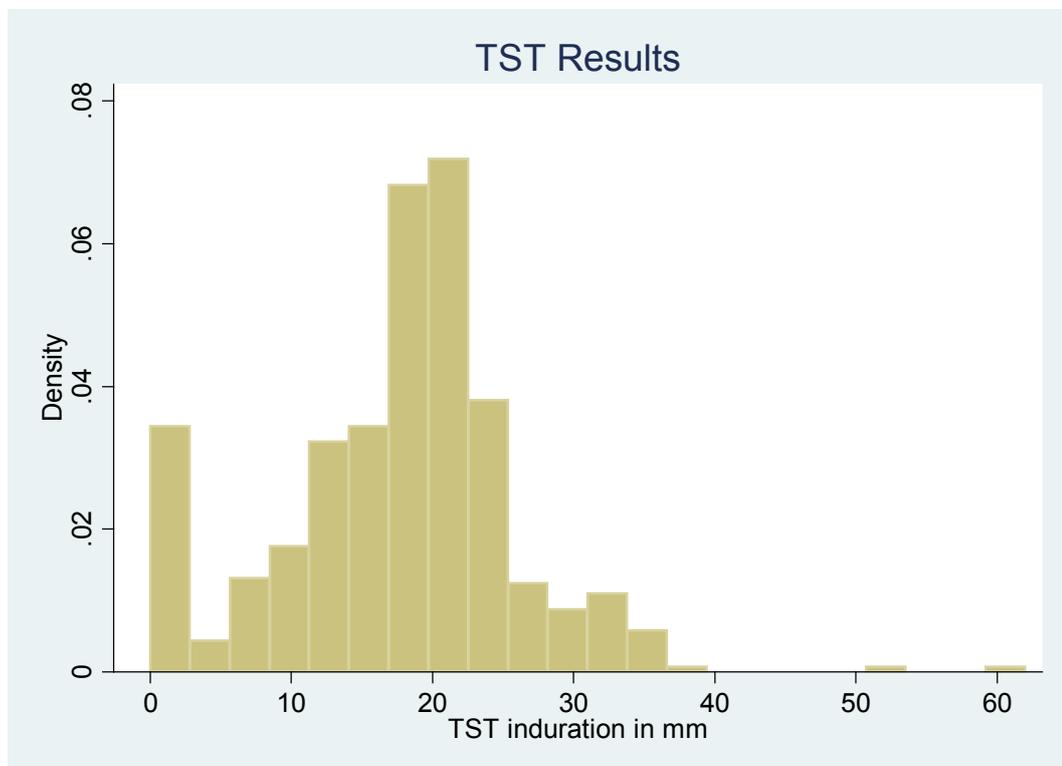
Whilst participants were requested to consent to the administration of three tests for LTBI, not all the test results could be used in the analysis. For TST, test results were used only if the test was read by the trained observer within the 72 hours cut-off in accordance with the protocol. TST reactions that were not read in the specified time were therefore not included in the analysis yielding 484 valid results. A graphic presentation of the frequency distribution of TST reactions is shown in figure 4.2. For QFT-GIT 497 participants had the test done with one indeterminate result which could not be used in the analysis leaving 496 valid test results. For T-SPOT.TB there was a lower yield of valid results. Of the 493 participants who had a T-SPOT.TB test result, 465 had a valid test result whilst 28 (6%) had indeterminate results.

With regard to test outcomes, there were marked differences between the LTBI test positivity prevalence for the different tests used. For TST 84% of participants were shown to be positive (skin induration of 10mm or higher). For the IGRA assays the prevalence was considerably lower at 65% for QFT-GIT and 60% for T-SPOT.TB.

There were differences between staff from primary and secondary level facilities with regard to the distribution of TB associated risk factors. Secondary level staff demonstrated greater BCG vaccination coverage (88% versus 80%;  $P=0.025$ ). More primary level staff reported ever having been on treatment for TB (18% VS 7%;  $P=0.000$ ) and HIV infection was also significantly higher (19% versus 2%  $P=0.000$ ) in this group. Chest x-ray abnormality was more frequent in this group with a larger number of x-rays reported as being compatible with inactive TB and active TB respectively (20% vs. 11% and 10% vs. 5%;  $P=0.001$ ).

Despite these marked differences, which are suggestive of increased risk, there were no significant differences with respect to the outcomes of latent TB infection as reflected in TST and IGRA test results. However, the prevalence of test positivity was consistently higher for primary level staff across all three tests. The median TST reaction was higher in primary level staff compared to secondary level staff (19mm versus 16mm; P=0.001).

**Figure 4.2. TST size and frequency distribution (n=484)**



#### **4.3.4 Occupational and environmental characteristics**

An assessment of the distribution of occupational and environmental risk factors for TB across the whole group revealed that most employees perceived their exposure as high risk with 90% reporting having daily contact with TB patients and 76% reporting having recent contact (in the last 3 months) with a newly diagnosed TB patient (Table 4.3). The majority of those who reported having had a recent exposure to TB patients indicated that such exposure occurred in the occupational setting (96%) rather than the household or another setting. An occupational exposure index was developed using a composite of measures related to proximity to infectious source, duration of exposure and infectivity of source, with a maximum exposure score equal to 11 points. The median occupational exposure score was 7 [IQR 5 – 9].

Large numbers of employees were engaged in occupational tasks directed at providing care to TB patients such as interviewing of patients (48%), counselling of patients (42%) and general nursing care (30%). High-risk procedures associated with increased TB risk such as sputum collection and bronchoscopy procedures were less frequently performed (21% and 1%, respectively) by staff.

Knowledge and practice of environmental measures were evaluated by gauging whether staff were aware of specific engineering, administrative and aware of infection control measures such as the presence safe working practices that were practiced or available in their facilities. A high proportion of employees reported being of natural/ mechanical ventilation (93%) measures in their facilities, provision of disposable masks for patients with suspected TB (96%), provision of N-95 masks to staff (93%) and teaching of cough etiquette to patients (79%). The reported awareness of the provision of on-site diagnostic services was high (78%), but administrative measures

such as knowledge of an infection control policy, separating TB patients from other patients and early triage of potential TB patients were less prevalent.

Notably the majority of employees perceived themselves as being at high risk of contracting TB (58%). However, a large proportion reported having had no training to protect themselves from infection (38%) or reported having had no training to prevent spread of TB between patients (43%).

#### **4.3.5 Univariate analysis of factors associated with Latent TB Infection**

Univariate analysis for the whole group was performed to explore the relationships between individual variables and latent TB infection test outcomes as reflected in test positivity for either of the IGRA assays and the TST (Table 4.4). Increasing age was positively associated with the presence of LTBI for all 3 tests. BCG vaccination was not significantly associated with test positivity. Staff involved in home-based care were more likely to have a positive TSPOT-TB (OR= 3.83, CI 1.56 – 9.37) as were those working in institutions where cough etiquette was advocated (OR= 1.73, CI 1.10 – 2.71). Duration of employment is a known risk factor for LTBI in a health care environment and this relationship showed a consistently positive association across all the tests, although not significant for any one of the tests. No significant associations were demonstrated for any of the other occupational and environmental risk factors and the prevalence of LTBI. Sputum positivity for active disease was associated with increased risk of test positivity for the IGRA assays. So too was having daily exposure to TB patients.

#### 4.3.6 Multivariate analysis of factors associated with LTBI

Multivariate analysis was conducted adjusting for age category and gender (Table 4.5). Separate models were developed for each variable to explore associations after adjustment. There were no significant associations with TST positivity after adjustment in the combined group.

For a positive test result on QFT-GIT, BCG vaccination emerged as a negative predictor [OR=0.43; (95% CI 0.20 – 0.91)]. Participants with a chest radiograph suggestive of active TB were more likely to test positive on QFT-GIT [OR=2.27; (95% CI 0.99 – 5.18)].

TSPOT positivity was associated with, being a Xhosa speaker (OR=1.49, CI 1.02 – 2.19) in the multivariate analysis. Being involved in home-based care continued to be an independent predictor of a positive test [OR=4.14; (95% CI 1.66 – 10.29)]. As was, being employed at a health facility that advocated cough etiquette [OR=1.68; (95% CI 1.06 – 2.66)].

Stratified analysis was performed which evaluated relationships between covariates and test outcomes separately for staff at primary and secondary level facilities. These multivariate models were then adjusted for age and gender. In all cases where significant associations had been demonstrated in unadjusted models, these remained with some associations also demonstrating increased strength following multivariate adjustment.

For TST outcome in primary level staff, participants with HIV positive status were less likely to test positive on TST [OR=0.41; (95% CI 0.17 – 0.95)] (Table 4.6.1) whilst those with long employment duration were more likely to have a positive TST [OR=4.17; (95% CI 1.12 – 15.62)]. In secondary level staff individuals who had undergone some training on self-

protection from TB infection, were also less likely to test positive [OR=0.38; (95% CI 0.16 – 0.91)].

In primary level staff a positive QFT-GIT test was more likely in individuals who had a history of ETS exposure [OR=2.37; (95% CI 1.34 – 4.22)], were involved in sputum collection [OR=3.25; (95% CI 1.28 – 8.09)] or were employed in healthcare for more than 20 years [OR= 2.42; (95% CI 1.09 – 5.38)](Table 4.6.2). As with TST, secondary level staff were less likely to have a positive test if they had undergone some training on self- protection from TB infection [OR=0.41; (95% CI 0.22 – 0.77)].

A positive TSPOT-TB test was strongly associated with being engaged in providing home-based care to TB patients [OR= 4.14; (95% CI 1.60 – 10.70)], working at a facility where cough etiquette was advocated [OR=2.06; 95% CI (1.04 – 4.10)] and where disposable surgical masks were provided to potentially infectious patients [OR=3.65; (1.16 – 11.51)]. These associations were however only applicable to staff working at a primary level facility (Table 4.6.3).

#### **4.3.7 Predictors of LTBI in logistic regression models**

Predictive models that best estimated TST positivity across both groups suggest that those of male gender who spoke an African home language and reported exposure to environmental tobacco smoke were most likely to test positive for TST (Table 4.7). By contrast, for QFT-GIT male gender, older age, a history of BCG vaccination and being engaged in sputum collection were most predictive of a positive test. For LTBI measured by TSPOT-TB those who were older, of male gender, had a history of BCG vaccination, who worked at facilities that practised cough etiquette and provide disposable masks were at greater risk of testing positive.

#### **4.3.8 Characteristics of newly diagnosed TB cases**

Two participants indicated that they were currently receiving TB treatment, one of whom was HIV positive. Of the 103 participants referred for sputum investigation, a further five tested positive for active disease. This translates into an overall prevalence of 7/505 or 14 / 1000 for active TB in this population. Of the five new cases detected none tested positive on sputum microscopy and all five were culture positive. Three tested positive on symptom screen , three on chest radiograph and three were HIV positive (Table 4.8). One case did not test positive on either chest radiograph or symptom screen, but was referred for sputum at her own request as she had felt unwell and had a colleague recently diagnosed with TB as a result of the study. This represented a deviation from the study protocol. All cases were TST positive, three were QFT and four were T-SPOT.TB positive.

**Table 4.1 Demographic and general health qualities of participants (N=505)**

<b>Participant characteristics</b>	<b>Number (%) of participants</b>	
<b>Gender</b>		
-Male	134 (26%)	
-Female	371 (74%)	
<b>Age</b>		
- <30 years	126 (25%)	
- 31-40 years	136 (27%)	
- 41-50 years	134 (27%)	
- >50 years	109 (22%)	
<b>Parental employment</b>		
-Parents not working	40 (8%)	
-Single parent working	215 (43%)	
-Both parents working	250 (50%)	
<b>Type of facility where employed</b>		
-Primary level	263 (52%)	
-Secondary level	242 (48%)	
<b>Home language</b>		
-English	74 (15%)	
-Afrikaans	148 (30%)	
-Xhosa	256 (51%)	
-Other	27 (5%)	
<b>Smoking history</b>		
-Current	110 (22%)	
- Ex-smoker	63 (12%)	
- Non-smoker	332 (66%)	
<b>Alcohol use</b>		
-Ever consume alcohol	280 (55%)	
-Current consumption of alcohol	160 (32%)	
<b>On treatment for other medical condition</b>		186 (37%)
<b>Diabetes</b>		
-Diagnosed	38 (8%)	
-On treatment	31 (6%)	

Data are presented as % or median (interquartile range) unless otherwise indicated.

**Table 4.1 (continued). Demographic and general health qualities of participants (N=505)**

Participant characteristics	Number (%) of participants
<b>HIV testing and status</b>	
-Ever tested for HIV	419 (83%)
-Willing to disclose result of test	405 (97%)
-Positive for HIV on previous test	49 (12%)
-Willing to be tested now	354 (70%)
<b>*Overcrowding</b>	
-No of people in house (median and IQR)	4 (3 – 5)
-No of bedrooms in house (median and IQR)	3 (2 – 3)
-No of people per room (median and IQR)	2 (1 – 2)
<b>Occupational status</b>	
Professional health qualification	190 (38%)
<b>Job category</b>	
-Management	13 (3%)
-Doctors	12 (2%)
- Nurses	137 (27%)
-Research	12 (2%)
-Support staff	137 (27%)
-Lay health care workers	85 (17%)
-Administration staff	71 (14%)
<b>Years of employment in a health environment</b>	<b>6 (2 -18)</b>

Data are presented as % or median (interquartile range) unless otherwise indicated.

**Table 4.2 Frequency and distribution of TB specific variables (N=505)**

<b>Variable</b>	<b>Number (%) of participants</b>
<b>History BCG vaccination</b>	
-Yes	423 (84%)
-No	26 (5%)
-Do not know	56 (11%)
<b>Vaccination scar</b>	398 (79%)
<b>Ever treated for TB</b>	65 (13%)
<b>Currently on TB treatment</b>	2 (0.4%)
<b>Previous TB screening</b>	
- All methods of screening	201 (40%)
- Screening by chest radiograph for TB (N=201)	160 (80%)
-Screening by sputum test for TB (N=201)	115 (57%)
<b>Current TB symptoms</b>	
-Current cough	96 (19%)
-Cough >2weeks	31 (6%)
- Haemoptysis	2(0.4%)
- Weight loss	56 (11%)
-Night sweats	28 (6%)
- Fever >2weeks	46 (9%)
-Chest pain>2 weeks	54 (11%)
<b>Chest radiograph</b>	
-Normal/other	381 (77%)
-Inactive TB	78 (16%)
-Active TB	37 (7%)
<b>Sputum positive (N=103)</b>	5 (5%)
<b>*TST reading</b>	18 (13 – 22)
<b>**TST Positive (N=484)</b>	405 [80% -87%]
<b>**QFT-GIT positive (N=496)</b>	324 [61% – 70%]
<b>**TSPOT-TB positive (N=465)</b>	277[55% - 64%]

\*For TST reading data presented as median and IQR.\*\* For test outcomes 95% CI shown.

**Table 4.3 Occupational and environmental risk factors for exposure to *M. tuberculosis***

Exposure	N (%) of participants
<b>Daily contact with suspected TB patients</b>	456 (90%)
<b>Recent contact with newly diagnosed TB patient (last 3 months)</b>	385 (76%)
<b>Involvement in occupational tasks directed at TB patients</b>	
-Clerking / interviewing	241 (48%)
-Counselling	213 (42%)
-Clinical examination	112 (22%)
-Sputum collection	104 (21%)
-General nursing care	153 (30%)
-Home-based care	1 (8%)
-Bronchoscopy procedures (N=502)	6 (1%)
<b>Knowledge and practice of infection control measures</b>	
-Awareness of a facility infection control policy	313 (62%)
-Presence of ventilation measures (natural / mechanical)	472 (93%)
-Presence of UV radiation	224 (44%)
-Teaching of cough etiquette to patients	397 (79%)
-Early triage of potentially infectious patients	321 (64%)
-Separating TB patients from other patients in clinic areas (N=501)	239 (47%)
-Provision of on-site TB diagnostic services	396 (78%)
-Provision of disposable surgical masks to infectious patients	485 (96%)
-Provision of N-95 masks for staff to wear	472 (93%)
- Provision of PPE	456 (90%)
<b>Recent exposure to TB</b>	
- History of recent exposure	385 (76%)
- Recent occupational exposure	367 (96%)
- Occupational contact score (max score=11)	7 (5 – 9)
<b>Probability of infection (perception of risk)</b>	
-Low	83 (16%)
-Moderate	127 (25%)
-High	296 (58%)
<b>Extent of training on self-protection</b>	
-No training	191 (38%)
-Some training	219 (43%)
-Extensive training	95 (19%)
<b>Extent of training to prevent spread between patients</b>	
- No training	216 (43%)
-Some training	221 (44%)
-Extensive training	68 (13%)

N=number. Data are presented as % or median (interquartile range) unless otherwise indicated.

**Table 4.4 Unadjusted logistic regression analysis of IGRA assays in comparison to TST (represented as odds ratios and 95% confidence intervals)**

VARIABLE	QFT-GIT	TSPOT-TB	TST
<b>Age category</b>			
<30	1.00	1.00	1.00
31-40	1.36 (0.82 – 2.25)	<b>1.91 (1.14 – 3.19) *</b>	<b>2.08 (1.04 – 4.17)*</b>
41-50	<b>2.18 (1.28 – 3.72) **</b>	<b>2.49 (1.46 – 4.23) **</b>	1.85 (0.93 – 3.67)
>50	1.31 (0.77 – 2.24)	<b>2.05 (1.19 – 3.55) *</b>	1.11 (0.58 – 2.13)
<b>Gender</b>			
Female	1.00	1.00	1.00
Male	1.40 (0.91 – 2.16)	1.39 (0.90 – 2.13)	1.53 (0.84 -2.81)
<b>Smoking</b>			
Non-smoker	1.00	1.00	1.00
Ex-smoker	0.90 (0.51 – 1.58)	1.06 (0.59 – 1.89)	1.14 (0.53 – 2.46)
Current smoker	0.95 (0.60 – 1.51)	0.78 (0.49 – 1.23)	1.03 (0.56 – 1.90)
<b>ETS exposure</b>			
	1.44 (0.96 – 2.16)	1.20 (0.80 – 1.81)	1.56 (0.93 – 2.60)
<b>Home language</b>			
Afrikaans, English, other	1.00	1.00	1.00
Xhosa	1.26 (0.87 – 1.82)	1.42 (0.98 – 2.06)	1.64 (1.00 - 2.69)

ETS: environmental tobacco smoke.

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

**Table 4.4 (continued). Unadjusted logistic regression analysis of IGRA assays in comparison to TSTs (represented as odds ratios and 95% confidence intervals)**

VARIABLE	QFT-GIT	TSPOT-TB	TST
<b>Type of facility</b>			
Primary level facility	1.00	1.00	1.00
TB hospital	0.95 (0.65 – 1.37)	0.82 (0.57 – 1.19)	0.87 (0.53 – 1.41)
<b>Risk factors for LTBI</b>			
BCG vaccination	0.53 (0.25 – 1.09)	0.96 (0.49 – 1.89)	0.84 (0.34–2.07)
Diabetes diagnosis	0.90 (0.45 – 1.79)	1.13 (0.56 – 2.25)	1.23 (0.46 - 3.27)
Current alcohol use	0.95 (0.64 – 1.41)	0.95 (0.64 – 1.42)	0.66 (0.40 -1.09)
Previous TB treatment	1.04 (0.60 – 1.81)	1.64 (0.91 – 2.95)	1.18 (0.56 – 2.51)
Current TB treatment	0.53 (0.03 – 8.52)	-	-
Symptom screen positive	1.29 (0.84 – 1.98)	1.19 (0.78 – 1.82)	0.88 (0.51 -1.52)
<b>HIV status (N=431)</b>			
Positive / reported as positive	0.69 (0.38 – 1.23)	1.18 (0.64 – 2.19)	0.66 (0.32 – 1.43)
<b>Sputum (N=96)</b>			
Positive	1.43 (0.89 – 2.31)	2.17 (0.23 – 20.24)	
<b>Chest radiograph (N=456)</b>			
Normal / other	1.00	1.00	1.00
Inactive TB	1.01 (0.61 – 1.68)	1.29 (0.77 – 2.16)	1.76 (0.80 -3.84)
Active TB	2.05 (0.91 – 4.60)	1.45 (0.68 – 3.08)	1.63 (0.55 – 4.77)

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

**Table 4.4 (continued). Unadjusted logistic regression analysis of IGRA assays in comparison to TSTs (represented as odds ratios and 95% confidence intervals)**

VARIABLE	QFT-GIT	TSPOT-TB	TST
<b>Occupational Factors</b>			
Health qualification	0.96 (0.65 – 1.40)	0.88 (0.60 – 1.29)	0.97 (0.59 – 1.59)
Clerking / interview TB patients	1.02 (0.70 – 1.48)	1.19 (0.82 – 1.73)	1.03 (0.63 – 1.68)
Counselling	0.83 (0.57 – 1.20)	0.94 (0.64 – 1.36)	0.84 (0.51 – 1.37)
Examination	0.82 (0.53 – 1.28)	0.81 (0.52 – 1.26)	0.95 (0.53 – 1.69)
Sputum collection	1.43 (0.89 – 2.31)	1.14 (0.72 – 1.82)	0.95 (0.53 – 1.71)
Nursing	1.09 (0.73 – 1.63)	0.89 (0.59 – 1.33)	0.91 (0.54 – 1.54)
Home-care	1.44 (0.70 – 2.96)	<b>3.82 (1.56 – 9.35) **</b>	1.27 (0.48 – 3.37)
Bronchoscopy	1.06 (0.19 – 5.86)	0.45 (0.07 – 2.71)	-
Infection Control Policy	1.02 (0.70 – 1.49)	1.03 (0.71 – 1.51)	0.80 (0.48 – 1.35)
Ventilation measures	1.14 (0.54 – 2.39)	1.14 (0.54 – 2.40)	1.35 (0.53 – 3.41)
UV lights	0.88 (0.61 – 1.28)	0.82 (0.57 – 1.19)	1.00 (0.61 – 1.63)
Cough etiquette	1.20 (0.77 – 1.87)	<b>1.69 (1.08 – 2.64)*</b>	1.35 (0.77 – 2.36)
Early triage	1.06 (0.72 – 1.56)	1.09 (0.74 – 1.61)	0.96 (0.58 – 1.60)
Separation TB patients	1.20 (0.83 – 1.74)	1.20 (0.83 – 1.74)	0.98 (0.60 – 1.60)
Diagnostic services	0.94 (0.60 – 1.49)	0.81 (0.51 – 1.29)	1.43 (0.81 – 2.52)
Disposable surgical masks	1.39 (0.55 – 3.52)	2.09 (0.82 – 5.30)	2.10 (0.73 – 6.07)
Provision of N95 Masks	1.42 (0.69 – 2.91)	1.74 (0.84 – 3.57)	0.97 (0.36 – 2.62)
PPE provision	1.39 (0.76 – 2.56)	1.39 (0.74 – 2.59)	1.12 (0.50 – 2.51)

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

**Table 4.4 (continued). Unadjusted logistic regression analysis of IGRA assays in comparison to TSTs (represented as odds ratios and 95% confidence intervals)**

<b>COVARIATE</b>	<b>QFT-GIT</b>	<b>TSPOT-TB</b>	<b>TST</b>
<b>Training on self-protection</b>			
No training	1.00	1.00	1.00
Some training	0.68 (0.45 – 1.03)	0.81 (0.54 – 1.23)	0.73 (0.42 – 1.25)
Extensive training	0.99 (0.57 – 1.70)	0.89 (0.53 – 1.50)	0.87 (0.43 – 1.76)
<b>Training patient protection</b>			
No training	1.00	1.00	1.00
Some training	1.10 (0.74 – 1.64)	1.45 (0.97 – 2.16)	1.29 (0.77 – 2.18)
Extensive training	1.25 (0.69 – 2.25)	1.11 (0.63 – 1.96)	1.10 (0.53 – 2.31)
<b>Employment in healthcare</b>			
< 10 years	1.00	1.00	1.00
10 – 19 years	1.09 (0.65 – 1.82)	1.19 (0.71- 2.01)	1.08 (0.55 – 2.12)
>20 years	1.40 (0.90 – 2.20)	1.23 (0.79 -1.91)	1.35 (0.74 – 2.46)
<b>Recent contact with TB patient</b>	1.14 (0.74 – 1.75)	1.16 (0.75 – 1.78)	0.97 (0.55 – 1.73)
<b>Daily contact with TB patient</b>	1.22 (0.66 – 2.23)	1.21 (0.66 – 2.23)	1.09 (0.49 – 2.44)
<b>Occupational contact score</b>	1.01 (0.93 – 1.10)	1.01 (0.93 – 1.10)	1.00 (0.89 – 1.11)

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

**Table 4.5 Adjusted logistic regression analysis of IGRA assays in comparison to TST (represented as odds ratios and 95% confidence intervals)**

VARIABLE	QFT-GIT	TSPOT-TB	TST
<b>Age category</b>			
<30	1.00	1.00	1.00
31-40	1.41 (0.85 – 2.34)	<b>1.98 (1.18 – 3.32) **</b>	<b>2.19 (1.09 – 4.41)*</b>
41-50	<b>2.29 (1.33 – 3.91) **</b>	<b>2.60 (1.51 – 4.46) ***</b>	1.95 (0.98 – 3.89)
>50	1.37 (0.80 – 2.34)	<b>2.15 (1.23 – 3.74)*</b>	1.17 (0.61 – 2.25)
<b>Gender</b>			
Female	1.00	1.00	1.00
Male	1.48 (0.95 – 2.30)	1.50 (0.97 – 2.34)	1.64 (0.89 -3.02)
<b>Smoking</b>			
Non-smoker	1.00	1.00	1.00
Ex-smoker	0.78 (0.42 – 1.43)	0.93 (0.49 – 1.75)	1.07 (0.47 – 2.43)
Current smoker	0.85 (0.52 – 1.38)	0.70 (0.43 – 1.14)	0.97 (0.51 – 1.86)
<b>ETS exposure</b>	1.43 (0.94 – 2.15)	1.18 (0.78 – 1.80)	1.48 (0.88 – 2.49)
<b>Home language</b>			
Afrikaans, English, other	1.00	1.00	1.00
Xhosa	1.29 (0.88 – 1.88)	<b>1.49 (1.02 – 2.19)*</b>	1.60 (0.97 - 2.2.66)

Data adjusted for age and gender. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001

**Table 4.5 (continued). Adjusted logistic regression analysis of IGRA assays in comparison to TST (represented as odds ratios and 95% confidence intervals)**

VARIABLE	QFT-GIT	TSPOT-TB	TST
<b>Type of facility</b>			
Primary level facility	1.00	1.00	1.00
TB hospital	0.91 (0.62 – 1.33)	0.77 (0.52 – 1.13)	0.90 (0.54 – 1.49)
<b>Risk factors for LTBI</b>			
BCG vaccination	<b>0.43 (0.20 – 0.91)*</b>	0.96 (0.49 – 1.89)	0.78 (0.30 - 1.98)
Diabetes diagnosis	0.80 (0.39 – 1.62)	0.95 (0.46 – 1.93)	1.23 (0.45 - 3.34)
Current alcohol use	0.90 (0.59 – 1.37)	0.94 (0.61 – 1.44)	0.61 (0.35 -1.04)
Previous TB treatment	1.08 (0.61 – 1.88)	1.66 (0.91 – 3.04)	1.14 (0.53 – 2.43)
Current TB treatment	0.84 (0.05 – 13.85)	-	-
Symptom screen positive	1.28 (0.83 – 1.99)	1.20 (0.78 – 1.86)	0.87 (0.50 -1.52)
<b>HIV status (N=431)</b>			
Positive / reported as positive	0.81 (0.44 – 1.50)	1.48 (0.77 – 2.84)	0.64 (0.29 – 1.42)
<b>Sputum (N=96)</b>			
Positive	0.15 (0.01 – 1.55)	0.69 (0.06 – 8.70)	-
<b>Chest radiograph (N=456)</b>			
Normal / other	1.00	1.00	1.00
Inactive TB	0.88 (0.52 – 1.49)	1.14 (0.67 – 1.94)	1.60 (0.72 -3.52)
Active TB	2.27 (0.99 – 5.18)	1.68 (0.77 – 3.64)	1.78 (0.60 – 5.26)

Data adjusted for age and gender. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

**Table 4.5 (continued). Adjusted logistic regression analysis of IGRA assays in comparison to TST (represented as odds ratios and 95% confidence intervals)**

VARIABLE	QFT-GIT	TSPOT-TB	TST
<b>Occupational Factors</b>			
Health qualification	0.99 (0.67 – 1.47)	0.88 (0.59 – 1.30)	1.05 (0.63 – 1.78)
Clerking / interview TB patients	1.06 (0.72 – 1.57)	1.20 (0.81 – 1.78)	1.02 (0.61 – 1.70)
Counselling	0.87 (0.59 – 1.28)	0.96 (0.65 – 1.42)	0.88 (0.53 – 1.46)
Examination	0.88 (0.56 – 1.38)	0.86 (0.54 – 1.35)	1.04 (0.56 – 1.87)
Sputum collection	1.54 (0.94 – 2.50)	1.18 (0.73 – 1.90)	1.03 (0.57 – 1.89)
Nursing	1.13 (0.74 – 1.73)	0.88 (0.58 – 1.34)	1.00 (0.58 – 1.72)
Home-care	1.48 (0.71 – 3.08)	<b>4.14 (1.66 – 10.29) **</b>	1.27 (0.47 – 3.44)
Bronchoscopy	0.95 (0.17 – 5.37)	0.38 (0.06 – 2.35)	-
Infection Control Policy	1.00 (0.67 – 1.47)	0.97 (0.66 – 1.44)	0.81 (0.48 – 1.38)
Ventilation measures	1.13 (0.54 – 2.40)	1.12 (0.52 – 2.39)	1.43 (0.56 – 3.65)
UV lights	0.84 (0.57 – 1.24)	0.75 (0.51 – 1.10)	1.02 (0.62 – 1.69)
Cough etiquette	1.19 (0.76 – 1.88)	<b>1.68 (1.06 – 2.66)*</b>	1.32 (0.74 – 2.36)
Early triage	1.01 (0.69 – 1.50)	1.03 (0.69 – 1.53)	0.95 (0.57 – 1.60)
Separation TB patients	1.20 (0.83 – 1.75)	1.20 (0.82 – 1.75)	0.99 (0.60 – 1.61)
Diagnostic services	0.93 (0.59 – 1.48)	0.78 (0.48 – 1.25)	1.40 (0.79 – 2.47)
Disposable surgical masks	1.22 (0.48 – 3.12)	1.83 (0.71 – 4.73)	1.98 (0.67 – 5.84)
Provision of N95 Masks	1.33 (0.64 – 2.77)	1.51 (0.72 – 3.17)	0.99 (0.36 – 2.73)
PPE provision	1.28 (0.69 – 2.38)	1.18 (0.62 – 2.25)	1.11 (0.49 – 2.52)

Data adjusted for age and gender. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

**Table 4.5 (continued). Adjusted logistic regression analysis of IGRA assays in comparison to TST (represented as odds ratios and 95% confidence intervals)**

<b>VARIABLE</b>	<b>QFT-GIT</b>	<b>TSPOT-TB</b>	<b>TST</b>
<b>Training on self-protection</b>			
No training	1.00	1.00	1.00
Some training	0.68 (0.45 – 1.04)	0.77 (0.50 – 1.17)	0.76 (0.44 – 1.31)
Extensive training	0.98 (0.57 – 1.71)	0.83 (0.49 – 1.42)	0.87 (0.42 – 1.77)
<b>Training patient protection</b>			
No training	1.00	1.00	1.00
Some training	1.11 (0.74 – 1.66)	1.42 (0.94 – 2.14)	1.33 (0.78 – 2.26)
Extensive training	1.24 (0.68 – 2.25)	1.03 (0.57– 1.85)	1.14 (0.54 – 2.41)
<b>Employment in healthcare</b>			
< 10 years	1.00	1.00	1.00
10 – 19 years	0.96 (0.55 – 1.68)	0.87 (0.49- 1.54)	1.03 (1.00 – 1.07)
>20 years	1.35 (0.78 – 2.33)	0.92 (0.53 -1.57)	1.68 (0.80 – 3.52)
<b>Recent contact with TB patient</b>	1.16 (0.75 – 1.80)	1.16 (0.75 – 1.78)	0.95 (0.53 – 1.71)
<b>Daily contact with TB patient</b>	1.12 (0.60 – 2.09)	1.05 (0.56 – 1.96)	0.98 (0.43 – 2.23)
<b>Occupational contact score</b>	1.02 (0.93 – 1.11)	1.02 (0.93 – 1.11)	1.02 (0.91 – 1.14)

Data adjusted for age and gender; \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

**Table 4.6.1 Adjusted logistic regression analysis of significant determinants of TST in primary and secondary facilities (represented as odds ratios and 95% confidence intervals)**

COVARIATE	UNADJUSTED	ADJUSTED
<b>TST</b>		
<b>PRIMARY</b>		
<b>HIV status (N=431)</b>		
Negative/ reported as negative	1.00	1.00
Positive / reported as positive	<b>0.41 (0.18 – 0.92)*</b>	<b>0.41 (0.17 – 0.95)*</b>
<b>Employment in healthcare</b>		
< 10 years	1.00	1.00
10 – 19 years	1.04 (0.44 – 2.47)	1.04 (0.44 – 2.47)
>20 years	<b>3.47 (1.01 – 11.97)*</b>	<b>4.17 (1.12 – 15.62)*</b>
<b>SECONDARY</b>		
<b>Training to protect yourself</b>		
Nil	1.00	1.00
Some	<b>0.38 (0.17 – 0.87)</b>	<b>0.38 (0.16 – 0.91)</b>
Extensive	0.63 (0.21 – 1.90)	0.69 (0.21 – 2.23)

Data adjusted for age and gender. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

**Table 4.6.2 Adjusted logistic regression analysis of significant determinants of QFT-GIT positivity in primary and secondary facilities (represented as odds ratios and 95% confidence intervals)**

VARIABLE	UNADJUSTED	ADJUSTED
<b>QFT</b>		
<b>PRIMARY LEVEL FACILITY</b>		
ETS exposure	<b>2.21 (1.27 – 3.85) **</b>	<b>2.38 (1.34 – 4.22) **</b>
Sputum collection	<b>3.16 (1.27 – 7.88) **</b>	<b>3.25 (1.28 – 8.09)*</b>
<b>Employment in healthcare</b>		
< 10 years	1.00	1.00
10 – 19 years	1.79 (0.88 – 3.65)	1.69 (0.78 – 3.65)
>20 years	<b>2.28 (1.11 -4.66)*</b>	<b>2.42 (1.09 – 5.38)*</b>
<b>SECONDARY LEVEL FACILITY</b>		
<b>Training to protect yourself</b>		
Nil	1.00	1.00
Some	<b>0.38 (0.17 – 0.88)*</b>	<b>0.41 (0.22 – 0.77) **</b>
Extensive	0.63 (0.21 – 1.93)	0.70 (0.29 – 1.65)

Data adjusted for age and gender. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

**Table 4.6.3 Adjusted logistic regression analysis of significant determinants of T-SPOT.TB positivity in primary and secondary facilities (represented as odds ratios and 95% confidence intervals)**

<b>VARIABLE</b>	<b>UNADJUSTED</b>	<b>ADJUSTED</b>
<b>TSPOT.TB</b>		
<b>PRIMARY</b>		
Years in health employment	<b>1.04 (1.00 – 1.07)*</b>	1.02 (0.98 – 1.06)
Home-care	<b>3.61 (1.44 – 9.06) **</b>	<b>4.14 (1.60 – 10.70) **</b>
Cough etiquette	<b>2.03 (1.05 – 3.93)*</b>	<b>2.06 (1.04 – 4.10)*</b>
Disposable surgical masks	<b>3.51 (1.16 – 10.63)*</b>	<b>3.65 (1.16 – 11.51)*</b>

Data adjusted for age and gender. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001.

**Table 4.7 Multiple logistic regression models of LTBI predictors among health care workers**

<b>Factor</b>	<b>Estimate (OR)</b>	<b>95% CI</b>	<b>p Value</b>
<b>TST</b>			
African Home language	1.57	0.96 – 2.57	0.072
Exposure to secondary smoke	1.47	0.88 – 2.46	0.144
Male gender	1.60	0.87 – 2.94	0.129
<b>AIC =430.97</b>			
<b>QFT-GIT</b>			
Exposure to secondary smoke	1.38	0.91 – 2.09	0.127
History of BCG vaccination	0.51	0.29 – 0.91	<b>0.022</b>
Sputum collection	1.77	0.95 – 2.56	0.077
Male gender	1.41	0.90 – 2.22	0.135
Age 31-40	1.53	0.91 – 2.58	0.108
Age 41–50	2.59	1.49 – 4.52	<b>0.001</b>
Age >50	1.55	0.89 – 2.70	0.125
<b>AIC = 633.19</b>			
<b>TSPOT.TB</b>			
History of BCG vaccination	0.60	0.34 – 1.06	0.078
Home care of TB patients	4.16	1.64 – 10.55	<b>0.003</b>
Advocate cough etiquette	1.59	0.99 – 2.56	0.056
Disposable masks available	2.11	0.78 – 5.69	0.139
Male gender	1.64	1.03 – 2.59	<b>0.036</b>
Age 31 – 40	2.07	1.21 – 3.55	<b>0.008</b>
Age 41 – 50	2.57	1.46 – 4.54	<b>0.001</b>
Age > 50	2.39	1.33 – 5.29	<b>0.003</b>
<b>AIC =601.35</b>			

**Table 4.8 Characteristics of newly diagnosed TB cases**

	TST	QFT	TSPOT.TB	Symptom Screen	CXR	HIV	Microscopy	Culture
<b>Case 1 (100)</b>	+	+	+	+	+	-	-	+
<b>Case 2 (115)</b>	+	+	+	-	+	-	-	+
<b>Case 3 (125)*</b>	+	+	+	-	-	+	-	+
<b>Case 4 (142)</b>	+	-	+	+	+	+	-	+
<b>Case 5 (220)</b>	+	-	-	+	-	+	-	+

Symptom screen: yes to any one cardinal symptom of TB.CXR: radiological picture of active TB.

\*Protocol deviation as symptom screen and CXR negative.

#### 4.4 KEY FINDINGS

1. Based on voluntary participation, a 67% participation rate was achieved for the study with staff equally drawn from primary and secondary level facilities.
2. Participants were predominantly female (74%) and Xhosa speaking with nurses(27%) and support staff (27%) comprising the biggest occupational categories.
3. BCG coverage was virtually universal and 13% of staff indicated that they had previously been treated for TB.
4. There were significant differences in the demographic profiles of staff employed at primary and secondary level facilities. Primary care staff were younger, predominantly female, Xhosa-speaking, less likely to smoke, had a shorter duration of employment,were more likely to be HIV positive and to have had treatment for previous TB.
5. LTBI prevalence as measured by TST, QFT -GIT and T-SPOT-TB were 84%, 65% and 60% respectively for the group overall and surprisingly were not significantly different between primary and secondary level staff.
6. Despite a high proportion of individuals testing positive on symptom screen (26%) and chest x-ray (7%), only five new cases of tuberculosis was diagnosed. This together with two co-prevalent cases already on treatment resulted in a TB disease prevalence of 7/505 or 14/1000.
7. All cases detected by screening were diagnosed on sputum culture. Three cases were HIV positive and three had radiographic evidence of TB whilst a further three were positive on symptom screen.

8. Occupational exposure to TB patients was highly prevalent, with good awareness of the practice of infection control measures demonstrated (44 – 93%) and awareness of personal protective equipment (90%).
9. Despite a perception by 58% of participants that they were at high risk for contracting TB, 38% have had no training to protect themselves against TB.
10. Following multivariate analysis that adjusted for age and gender a positive TST was significantly associated with long duration of employment (>20 years) and negatively associated with a positive HIV status in primary level staff. In secondary level staff, training in infection control was negatively associated with TST positivity.
11. For QFT-GIT significant predictors of a positive test in primary level staff were long duration of employment (>20 years), performing sputum collection and exposure to environmental tobacco smoke. In secondary level staff, training in infection control was negatively associated with QFT-GIT positivity.
12. T-SPOT.TB positivity was associated with the performance of home-care to TB patients and paradoxically with the practice of infection control measures such as practice of cough etiquette and provision of disposable surgical masks by staff at primary level facilities.
13. Models generated to best predict test outcome shows male gender to be a consistent positive predictor across all test outcomes, whilst increasing age showed a positive, and BCG vaccination a negative association with IGRA outcomes.

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## **CHAPTER 5**

### **AGREEMENT BETWEEN IGRA ASSAYS AND TST AND A COMPARISON OF TEST PERFORMANCE USING LATENT CLASS ANALYSIS**

## CHAPTER 5

### 5.1 INTRODUCTION AND OBJECTIVES

Health care workers (HCWs) are known to be at increased risk of Tuberculosis (TB) infection and disease on account of occupational exposure to infectious TB patients (Baussano et al., 2011; Joshi et al., 2006; Menzies, Joshi & Pai, 2007). TB infection prevalence among HCWs in low and middle-income countries has been estimated to be 54% (range 33 – 79%) with an annual incidence of infection of 8.4% in high burden countries (i.e. >100/100 000 TB cases per population). In high burden communities within South Africa, the community prevalence of TB infection has been shown to be greater than 80%, posing a challenge for TB control in this country (Mahomed et al., 2006).

Until recently the tuberculin skin test (TST) was the only test available for evaluating TB infection. The TST measures a hypersensitivity response to purified protein derivative (PPD), a mixture of antigens some of which are common to *Mycobacterium tuberculosis*, *M Bovis* (the source of the Bacille Calmette-Guerin (BCG) and several nontuberculous Mycobacteria (NTM). Whilst the test has acceptable sensitivity, its specificity is suboptimal, in populations with BCG vaccination and significant infection with NTM.

Recent advances in immunology have led to promising alternatives to the TST, for diagnosis of LTBI (Menzies, Pai & Comstock, 2007; Pai, 2005; Pai, Kalantri & Dheda, 2006). These are the in-vitro interferon-gamma (IFN- $\gamma$ ) assays which use antigens such as the early secreted antigenic target 6 (ESAT-6) and culture filtrate protein (CFP-10). These proteins are encoded within the region of difference (RD1) of the *M tuberculosis* genome and are therefore more specific than PPD. Furthermore they are not shared by BCG strains or other NTM species that may lead to non-specific sensitization and

false positive reactions as with TST. IGRA assays have other advantages in that they require one visit, do not rely on trained readers, provide an objective laboratory measure of LTBI and may be used in serial testing without causing boosting. These assays are however more expensive and require laboratory infrastructure for processing. Furthermore there is limited research on the utility of IFN- $\gamma$  assays in high TB incidence countries.

Two IFN- $\gamma$  assays for diagnosing LTBI have been developed and licensed for commercial distribution. These are the QuantiFERON-TB Gold-In-Tube (QFT-GIT) (Cellestis Limited, Victoria, Australia) and the T-SPOT.TB (Oxford Immunotec, Oxford, UK). The QFT-GIT uses an enzyme-linked immunosorbent assay (ELISA) to measure antigen specific production of IFN- $\gamma$  by circulating T-cells in whole blood whilst the T-SPOT.TB measures peripheral blood mononuclear cells (PMBCs) that produce IFN- $\gamma$ , using an Elispot technique (Pai, Zwerling & Menzies, 2008). Research in low incidence settings have suggested that IFN- $\gamma$  assays have superior specificity, better correlation with recent TB exposure and are less affected by cross reactivity due to BCG vaccination (Pai, 2005). In such settings where serial screening for LTBI is generally implemented, IFN- $\gamma$  assays have been incorporated into screening protocols for HCWs (Mazurek et al. 2010; National Collaborating Centre for Chronic Conditions (UK) & Centre for Clinical Practice at NICE (UK), 2011).

The use of IGRAs instead of TST for one-time screening has resulted in a lower prevalence of positive tests than with TST and fewer HCWs who require LTBI treatment in low TB incidence settings. Studies among HCWs in low TB incidence countries that evaluated head-to-head comparison between TST and IFN-assays have shown varying levels of agreement and discordance between tests (Lien et al., 2009; Nienhaus et al., 2008; Zwerling et al., 2012a; Vinton et al., 2009). Whilst some of the discordance is driven by BCG vaccination, this in itself is not sufficient explanation for the discordance and has raised questions about their application in clinical

practice (Zwerling et al., 2012b). Very few studies involving a head-to-head comparison between IGRAs and TST have been conducted among HCWs in high TB incidence settings and whilst these have shown moderate to substantial agreement, the evidence base is currently too limited to support any recommendations for their routine use (Pai et al., 2009; Zwerling et al., 2012b; World Health Organization, 2010).

Meta-analyses and systematic reviews evaluating test performance have supported comparable sensitivity and superior specificity of IGRAs compared to TST. The context of such studies have in the main been low TB incidence settings (Pai, Zwerling & Menzies, 2008). Test performance has varied depending on BCG coverage of the study population. Data from high TB incidence settings are scarce and limited for high risk populations and immunocompromised persons.

In the absence of a gold standard for LTBI, studies have relied on active TB as a proxy measures for LTBI. These two disease states occur at opposite ends of the TB infection spectrum and are considered two completely different clinical entities. It is known that cellular immunity is compromised in TB disease. This may influence TST test response resulting in a falsely negative result. Latent class analysis (LCA) is a statistical method that has been widely used to estimate prevalence and diagnostic test sensitivity in the absence of results from a perfect diagnostic test or gold standard. It therefore has particular application in the context where multiple tests may be used to determine disease status in the absence of a true gold standard as in latent TB infection (Dendukuri, Hadgu & Wang, 2009; Dendukuri & Joseph, 2001; Pai et al., 2008). LCA modelling also allows for the consideration of conditional dependency as tests may not be independent of each other and may exhibit a degree of correlation, e.g. as between two IGRA assays, which may affect estimates.

This study conducted LTBI screening of a local HCW population, using a head-to-head comparison between TST and IGRA tests administered in a high TB incidence setting. This allowed for the determination of agreement and discordance between tests and factors associated with concordance and discordance. The application of LCA furthermore allowed for a comparison between the relative sensitivity and specificity of the tests for the diagnosis of LTBI, in the absence of a gold standard.

The data generated allowed for the calculation of estimates for the main outcomes of interest as previously outlined in the thesis objectives:

1. To determine the level of agreement between TST and IGRA.
2. To identify factors associated with discordance in health care workers in a high TB and HIV prevalence community.
3. To compare sensitivity and specificity of the LTBI diagnostic tests in a high TB incidence population.

## **5.2 METHODS**

### **5.2.1 Population and test outcomes**

All participants were required to undergo TST and venesection for QFT-GIT and T-SPOT.TB. TST was performed using 1 TU dose of PPD RT23 (Statens Serum Institut, Copenhagen, Denmark). Tuberculin was administered by a nurse experienced in administering the tuberculin skin test using the Mantoux method and skin induration was read after 48-72 hours. To prevent inter-reader variability only one person read the TST reaction, using a ruler and ballpoint method. An induration of at least 10mm was considered positive. In the case of known HIV infected individuals 5mm or more was considered a positive reading.

Participants were tested with the QFT-GIT and the T-SPOT.TB. Blood specimens for the IGRA assays were drawn concurrently with or within three days of administering the TST to eliminate the effect of potential boosting (van Zyl-Smit et al., 2009a; van Zyl-Smit et al., 2009b). The QFT-GIT test was considered positive if the interferon-gamma response minus the nil antigen was  $\geq 0.35$  IU/ml. The TSPOT.TB assay was performed in accordance with the manufacturer's instructions. The number of IFN- $\lambda$  spot forming T cells (SFC) per million peripheral blood mononuclear cells (PBMCs) was determined using an AIM ELISPOT reader and Oxford Immunotec software. A cut-off of six or more spots was treated as a positive result. To minimize inter-operator and inter-laboratory variability, all assays were done by the same operator in the same laboratory. Indeterminate results were treated as missing data and observations were not used in the agreement analyses.

### **5.2.2 Agreement statistics**

Statistical analyses were performed using Stata version 11 (Stata Corp, College Station, Texas). Outcomes that were evaluated included agreement between the tests and factors associated with discordance. The kappa statistic ( $\kappa$ ) was used to quantify agreement between tests and 95% confidence intervals were calculated for each kappa value. The kappa statistic measure of agreement is scaled to be 0 when the amount of agreement is equal to what would be expected to be observed by chance and 1 when there is perfect agreement. Kappa categories of agreement according to Landis and Koch are therefore: poor if below 0; slight if  $\geq 0.21$ ; fair if 0.21 – 0.40; moderate if equal to 0.41-0.60, substantial if 0.61 -0.80 and almost perfect if 0.81-1.00 (Munoz & Bangdiwala, 1997). In view of the variability observed in studies with serial administration of IGRAs and the potential impact of boosting of IGRA test response by TST, agreement analyses using higher cut-offs for test positivity were also performed on baseline results.

Agreement analyses were also carried out on the test outcomes generated from repeat testing of the cohort one year later. This agreement was also calculated on absolute values using standard cut-offs for test positivity and did not take into consideration the baseline test results.

Factors associated with discordance were explored using a multinomial logistic regression model that compared discordant groups with a comparison group displaying perfect agreement between test outcomes. Discordant groups for both IGRA assays were defined as having a positive TST/negative IGRA or a negative TST/ positive IGRA).

### **5.2.3 Latent Class analysis**

To estimate the sensitivity and specificity of different diagnostic tests, latent class analysis (LCA) was used. This is a statistical modelling technique that is based on the premise that the results of various imperfect tests for the same condition are influenced by a common underlying latent variable, which represents the true disease status (Christopher et al., 2010; Dendukuri, Hadgu & Wang, 2009; Pai et al., 2008). A pre-condition for a meaningful LCA model (i.e. for the model to be identifiable) is that the number of diagnostic tests used on the study sample must provide at least as many degrees of freedom as the number of parameters to be estimated. If not, then a Bayesian estimation approach can be used to place reasonable bounds on some parameters about which prior information is available. This would allow for the estimation of the remaining parameters conditional on this prior information.

For this study design that involved 3 dichotomous tests, the problem is identifiable if it is assumed that the results of individual diagnostic tests for a given disease status are independent (conditional independence) and that observed associations between tests are due solely to the latent variable.

However, this assumption is questionable as there are similarities in the technological properties and immunological mechanism underlying the two IGRA assays, which could potentially result in correlation between test results (conditional dependence). Therefore, an alternative model was also considered allowing for conditional dependence between QFT and TSPOT given subjects truly positive, and conditional dependence between QFT and TSPOT given subjects truly negative. The conditional dependence is assumed to be positive in both groups. This conditional dependence model is no longer identifiable necessitating the use of prior information.

In the analysis, a non-informative prior is used for the prevalence of LTBI which is unknown. Prior information on the accuracies of the three tests is elicited from Pai, Zwerling and Menzies (2008) using data on the sensitivity and specificity of TST and QFT-GIT based on systematic reviews and meta-analyses. The prior 95% CI of each parameter is constructed in such a way that the prior 95% CI covers all the 95% CIs from individual studies included in the meta-analysis. Therefore it is equivalent to the prediction interval resulting from the meta-analysis. Non-informative priors are used for the conditional dependence terms and the method introduced by Dendukuri and Joseph (2001) is used to construct the priors. The prior set is presented in Table 5.1.

In applying a latent class model, subjects with both determinate and indeterminate results were included as participants with indeterminate results in one test may still have relevance in the analysis on account of determinate results in the remaining two tests. A fixed effects model allowing for conditional dependence between QFT-GIT and TSPOT.TB was used to fit the data (Dendukuri & Joseph, 2001). WinBUGS software was used to analyse the data. Twenty thousand samples were drawn from the posterior distribution after discarding a burn-in of 1000 iterations. Convergence of the Monte Carlo Markov chain was assessed using the Gelman-Rubin statistic which is named BGR within WinBUGS.

**Table 5.1 Prior information on sensitivity and specificity of tuberculin skin-test and IGRA assays\***

Parameter	Prior distribution range (95% CI)
TST sensitivity	43 – 100
TST specificity	27 – 100
QFT-GIT sensitivity	52 – 99
QFT-GIT specificity	67 – 100
TSPOT.TB sensitivity	63 – 100
TSPOT.TB specificity	77 – 100

**\*Dendukuri and Joseph, 2001**

## **5.3 RESULTS**

### **5.3.1 Participants with TST and IGRA assay outcomes**

At baseline a valid outcome measure for TST was available for 484 participants who had undergone TST and had a reading recorded. For participants who had a QFT-GIT test administered, valid results were available for 496 as one test result was indeterminate. For T-SPOT.TB the indeterminate rate was higher and only 465 test results were valid. Valid test pairs were available for comparison between TST and QFT-GIT for 482 participants and between TST and T-SPOT.TB for 450 participants. At follow-up 339 participants had repeat IGRA assays done but only 147 had repeat TST administered as many participants refused repeat TST on account of discomfort experienced following the TST administered at baseline. This resulted in 147 participants with both TST and QFT-GIT and 138 participants with both TST and T-SPOT.TB that could be used in agreement analyses at follow-up. For T-SPOT.TB and QFT-GIT there were 315 participants with valid test results for both assays at follow-up.

### **5.3.2 Agreement and discordance between TST and IGRA assays**

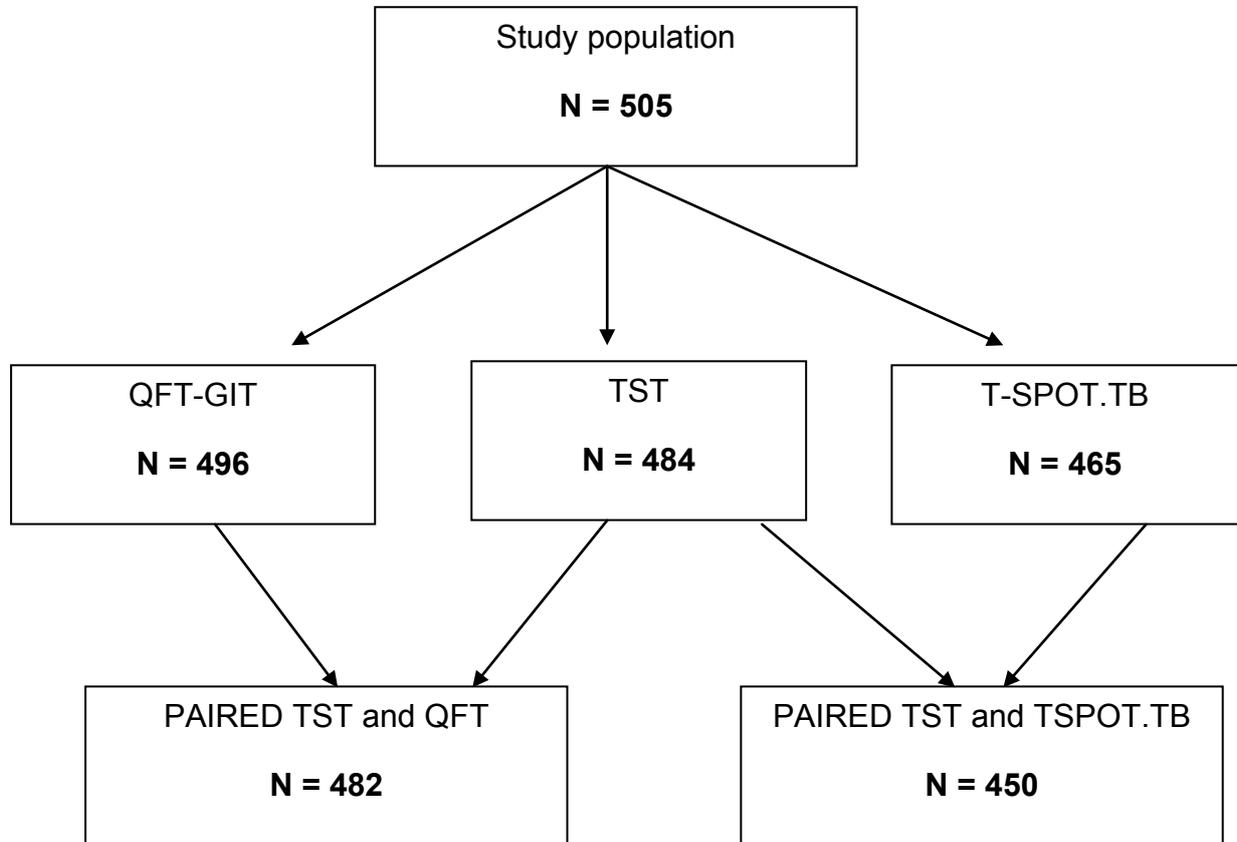
There was only fair agreement between TST and QFT – GIT [ $\kappa=0.28$ , CI 0.19 – 0.39] as well as between TST and T-SPOT.TB [ $\kappa =0.25$ , CI 0.18 – 0.33] (Table 5.2). Agreement between the two IGRA assays as expected was far higher at 83.7% [ $\kappa = 0.65$ , 95% CI 0.56 – 0.74] which is indicative of substantial agreement. Participants were followed up at one year interval and TST and IGRA assays were repeated, allowing for agreement comparison of tests performed at follow-up. Upon repeat testing at one year, agreement between QFT-GIT and TST improved from fair to moderate. This was however calculated on absolute values using standard cut-offs for test positivity and did not take into consideration prior test results.

### **5.3.3 Agreement and discordance between TST reactions and IGRA assays**

At baseline agreement analysis was performed between the IGRA assays and three different cut-offs for TST ( $\geq 5$ ,  $\geq 10$  and  $\geq 15$  mm) without taking HIV status into consideration (Table 5.3). Agreement between QFT-GIT was fair and remained marginally unchanged across the different cut-offs for TST with slight improvement in kappa values from 0.25 – 0.29. Agreement between TST and TSPOT.TB was fair with slight improvement as TST induration increased ( $\kappa = 0.21 - 0.30$ ).

Because of uncertainty around IGRA cut-offs and a high degree of natural variability in test results obtained from sequential screening, agreement analyses were performed using different cut-offs. Agreement between TST  $\geq 15$ mm and QFT-GIT of 0.70 IU/ml which is twice the cut-off currently recommended as indicative of a positive test, did not show any improvement [ $\kappa = 0.27$ , 95% CI 0.18 – 0.36]. Similarly using the more conservative cut-off of an 8 spot increment to denote a positive T-SPOT.TB and comparing this to a TSTS  $\geq 15$  mm did not alter the level of agreement [ $\kappa = 0.28$ , 95% CI 0.19 – 0.37] when compared to conventional cut-offs that denote test positivity.

**Figure 5.1. TST and IGRA tests administered at baseline**



**Table 5.2 Agreement and discordance between TST and IGRA assays at baseline and follow-up**

	BASELINE		FOLLOW-UP	
	QFT-GIT (N=482)	TSPOT.TB (N=450)	QFT-GIT (N=147)	TSPOT.TB (N=138)
Positive TST and positive IGRA assay	293	249	97	81
Negative TST and negative IGRA assay	53	55	20	19
Positive TST and Negative IGRA assay	112	126	26	33
Negative TST and positive IGRA assay	24	20	4	5
Agreement (%)	71.8%	67.6%	79.6%	72.5
Kappa (95% CI)	0.28 [0.20 – 0.36]	0.25 [0.18 – 0.33]	0.45 [0.31 – 0.60]	0.34 [0.20 - 0.49]

TST≥10mm or ≥5mm if HIV positive used to denote a positive TST test response at baseline.

**Table 5.3 Agreement and discordance between TST reactions and IGRA assays at baseline using different cut-offs**

	<b>TST<math>\leq</math>5</b>	<b>TST<math>\leq</math>10</b>	<b>TST<math>\leq</math>15</b>
<b>QFT-GIT (N=482)</b>			
Positive TST & positive IGRA assay	307	293	257
Negative TST & negative IGRA assay	39	53	77
Positive TST & Negative IGRA assay	126	112	88
Negative TST & positive IGRA assay	10	24	60
Agreement (%)	71.8%	71.8%	69.3%
Kappa (95% CI)	0.25 [0.18 – 0.31]	0.28 [0.20 – 0.36]	0.29 [0.20 – 0.37]
<b>TSPOT.TB (N=450)</b>			
Positive TST and positive IGRA assay	259	249	221
Negative TST and negative IGRA assay	40	55	84
Positive TST and Negative IGRA assay	141	126	97
Negative TST and positive IGRA assay	10	20	48
Agreement (%)	66.4%	67.8%	67.8%
Kappa (95% CI)	0.21 [0.14 – 0.28]	0.25 [0.16 – 0.33]	0.30 [0.21 – 0.39]

### 5.3.4 Agreement and discordant test responses

Discordant test responses were evaluated using a multinomial regression analysis that compared discordant with concordant test responses which served as the reference group. For a TST positive/QFT-GIT negative discordant test response there were no significant predictors and specifically no association with BCG vaccination. Whilst there was a significantly negative association between years in healthcare employment and a discordant (TST - / QFT-GIT+) test response (OR=0.94, 95% CI 0.89 – 0.99) i.e. longer serving employees were more likely to have a discordant test response (TST- / QFT-GIT+). Recent exposure defined as a recent contact with an infectious TB patient was not significantly associated with discordance.

Negative predictors of discordance (TST+/TSPOT.TB-) were older age (OR=0.97, CI 0.95 – 0.99) and being engaged in home-based care (OR=0.32, 95% CI 0.11 – 0.94). Participants with a discordant test response (TST-/ TSPOT.TB +) were more likely to be HIV positive, have had TB treatment previously and to test positive on symptom screen when compared to those with concordant tests responses. They were also more likely to be working in a facility that practiced triaging of TB patients as a measure of infection control. As with TST-/ QFT+ discordance, TST-/ TSPOT.TB + discordance were negatively associated with years in healthcare employment.

Discordance between IGRA assays were similarly evaluated using regression analysis. Being HIV positive was strongly associated with a discordant (T- SPOT.TB+/ QFT-GIT-) test response as was being engaged in home-based care and being a Xhosa speaker (Table 5.6). Negative predictors of this type of discordance were being engaged in occupational tasks such as counselling of TB patients, sputum collection and nursing care of TB patients. Having a health qualification and working at a facility that

provided personal protective equipment (PPE) (*during performance of high risk procedures*) were similarly negatively associated with this pattern of discordance.

No positive associations were found for QFT+/ T-SPOT.TB- discordance. Negative associations were demonstrated for age and for certain occupational factors such as being engaged in clerking of TB patients, working at facility that practices cough etiquette and provision of 95 respirators and PPE (*during performance of high risk procedures*).

### **5.3.5 Latent class analysis**

A comparison between test performance using a traditional latent class analysis model assuming conditional independence and one that takes account of conditional dependency between IGRA assays (fixed effect model) is shown in Table 5.7. In the fixed effect model sensitivity is highest for TST (93.3%), followed by QFT-GIT (84.1%) and T-SPOT.TB (78.6%). Specificity on the other hand is lowest for TST (54.7%), followed by QFT-GIT (93.2%) and T-SPOT.TB (96.1%).

The estimate of LTBI in this model was 77% (95% CI 63.8 – 91) and slightly higher than the prevalence generated by the traditional LCA model at 69.3% [95% CI 63.1 -74.6]. TST test performance showed an improvement in sensitivity and specificity with the application of a fixed effects model, whilst the values for both IGRA assays declined. In general, the confidence intervals for test sensitivity and specificity generated from the application of a fixed effects model all fell within the prior distributions, which were estimated from previous studies.

**Table 5.4 Results of multinomial regression for discordant QuantiFERON-TB Gold-in-tube and tuberculin skin test**

Risk Factor	Positive TST and negative QFT-GIT test result	Negative TST and positive QFT-GIT test result
	OR (95% CI)	OR (95% CI)
	N=112	N=24
Older age, per each additional year	0.99 (0.70 – 1.01)	1.01 (0.98 – 1.05)
Male gender	0.93 (0.57 -1.52)	1.15 (0.46 – 2.87)
BCG Vaccination	1.93 (0.79 – 4.71)	1.20 (0.27 – 5.32)
HIV Positive / reported as positive	1.12 (0.55 – 2.25)	1.97 (0.63 –6.16)
Previous TB Treatment	1.03 (0.55 – 1.94)	1.34 (0.44 – 4.09)
Symptom screen positive	0.67 (0.40 -1.13)	1.56 (0.66 – 3.69)
Years in healthcare	0.99 (0.97 – 1.01)	<b>0.94 (0.89 – 0.99)*</b>
Home care of TB	0.73 (0.31 – 1.71)	0.99 (0.22 – 4.44)
Separation of TB patients	1.00 (0.65 – 1.53)	2.30 (0.96 – 5.51)
Recent contact with TB patients	0.75 (0.46 – 1.2 )	0.70 (0.28 – 1.74)
Daily contact with TB patients	0.78 (0.39 – 1.5	1.12 (0.25 – 4.99)

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001

**Table 5.5 Results of multinomial regression for discordant TSPOT.TB and tuberculin skin test**

Risk Factor	Positive TST and negative TSPOT.TB test result	Negative TST and positive TSPOT.TB test result
	OR (95% CI)	OR (95% CI)
	N=126	N=20
Older age, per each additional year	<b>0.97 (0.95 – 0.99) **</b>	1.00 (0.96 - 1.04)
Male gender	0.93 (0.58 -1.50)	1.22 (0.45 – 3.29)
BCG Vaccination	1.22 (0.55 – 2.67)	1.78 (0.23 – 13.81)
HIV Positive / reported as positive	1.03 (0.50 – 2.11)	<b>4.72 (1.64 – 13.59) **</b>
Previous TB Treatment	0.88 (0.46 – 1.68)	<b>3.00(1.09 – 8.28)*</b>
Symptom screen positive	1.05 (0.65 -1.68)	<b>2.95 (1.18 – 7.35)*</b>
Years in healthcare	0.98 (0.96 – 1.0)	<b>0.94 (0.88 – 1.00)*</b>
Home-care	<b>0.32 (0.11 – 0.94)*</b>	1.74 (0.48 – 6.30)
Separation TB patients	1.00 (0.66 – 1.52)	2.66 (1.00 – 7.11)
Recent contact with TB patient	0.74 (0.46 – 1.19)	0.63 (0.23 – 1.72)
Daily contact with TB patient	0.88 (0.45 – 1.71)	2.08 (0.27 – 16.09)

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001

**Table 5.6 Results of multinomial regression for discordant QFT-GIT and T-SPOT.TB**

Risk Factor	Positive QFT-GIT and negative T-SPOT.TB test result	Negative QFT-GIT and positive T-SPOT.TB test result
	OR (95% CI)	OR (95% CI)
	N=47	N=29
Older age, per each additional year	<b>0.97 (0.95 – 1.00)*</b>	0.97 (0.92 - 1.01)
Male gender	0.98 (0.50 -1.97)	1.09 (0.47 – 2.55)
BCG Vaccination	0.46 (0.19 – 1.12)	1.09 (0.25 – 4.83)
HIV Positive / reported as positive	1.18 (0.44 – 3.18)	<b>5.11 (2.11 – 12.33) ***</b>
Previous TB Treatment	0.29 (0.07 – 1.22)	1.34 (0.49 – 3.67)
Symptom screen positive	1.35 (0.70 -2.59)	1.09 (0.47 – 2.55)
Years in healthcare	0.97 (0.93– 1.00)	0.97 (0.93 – 1.01)
Home-care	0.26 (0.03 – 1.94)	<b>3.10 (1.17 – 8.21)*</b>
Separation TB patients	1.29 (0.71 – 2.37)	1.61 (0.75 – 3.47)
Recent contact with TB patient	0.77 (0.39 – 1.52)	0.77 (0.33 – 1.80)
Daily contact with TB patient	0.59 (0.24 – 1.40)	0.64 (0.21 – 1.94)
<b>Xhosa home language</b>	1.15 (0.63 – 2.12)	<b>2.26 (1.00 – 5.08)*</b>
<b>Health qualification</b>	0.64 (0.33 – 1.24)	<b>0.32 (0.12 – 0.84)*</b>
<b>Clerking TB patients</b>	<b>0.43 (0.22 – 0.82)*</b>	0.61 (0.28 – 1.33)
<b>Sputum collection</b>	0.51 (0.21 – 1.25)	<b>0.13 (0.02 – 0.93)*</b>
<b>Nursing care</b>	0.66 (0.32 – 1.33)	<b>0.25 (0.07 – 0.83)*</b>
<b>Cough Etiquette</b>	<b>0.48 (0.25 – 0.92)*</b>	0.95 (0.37 – 2.42)
<b>N-95 masks</b>	<b>0.33 (0.13 – 0.82)*</b>	0.36 (0.11 – 1.13)
<b>PPE provision</b>	<b>0.34 (0.15 – 0.78)*</b>	<b>0.31 (0.12 – 0.82)*</b>

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001

**Table 5.7 Sensitivity and specificity of three diagnostic assays for tuberculosis infection estimated among healthcare workers in Cape Town, South Africa by using a latent class model (LCA) with and without conditional dependency (N=472)**

LTBI test	Sensitivity [95% CI]		Specificity [95%CI]	
	LCA	LCA with fixed effect	LCA	LCA with fixed effect
Tuberculin skin test	93.1 [89.9 – 95.8]	93.3 [90 – 96.4]	38.1 [30 – 46.3]	54.7 [32.0 – 99.7]
QuantiFERON-GIT	92.3 [88.4 – 96.2]	84.1 [71 – 94.5]	94.4 [84 – 100]	93.2 [79.4 – 100]
T-SPOT.TB	86.9 [81.7 – 93]	78.8 [65.7 – 90.9]	97 [89.6 – 100]	96.1 [85.1 – 100]

Covariates incorporated into the model to account for conditional dependency between QFT-GIT and TSPOT.TB. Covariate 1 for conditional dependence for subjects truly positive [mean= 0.058, 95% CI 0.0015 – 0.134] and covariate 0 for conditional dependency for subjects truly negative [mean=0.012, 95%CI 1.0 e<sup>-5</sup> – 0.066]

## 5.4 KEY FINDINGS

1. There was only fair agreement between TST and QFT-GIT as well as between TST and TSPOT.TB as reflected in  $\kappa$  values of 0.28 and 0.25 respectively.
2. Agreement between the two IGRA assays as expected was substantial with  $\kappa=0.65$ .
3. Varying the TST cut-off did not enhance agreement with the IGRA assays.
4. Varying the IGRA cut-offs to higher levels than what is conventionally used to denote a positive test did not enhance agreement with  $TST \geq 15\text{mm}$ .
5. Contrary to other studies, discordant test responses were not associated with BCG vaccination or recent TB infection.
6. TST negative / IGRA positive discordance was negatively associated with duration of employment in healthcare for both IGRA assays suggesting that participants with long duration of employment would tend to have concordant results.
7. Negative predictors of discordance (TST+ / T-SPOT.TB-) were older age and being engaged in home-based care.

8. Positive predictors of a discordant test response (TST- / T-SPOT.TB +) were HIV infection, a history of previous TB treatment, testing positive on symptom screen or working in a facility separated TB patients from others, as part of infection control.

9. There was also discordance between IGRA assays primarily related to HIV status, being engaged in home-based care and being a Xhosa speaker. Negative predictors of IGRA discordance were in the main related to occupational tasks and the practice of infection control measures.

10. The markedly increased risk of a discordant test response in HIV-infected participants (OR=4.72) has been replicated in other studies in similar settings and suggests that T-SPOT.TB test performance is relatively unimpaired by HIV infection and potentially more sensitive than TST for detecting LTBI in HIV-infected participants (Rangaka et al., 2007; Mutsvangwa et al., 2010). The impairment of TST reaction may also be due to impaired immunity manifesting as anergy.

11. The finding that those participants with previous TB or who were symptom screen positive were also more likely to have a discordant test response (TST- / T-SPOT.TB+) is unexplained. Previous TB may be considered a marker of remote exposure (whilst also a risk factor for developing TB) and therefore one would expect less correlation with T-SPOT.TB as positive IGRAs are generally considered to be indicative of recent exposure. Unexplained discordance has been described in studies from both low and high TB incidence settings. It is postulated that both the epidemiological context and the use of standard cut-offs may contribute to such discordance (Pai, Kalantri & Menzies, 2006; Pai & Menzies, 2007).

12. Using Latent class analysis and a fixed effect model showed TST to have the highest sensitivity, followed by QFT-GIT and T-SPOT.TB with specificity lowest for TST followed by QFT-GIT and T-SPOT.TB. The differences in these estimates of sensitivity and specificity cannot however be considered statistically significant as there was still some overlap in confidence intervals for the three tests with the largest difference demonstrated between the sensitivity of TST and T-SPOT.TB.

13. The use of a fixed effects model was associated with some improvement in specificity and sensitivity for TST but not for IGRAs.

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## **CHAPTER 6**

### **ANNUAL INCIDENCE OF TB INFECTION IN HEALTH CARE WORKERS AND FACTORS ASSOCIATED WITH INCREASED RISK OF TEST CONVERSION**

## CHAPTER 6

### 6.1 INTRODUCTION AND OBJECTIVES

Health care workers (HCWs) are globally considered a high risk occupational grouping for contracting tuberculosis (TB)(Baussano et al., 2011; Christopher et al., 2011; Joshi et al., 2006; Menzies, Joshi & Pai, 2007). Whilst TB in HCWs is a growing public health problem, data from high TB incidence countries (> 100/ 100 000 TB cases per population) is limited. In such countries, the median annual risk of new latent tuberculosis infection (LTBI) in HCWs is estimated at 7.2 % [Interquartile range (IQR) 4.1 - 14.3%], whilst the median incidence of TB disease has been estimated to be 1180 / 100 000 (IQR 91-3 222) (Baussano et al., 2011). When compared to a population incidence of 311/ 100 000, the median estimated annual incidence rate ratio for active TB disease is 5.4 (IQR 1.7 -9.1) whilst the median difference is 409/100 000 (IQR 166 – 2817).These findings suggest that in high incidence settings 81% of TB cases in health care settings are occupationally acquired.

In South Africa, a high TB incidence country, there are limited data to quantify HCWs' risk of TB. Serial screening for LTBI is not routinely performed. Despite the proven efficacy of Isoniazid prophylaxis (IPT) as a means to prevent progression from LTBI to TB disease, this is also not routinely offered to HCWs in South Africa (Akolo et al., 2010; Lobue & Menzies, 2010; Tshitangano, 2013). Under the South African National TB program, those at highest risk of progression from LTBI to TB disease are offered IPT viz. children under the age of five years (who has had contact with a TB case) and HIV infected individuals. This is not necessarily always accompanied by testing for LTBI (Department of Health, South Africa, 2009; Department of Health, South Africa, 2013).

Whilst early studies conducted among South African HCWs showed no increased risk of tuberculosis compared to background, an increasing TB incidence in this population has been demonstrated in tandem with a growing HIV epidemic (Balt, Durrheim & Weyer, 1998; Naidoo & Jinabhai, 2006; Wilkinson & Gilks, 1998). More recent studies have demonstrated a consistently higher incidence of TB and markedly greater risk of drug-resistant tuberculosis in HCWs than the general population (Jarand et al., 2010; Naidoo & Jinabhai, 2006; O'Donnell et al., 2010, Claassens et al., 2013). This finding has been replicated in smaller studies conducted among specific groups working in health care settings such as TB field researchers and community HCWs in the Western Cape (Claassens et al., 2010; Kranzer et al., 2010).

There is increasing recognition that retention of skilled HCWs in the public health system is crucial to the implementation of programmes that address the large TB and HIV burden (Department of Health, South Africa, 2011). This has resulted in global advocacy for better protection of HCWs in the prevention, diagnosis and management of tuberculosis and HIV (WHO, UNAIDS & ILO, 2010). However, implementation of infection control measures, allocation of resources, and rational planning of intervention strategies require accurate estimates of the scale of the problem. Moreover, the success of preventative strategies, including infection control measures and targeted prophylaxis, needs to be measured through the use of validated tools and accurate estimates of the incidence of LTBI and disease progression in high risk populations or settings (Claassens et al., 2013; Farley et al., 2012). There are, however, a lack of data to inform prevention, diagnostic, and management strategies in this high-risk population in TB-endemic countries such as South Africa (Zungu & Malotle, 2011; Adams et al., 2012).

Traditionally, screening for LTBI in HCWs in low burden settings was done using the tuberculin skin test (TST). More recently the interferon-gamma release assays (IGRAs) have been the focus of interest for the diagnosis of LTBI. There are two commercially available IGRA assays: the T-SPOT.TB assay (Oxford Immunotec, Abingdon, UK) and the QuantiFERON-TB Gold-In-Tube (QFT-GIT) assay (Cellestis Ltd, Carnegie, Australia). These in-vitro assays have some advantages over the TST in that they only require one visit, are relatively easy to administer, are not subject to boosting, and deliver objective results. Their drawback is the cost and requirement for laboratory infrastructure, which may not be readily available in resource-constrained settings (Pai, 2005). IGRAs have been incorporated into testing guidelines in countries such as the USA and UK where serial LTBI testing of HCWs is routine (Mazurek et al., 2010; National Collaborating Centre for Chronic Conditions (UK) & Centre for Clinical Practice at NICE (UK), 2011). Serial screening with IFN- $\gamma$  assays have however been plagued by the problem of variability in results obtained from sequential administration (van Zyl-Smit et al., 2009a; van Zyl-Smit et al., 2009b). This has raised questions about their short-term reproducibility and whether current definitions of conversion is indicative of new infections or a reflection of test variability (Pai et al., 2006b; Pai & O'Brien, 2007; Zwerling et al., 2013; Gran, Assmuss & Dyrholm-Riise, 2013; Slater et al., 2013; Fong et al., 2012). This has led to many advocating for a review of the current cut-offs for test positivity so that it better reflects new infections versus false positives. This has resulted in the proposal of borderline zones of uncertainty around the current recommended assay cut-offs for both QFT-GIT (0.2 -0.7 IU/ml versus 0.35 IU/ml) and for T-SPOT.TB (5-7 spots versus 6 spots) (Mazurek et al., 2010; Zwerling et al., 2013). IGRA assays are subject to boosting by TST, if concurrently administered. This may potentially confound subsequent IGRA test responses. Data are currently very limited on serial screening using T-SPOT.TB as most studies have utilized QFT-GIT for serial screening of HCWs (Zwerling et al, 2012).

IFN- $\gamma$  responses over time appear to be influenced by the epidemiologic setting and it is unlikely that a uniform threshold would apply to all populations and settings. There is a need for further research focusing on within subject variability and test performance in serial screening. Specifically the utility of serial screening using IFN- $\gamma$  assays in high TB incidence settings with near universal BCG coverage is not known. TB infection incidence, if accurately measured, might provide some indication of TB risk in healthcare populations and the need for infection control measures in health care settings.

This chapter includes data from the follow-up phase of the study and represents the first prospective TB infection study among HCWs in South Africa. In fulfilment of the thesis objectives, this chapter of the study addresses the following objectives:

1. To document the prevalence and annual incidence of latent TB infection based on conversion rates using both IGRAs and TST in serial testing of health care workers.
2. To evaluate the occupational and environmental risk factors associated with incident TB infection (based on LTBI test conversion) in health care workers and explore the potential role of occupational exposure as a contributing factor to high TB infection rates.

## **6.2 METHODS**

### **6.2.1 Participation in the follow-up phase of the study**

This phase of the study was performed over 1 year from July 2010 to July 2011 and represents the follow-up phase of the baseline study that was conducted in 2009-2010 to evaluate determinants of prevalent latent TB

infection (discussed in chapter 4) in HCWs employed at seven health care facilities in Cape Town, South Africa. All 505 participants were eligible for participation at follow-up and were required to complete a short follow-up questionnaire including a TB symptom screen, and undergo repeat testing with TST and IGRA assays (QFT-GIT and T-SPOT.TB) to evaluate interval test response over a one year period. A chest radiograph was also offered as an additional screening tool to exclude active TB and participants were invited to undergo a repeat HIV test. Due to the non-availability of the mobile clinic from which to conduct the research, the follow-up phase of the study was in the main conducted from a dedicated space at the health facilities that formed part of the study.

Multiple attempts were made to contact participants who had participated in the baseline of the study to continue their participation in the follow-up phase of the study. These include bulk text messages sent via mobile phone technology, individual telephone calls to work telephones and personal cellphone numbers where these were available. Cross referencing was done with the facility human resource administration to try and obtain contact details of participants who had left the facility to minimize loss to follow-up. HCWs' right to refuse further participation was however respected.

### **6.2.2. Structured questionnaire**

A short structured questionnaire was administered by a trained nurse interviewer, who had initially also conducted the baseline interview. The questionnaire included a TB symptom screen and elicited updated personal and employment details. Participants were also asked about recent TB diagnosis, interim TB symptoms that may not have been present at baseline and HIV status. In addition questions regarding preferred models of occupational health service provision to HCWs with a specific focus on HIV and TB, were also included (This questionnaire is included as appendix 2).

### **6.2.3 TST testing and interpretation**

The one-step TST protocol was employed using two tuberculin units (0.1ml) of RT23 Purified Protein Derivative (PPD) (Statens Serum Institut Copenhagen), injected intradermally on the volar aspect of the forearm. The induration was measured by a trained reader after 48 – 72 hours using the ballpoint and ruler method. An induration of  $\geq 10$ mm, or in the case of an HIV positive individual  $\geq 5$ mm, was considered a positive test at baseline. As the study aim was to evaluate serial test responses, all participants regardless of TST status at baseline were requested to undergo repeat testing. A TST conversion was defined as a previously negative test ( $< 10$  mm, or  $< 5$ mm if HIV infected) that changed to a positive test together with at least a  $\geq 10$ mm increment, as per American Thoracic Society/Centers for Disease Control and Prevention and Infectious Diseases Society of America standards standard guidelines (American Thoracic Society, 2000). A reversion was defined as a positive test at baseline changing to a negative test at follow-up.

### **6.2.4 IGRA testing (QFT-GIT and T-SPOT.TB)**

Participants were re-tested with the QFT-GIT and the T-SPOT.TB tests irrespective of baseline IGRA test result. Bloods for the IGRA assays were drawn concurrently with or within three days of administering the TST to eliminate the effect of potential boosting, which may manifest after this initial period (van Zyl-Smit et al., 2009b). The QFT-GIT test was considered positive if the interferon-gamma response minus the nil antigen was  $\geq 0.35$  IU/ml. A QFT-GIT conversion was defined as a change from a negative test (IFN- $\gamma < 0.35$  IU/ml) to a positive test (IFN- $\gamma > 0.35$  IU/ml) as per the manufacturer's instructions. Reversion was defined as change from a positive test at baseline to a negative test at follow-up.

The T-SPOT.TB assay was performed in accordance with the manufacturer's instructions. The number of IFN- $\lambda$  spot forming T cells (SFC) per million peripheral blood mononuclear cells (PBMCs) was determined using an AIM ELISPOT reader and Oxford Immunotec software. A cut-off of six or more spots was treated as a positive result. To minimize inter-operator and inter-laboratory variability, all assays were done by the same operator in the same laboratory. For T-SPOT.TB, a negative test at baseline changing to a positive test ( $\geq 6$  spots increment in either Panel A or B) on follow-up testing was required. Reversion was defined as a baseline positive test with a negative test at follow-up. Various cut points for conversion were explored for both IGRAs and for TST.

#### **6.2.5 Statistical analysis**

Statistical analyses were performed using Stata version 11 (Stata Corp., College Station, Texas). Outcomes evaluated included test positivity at follow-up for TST, QFT-GIT and T-SPOT.TB. The prevalence of TB associated risk factors, occupational and environmental factors and test outcomes was calculated at baseline for this cohort. At follow-up the main outcome of interest was annual rate of TST and IGRA conversion which was used to define annual incidence of infection. Differences between participants included in the follow up and those lost to follow-up were explored using Chi-squared statistics, to evaluate the likely presence of attrition bias. Further analysis focused on identifying predictors of TST and IGRA conversion. Univariate and multivariate logistic regression analysis to explore the strength of associations with conversion were performed. An annual incidence of infection on the different tests was calculated.

## **6.3 RESULTS**

### **6.3.1 Demographic characteristics and participation**

The follow-up phase of the study commenced 1 year after the baseline. A total of 339 health care workers were followed up from the original cohort of 505, a follow-up rate of 67%. The main reason for participants being lost to follow-up was leaving the facility (91 participants). Most had left for unknown reasons (68), three had resigned, three had retired and six were students who had completed their rotation, whilst another eleven had been transferred out (Figure 1). There were significant differences between participants who were followed up and those lost to follow up. Participants who continued with the second phase of the study were older ( $p=0.007$ ), less likely to smoke ( $p=0.006$ ), less likely to be HIV positive ( $p=0.026$ ) and more likely to have had long duration of employment ( $>20$  years) in a health care environment ( $p=0.004$ ).

All participants in this phase completed the questionnaire. HIV results were available for 329 participants and chest radiographs for 288 participants. A substantial proportion (56%) declined to have a repeat TST administered so that only 142 participants had both a baseline and follow-up TST reading.

Uptake of repeat interferon gamma release assays was excellent, with 99% of participants undergoing repeat testing. This resulted in 332 participants having valid paired results for QFT-GIT and 292 for T-SPOT.TB.

The population consisted of predominantly females (71%) with 58% over the age of forty years (Table 6.1). Duration of employment in a health care environment was less than 10 years for the majority of participants (53%) whilst 30% reported a long duration of employment ( $>20$  years).

### **6.3.2 Occupational and environmental characteristics**

Involvement in rendering health care services to TB patients ranged from 2% for bronchoscopy procedures to 49% for interviewing patients (Table 6.2). High-risk procedures such as nursing of TB patients and collection of sputum samples were performed by 30% and 22% of respondents respectively.

There was varying reported facility compliance with environmental control measures for TB infection control with 64% of participants reporting that their facility had a specific infection control policy. Most displayed an awareness of ventilation measures being implemented at their facility (93%), whilst reported institutional compliance with the provision of personal protective equipment was high (89 to 95%).

### **6.3.3 Prevalence of TB associated risk factors and LTBI at follow-up**

The prevalence of BCG vaccination was high at 92% (Table 6.3). Three participants who had previously been negative at baseline, had a positive HIV test at follow-up representing an annual HIV infection incidence of 1% (95% CI 0.02 – 3). Two new cases of TB were diagnosed at follow-up and one participant not previously diagnosed had died of TB in the interval, yielding a TB incidence of 9/1000 [1%; 95% CI 0.2 – 2.6]. LTBI prevalence was high at 84% for TST, and 69% and 62% for QFT-GIT and T-SPOT.TB respectively.

### **6.3.4 TST and IGRA Conversions and Annual Incidence of TB infection**

The conversion rate represents the annual incidence of new TB infection in this group as it measures new infections in those at risk after 1 year of observation. At follow-up testing 13 out of 34 participants converted from a

negative to a positive TST, representing an annual rate of infection of 38% (95% CI: 22 – 56). One percent (4 participants) reverted from a previously positive to a negative test result (Table 6.4).

For QFT-GIT, of those who were negative at baseline, 25/113 converted to a positive test representing an annual rate of infection of 22% (95% CI: 15 – 31). The reversion rate was 7%.

For T-SPOT.TB, owing to a higher yield of indeterminate results, only 292 participants test results were utilized to generate conversion and reversion estimates. Of those who tested negative at baseline, 25/114 converted their test response to positive one a year later, representing a conversion rate of 22% (95% CI: 15 – 31). The reversion rate for this IGRA assay at 16% was considerably higher than that demonstrated by TST (Table 6.4).

Different cut-offs were explored for IGRA conversion on account of the natural variability inherent in repeat use of these tests. For QFT-GIT, using the most stringent definition with a cut-off of 0.70 IU/ml, the conversion rate declined to 13%, whilst for T-SPOT.TB using an eight spot increment, it declined to 18% (Table 6.5).

### **6.3.5 Univariate and Multivariate Analysis of Determinants of TST and IGRA Conversion**

Adjusted logistic regression models were applied to evaluate the relationship between a number of variables of interest and the conversion outcomes, treating age and gender as covariates (Table 6.6). In univariate analysis strongly positive associations ( $OR \geq 2$ ) were shown for TST conversion in those participants with the following characteristics: age > 40 years, exposure to environmental tobacco smoke, engaging in collection of sputum, TB symptom screen positive at follow-up and those who had a referral for

HIV care and or TB investigations in the screening interval. However, none of these associations had confidence intervals which excluded the null. Following multivariate analysis, only the following associations remained significant for TST. Individuals engaged in counselling TB patients were less likely to have a TST conversion [OR=0.12; (95% CI 0.15 – 0.92)].

This same factor was however strongly associated with an increased risk of QFT conversion [OR=3.04; (95% CI 1.01 – 9.15) P=0.047]. Males were also four times more likely to have a QFT-GIT conversion than female participants in the study [OR=4.02 (95% CI 1.40 – 11.58)].

Positive predictors of T-SPOT.TB conversion in adjusted analysis were employment sector-individuals in the local authority employ were 14 times more likely to convert than those working for the provincial health department [OR=14.19 (95% CI 1.28 – 157.75) P=0.031] and having a positive TST at baseline [OR=3.40; (95% CI; 1.02 – 11.34) P=0.047].

Comparison with community based studies among adolescents in the Western Cape showed that health care workers have a greater than threefold risk of annual infection as measured by TST conversion. This risk is fivefold greater than healthcare workers in other high TB incidence populations (Middelkoop et al., 2008) (Table 6.7).

**Table 6.1 Characteristics of participants who underwent repeat testing for latent TB infection by either TST and/or IGRA (N=339)**

Variable*	N= 339	%
<b>Gender</b>		
Female	241	71
Male	98	29
<b>Age group (years)</b>		
≤30	54	16
31- 40	90	27
41 – 50	98	29
>50	97	29
<b>Type of facility</b>		
Primary	173	51
Secondary	166	49
<b>Employment in health care</b>		
<10 years	178	53
10 – 19 years	60	18
≥20	101	30
<b>*Employment sector (N=334)</b>		
Provincial Health Dept.	209	63
City of Cape Town Health Dept.	23	7
NGO	63	19
Other	39	12
Formal Health Qualification	131	39
Diabetes (current)	29	9
Current alcohol consumption	109	32
<b>*Smoking history (N=338)</b>		
Non-smoker	216	64
Ex-smoker	46	14
Current smoker	76	22
Exposure to environmental tobacco smoke	243	72

\*All variables as measured at baseline

**Table 6.2 Prevalence of occupational and environmental risk factors among health care workers N=339**

<b>Variable*</b>	<b>N</b>	<b>%</b>
<b>Occupational tasks</b>		
Interviewing TB patients	166	49
Counselling TB patients	145	43
Examining TB patients	74	22
Collecting sputum	73	22
Nursing TB patients	103	30
Homecare of TB patients	29	9
Bronchoscopy	6	2
<b>Facility factors (Self-reported as present)</b>		
Infection control policy	220	64
Ventilation measures	315	93
Ultraviolet lights	157	46
Cough etiquette	265	78
Early triage	205	60
Separation of TB patients	157	46
Diagnostic services	262	77
Disposable surgical masks	322	95
N-95 masks	317	94
Personal protective equipment	303	89
Training (on protection of self)	219	65
Training (on protection of patients)	201	59

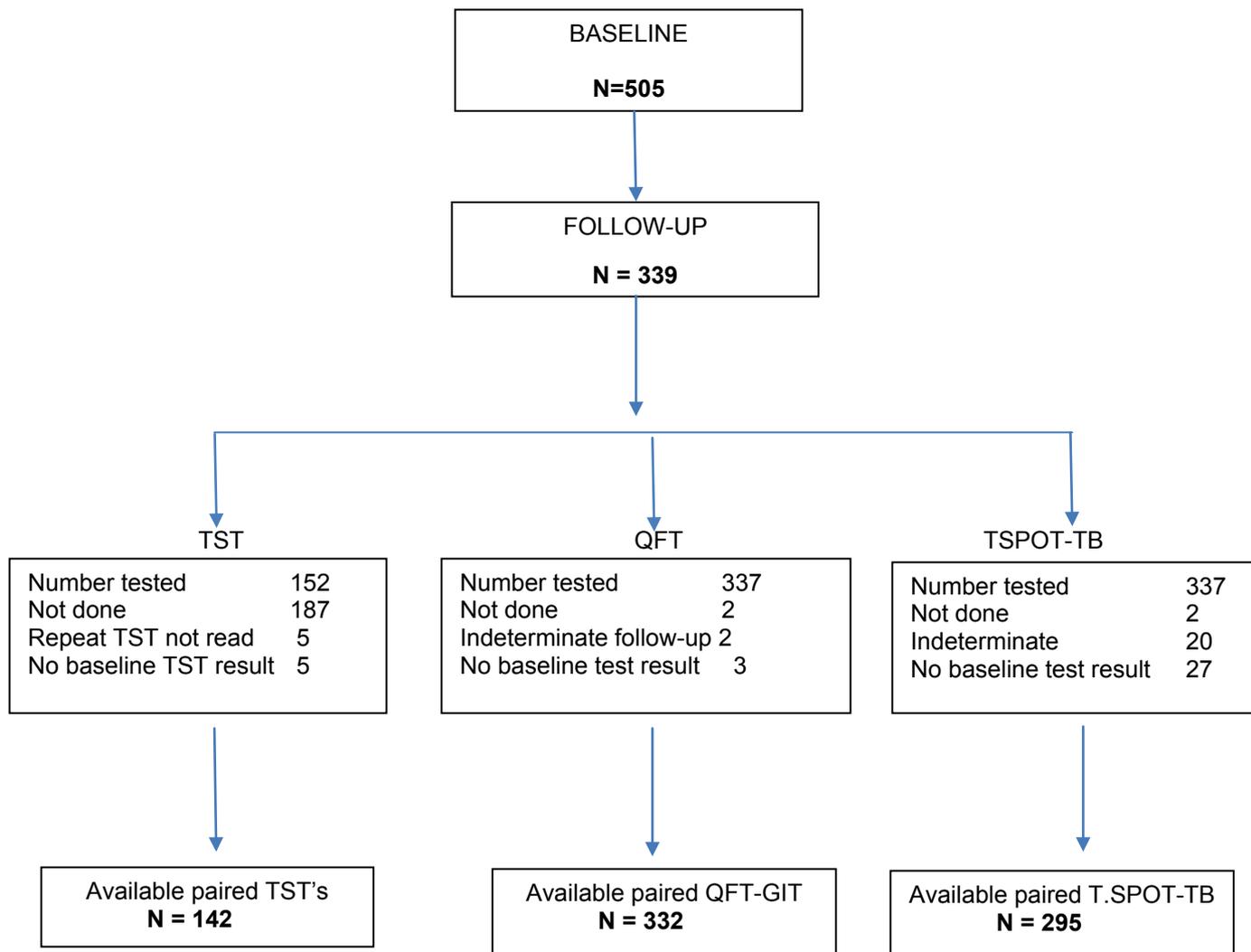
\*All variables as measured at baseline

**Table 6.3 Prevalence of TB associated risk factors at follow-up**

<b>Variable</b>	<b>N</b>	<b>%</b>
BCG vaccination*	313	92
Daily contact with TB patients*	308	91
HIV status check follow-up data(N=329)		
Positive	33	10
Negative	296	90
TB symptom screen positive [at F/U]	55	16
CXR [at F/U](N=288)		
Normal	242	84
Inactive TB	41	14
Active TB	5	2
Interval Referral for TB / HIV	44	13
History of TB Treatment (ever TB)*	44	13
Interim TB Diagnosis	7	2
Current TB diagnosis	2	1
Baseline TST positive (n=329)*	275 / 329	84
TST positive at follow-up	123 /147	84
QFT-GIT test positive at follow-up	231/335	69
TSPOT.TB positive at follow-up	198/317	62

\*Measured at baseline. F/U=Follow-up.

**Figure 6.1. Healthcare worker cohort screened at follow-up for LTBI**



**Table 6.4 Results of latent tuberculosis infection testing at baseline and follow-up including annualized rates of conversion**

<b>LTBI tests N (%)</b>	<b>TST</b>	<b>QFT</b>	<b>TSPOT.TB</b>
<b>Variable</b>			
<b>Test status at baseline</b>			
Number with follow-up test result	142	332	292
Number tested positive	108 (76%)	219 (66%)	180 (62%)
Number tested negative	34 (24%)	113 (34%)	115 (39%)
<b>Conversion and reversion</b>			
Conversion rate	13 / 34 (38%)	25/113 (22%)	25 / 115 (22%)
Reversion rate	4/ 108 (1%)	15/219 (7%)	28 /180 (16%)
Annual Rate of Conversion*	38% [22 – 56]	22% [15 – 31]	22% [15 – 31]

\*Annual rate based on conversion rates in uninfected sample with repeat testing done 1 year later and shown with 95% confidence intervals.

**Table 6.5 IGRA conversion using different definitions**

Conversion variables	Number	%
<b>QFT-GIT (N=113)</b>		
Baseline IFN- $\gamma$ <0.35 IU/ml and follow-up IFN- $\gamma$ >0.35 IU/ml	25/113	22%
Baseline IFN- $\gamma$ <0.35 IU/ml and follow-up IFN- $\gamma$ >0.35 IU/ml, plus a 30% increase in IFN- $\gamma$ over the baseline value	20/113	18%
Baseline IFN- $\gamma$ <0.35 IU/ml and follow-up IFN- $\gamma$ >0.35 IU/ml, plus an absolute increase of 0.35 IU/ml over the baseline value	16/113	14%
Baseline IFN- $\gamma$ <0.35 IU/ml and follow-up IFN- $\gamma$ >0.70 IU/ml	15/113	13%
<b>TSPOT TB (N=114)</b>		
Baseline TSPOT.TB negative and follow-up positive using the $\geq$ 6 spot increment	25/115	22%
Baseline TSPOT.TB negative and follow-up positive using the $\geq$ 8 spot increment	23/128	18%

Test outcomes for analytic purposes were based on prevailing manufacturer's instructions and for QFT-GIT was defined as baseline IFN- $\gamma$  <0.35 IU/ml and follow-up IFN- $\gamma$ >0.35 IU/ml and for T-SPOT.TB a negative baseline T-SPOT.TB with a follow-up positive  $\geq$  6 spots increment.

**Table 6.6 Adjusted Associations between potential predictors and TST and IGRA conversions**

Variable	TST [OR;95%CI] N= 13	QFT N=25	TSPOT.TB N=25
<b>Demographic</b>			
Employment sector			
Provincial Health Dept.	1.00	1.00	1.00
Local authority Health Dept.	-	1.16 [0.10 – 13.45]	<b>14.14 [1.30 – 160.27]*</b>
NGO	0.86 [0.06 – 12.50]	1.33 [0.35 – 5.13]	1.17 [0.32 – 4.27]
Other	0.50 [0.04 – 6.91]	1.17 [0.24 – 5.91]	0.90 [0.17 – 4.72]
Formal health qualification	0.30 [0.04 – 2.05]	1.28 [0.46 – 3.52]	0.84 [0.32 – 2.25]
Diabetes	1.58 [0.10 – 24.41]	2.21 [0.45 – 10.77]	-
Current alcohol consumption	0.27 [0.43 – 1.68]	0.62 [0.21 – 1.82]	1.68 [0.64 – 4.42]
Smoking history			
Non-smoker	1.00	1.00	1.00
Ex-smoker	1.07 [0.08 – 14.20]	0.97 [0.22 – 4.66]	0.19 [0.02 – 1.80]
Current smoker	1.36 [0.16 – 11.47]	0.64 [0.19 – 2.18]	1.45 [0.51 – 4.10]
ETS exposure	2.58 [0.47 – 14.11]	1.25 [0.45 – 3.45]	2.83 [0.88 – 9.10]

\*Bolded variables reached statistical significance (p<0.05)

**Table 6.6 (continued). Adjusted Associations between potential predictors and TST and IGRA conversions (Bolded variables reached statistical significance)**

Variable	TST [OR; 95%CI]	QFT	TSPOT.TB
<b>Occupational tasks</b>			
Interviewing TB patients	0.29 [0.05 – 1.85]	2.96 [0.99 – 8.85]	0.38 [0.14 – 1.03]
Counselling TB patients	<b>0.12 [0.15 – 0.92]</b>	<b>3.04 [1.01 – 9.15]*</b>	0.97 [0.37 – 2.52]
Examining TB patients	0.39 [0.03 – 4.51]	2.46 [0.80 – 7.51]	0.30 [0.06 – 1.44]
Collecting sputum	2.75 [0.25 – 29.97]	1.47 [0.42 – 5.09]	1.15 [0.36 – 3.62]
Nursing TB patients	1.07 [0.14 – 8.31]	1.49 [0.49 – 4.50]	1.04 [0.37 – 2.92]
Home-care of TB patients	-	6.74 [0.83 – 54.97]	-
Bronchoscopy	-	8.21 [0.39 – 174.17]	1.92 [0.15 – 24.00]
<b>Presence of facility factors</b>			
Infection control policy	-	1.16 [0.39 – 3.47]	1.16 [0.44 – 3.07]
Ventilation measures	0.96 [0.05 – 20.19]	0.69 [0.14 – 3.42]	3.19 [0.38 – 26.73]
UV lights	1.25 [0.22 – 7.10]	1.20 [0.45 – 3.16]	2.23 [0.86 – 5.78]
Cough etiquette	0.54 [0.09 – 3.12]	0.91 [0.31 – 2.71]	1.42 [0.50 – 4.06]
Early triage	0.19 [0.03 – 1.31]	0.54 [0.20 – 1.47]	0.86 [0.34 – 2.15]
Separation of TB patients	1.10 [0.20 – 5.97]	1.37 [0.52 – 3.62]	0.87 [0.34 – 2.22]
Diagnostic services	0.71 [0.12 – 4.19]	0.84 [0.26 – 2.67]	2.92 [0.76 – 11.29]
Disposable surgical masks	0.28 [0.02 – 4.44]	2.88 [0.27 – 30.73]	1.01 [0.18 – 5.55]
N-95 masks	0.21 [0.01 – 8.34]	0.26 [0.05 – 1.24]	1.11 [0.19 – 6.42]
PPE Provision	0.34 [0.02 – 4.98]	0.42 [0.11 – 1.58]	4.06 [0.45 – 36.27]

**Table 6.6 (continued). Adjusted Associations between potential predictors and TST and IGRA conversions**

Variable	TST [OR; 95%CI]	QFT	TSPOT.TB
<b>TB associated variables</b>			
BCG vaccination*	-	0.52 [0.03 – 8.62]	0.81 [0.14 – 4.79]
Employment in health care			
< 10	1.00	1.00	1.00
10 – 20	0.51 [0.04 – 6.41]	0.15 [0.02 – 1.06]	0.32 [0.07 – 1.49]
≥20	1.54 [0.98 – 24.27]	0.61 [0.13 – 2.89]	0.78 [0.18 – 3.45]
HIV positive status	1.06 [0.10 – 11.50]	1.20 [0.27 – 5.35]	0.60 [0.12 – 3.00]
TB symptom screen positive [at F/U]	7.31 [0.55 – 97.03]	1.22 [0.35 – 4.28]	0.90 [0.26 – 3.06]
CXR			
Normal	1.00	1.00	1.00
Inactive TB	-	2.01 [0.40 – 10.16]	1.49 [0.39 – 5.65]
Active TB	-	-	-
Interval Referral for TB / HIV	4.43 [0.51 – 38.33]	1.88 [0.41 – 8.61]	1.56 [0.36 – 6.77]
History of TB treatment (ever TB)*	0.72 [0.02 – 22.85]	0.25 [0.03 – 2.29]	0.32 [0.04 – 2.64]
Interim TB diagnosis	-	-	-
Current TB diagnosis	-	-	-
Baseline positive TST	-	2.03 [0.67 – 6.20]	<b>3.31 [0.99 – 11.03]*</b>

**Table 6.7. Comparison across different populations: LTBI test response and incidence of infection as measured by TST conversion rate**

Study author (year)	Country	N	Annual incidence of TB infection% (95% CI)	Incidence rate ratio
<b>Health care workers in this study</b>				
Adams(2014)	South Africa	34	38% (22-56)	<b>1.00</b>
<b>Health care workers in high incidence settings</b>				
Pai (2006)*	India	147	4.1% (2 – 9)	<b>0.11(0.03 – 0.25)</b>
Christopher (2011)**	India	179	7.8% (4 -13)	<b>0.21(0.10 -0.37)</b>
<b>Community</b>				
Middelkoop (2008)***	South Africa	83	11% (5 – 20)	<b>0.29(0.15 – 0.56)</b>

\*Study population comprise nursing and medical students; \*\*Study population comprise nursing students; \*\*\*Study population comprise adolescents aged 13 -22 in high HIV prevalent area

## 6.4 KEY FINDINGS

1. The prevalence of LTBI based on TST at baseline and follow-up remained consistent at 84%. This prevalence is similar to a population-based study of LTBI in South Africa using a TST value of  $\geq 10$  mm as cut-off (Mahomed et al., 2006). Lower rates of TST positivity have been reported in HCWs in high-burden TB countries such as India, Russia and Vietnam, ranging from 41% - 61% (Pai et al., 2005; Drobniewski et al., 2007; Lien et al., 2009; Christopher et al., 2010)
2. The LTBI prevalence for QFT-GIT and T-SPOT .TB increased marginally from baseline to follow-up. For QFT-GIT prevalence changed from 65% to 69%, whilst for T-SPOT.TB it changed from 60% to 62%.
3. The high background community rate of infection renders it difficult to ascertain the true contribution that occupational exposure makes to TB transmission rates in HCWs.
4. The annual incidence of infection in this population was extraordinarily high at 38% and 22% using TST and IGRAs assays, respectively..
5. Serial testing with IGRAs was characterized by high conversion rates of 22% in this study.
6. There is considerable uncertainty as to what the best cut-point for conversion should be when using IGRAs for serial screening (van Zyl-Smit et al., 2009a; Zwerling et al., 2013). This is true for both high and low incidence settings. Varying the conversion cut-points by using more stringent definitions resulted in a modest decrease in the conversion rate in our study.

7. Serial IGRA test response was characterised by a higher rate of reversion than demonstrated for TST in our study (7-16%vs. 1%).

8. In this study there were no factors consistently associated with test conversion for both the TST and IGRA assays. There were also no consistent associations for test conversion for the two IGRA assays. The threefold increased risk for QFT-GIT conversion associated with counselling of TB patients was the reverse of the negative association with TST conversion.

9. The strong association between a baseline positive TST result and a later T-SPOT.TB conversion points to possible suboptimal sensitivity of the T.SPOT.TB in this population.

10. In the analysis, the use of a borderline zone was considered and a multivariate analysis was conducted using an eight spot (5-7 spots increment considered borderline zone) increment for T-SPOT.TB but this did not change the associations.

11. The provision of TB services is a function of local government clinics and staff and may explain the strong association shown between T-SPOT.TB conversion (OR=14.19) and employment by the local authority.

12. Multivariate analysis using more stringent definitions of IGRA conversion showed QFT conversion to be associated with work in facilities that practiced separation / isolation of TB patients from other patients [OR 3.77 (95% CI 1.03 – 13.73)].

13. For most of the occupational and environmental control measures, no demonstrable protective effect was demonstrated against conversion.

14. There was no association between BCG vaccination and TST positivity in this study. The lack of a potentially confounding role for BCG on LTBI prevalence has been demonstrated in other studies and suggests that in communities vaccinated at birth BCG has little impact on subsequent TST response (Farhat et al., 2006).

15. In this study there was very low uptake of repeat TST, but excellent uptake of the repeat IGRA assays suggesting that despite being relatively invasive, the ease of administration (one visit), and absence of side effects or clinical reactions rendered it a better tolerated serial screening tool in this population.

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## **CHAPTER 7**

### **DISCUSSION**

## **CHAPTER 7**

### **7.1 INTRODUCTION**

This prospective study was undertaken to evaluate the use of IGRAs for LTBI screening in HCWs in a high TB incidence settings, against a background of high HIV prevalence. It measured LTBI prevalence using both IGRA assays in a head-to-head comparison with TST, together with host and occupational and environmental factors associated with LTBI prevalence at baseline (Chapter 4). Analyses were carried out to measure test agreement and perform a direct comparison of test performance (sensitivity and specificity) using a latent class analysis model. In addition, factors associated with test discordance were studied (Chapter 5). At one year follow-up, the incidence of TB infection using all three test modalities was calculated. Risk factors associated with incidence of infection (conversion) were explored to identify potentially modifiable factors to limit LTBI risk in HCWs (Chapter 6).

### **7.2 MAIN FINDINGS OF THE STUDY**

#### **7.2.1 PREVALENCE AND DETERMINANTS OF LATENT TUBERCULOSIS INFECTION IN HEALTH CARE WORKERS**

##### **Participation**

The study achieved a 67% participation rate (based on voluntary participation) with staff equally drawn from primary and secondary level facilities. Proportionately more staff from secondary facilities participated than those from primary facilities (72% vs. 63%). Reasons for this greater participation may be greater awareness among such staff of the hazards of TB as both secondary facilities were dedicated TB hospitals. By contrast,

primary facility staff provided a broad range of primary care services, some outside the facilities, resulting in increased mobility and time away from the facility. Staff employed at primary level facilities was also of younger age, predominantly female, Xhosa-speaking, had shorter duration of employment, were more likely to be HIV positive and to have had treatment for previous TB than secondary level staff. This is in keeping with a risk profile among primary level staff that is reflective of lower socio-economic status as well as increased TB risk. Socio-economic status has been shown to be strongly associated with TB in a study evaluating the social epidemiology of TB in South Africa (Harling, Ehrlich & Myer, 2008). Whilst a proxy measure for socio-economic status viz. parental employment (one or either parents employed versus no employment) during childhood was used, this did not show significant differences between the two groups. It is likely that this measure of socio-economic status was too remote to be strongly associated with current TB risk in this group of HCWs.

### **Non-occupational factors influencing TB risk**

Past BCG coverage was very high in HCWs. This is to be expected as it has been offered as part of the South African Department of Health's Expanded Immunization Programme (EIP) since 1960 to children at birth, with reportedly 95% population coverage attained (Department of Health, South Africa, 2003).

A high proportion of HCWs (13%) indicated that they had previously been treated for TB. This estimate represents a fourfold excess when compared to the South African adult population estimate (based on the 1998 Demographic and Household survey) which reported 2.8% of a representative sample as having been diagnosed with tuberculosis disease in their lifetime (Harling, Ehrlich & Myer, 2008). TB risk is further increased by the high prevalence of smoking (22%) among HCWs in this study, which is similar to estimates from a general population study (26%). Although the

causal pathways are not clearly understood, smoking has been shown to be associated with increased risk for both TB infection and disease (Brunet et al., 2011; van Zyl Smit et al., 2010; Harling, Ehrlich & Myer, 2008; Bates et al., 2007).

### **HIV prevalence**

HIV infection greatly increases the risk of TB disease by accelerating the progression to active TB in those who are latently infected. The HIV prevalence among HCWs in this study (11%) is lower than that of the population prevalence for adults aged 15 – 49 years in the Western Cape in 2008 (16.9%) (Avert. South Africa HIV & AIDS statistics, 2014). The high percentage of HCWs who had undergone HIV testing is encouraging and suggests that the stigma in this population associated with HIV is waning and prevention efforts are bearing fruit, with large numbers willing to be tested and or willing to disclose the result of their tests. No other studies among HCWs from other high burden settings have evaluated TB risk in relation to HIV. The HIV prevalence estimate, however, means that one out of ten HCWs in this study with LTBI face an exceptionally high risk of progression from LTBI to active TB. Current recommendations for HCW surveillance in South Africa thus recommend the determination of HIV status as part of screening for TB at pre-placement screening (Department of Health, South Africa, 2014).

### **Occupational exposure factors**

In this group of HCWs reported exposure to newly diagnosed TB patients (in the past three months) was high (76%), with 90% reporting daily contact with a suspected TB patient. This high background rate of exposure was not objectively verified, but is plausible given the high rates of TB in communities serviced by these facilities. HCWs displayed some awareness of the practice

of infection control measures (44 – 93%). However, more than a third had not had any training on protecting themselves or fellow patients against contracting TB, despite 58% of them perceiving themselves to be at high risk of infection. Farley et al (2012), in a review of infection control measures of South African hospitals providing treatment for drug-resistant tuberculosis, reported that annual infection control training was available in only 38% facilities, whilst written infection control plans were available in 54% facilities. Claassens et al (2013), in an audit of primary care, facilities reported that of facilities who had cases of TB among HCWs, only 20% had a written infection control plan in place, although 91% of HCWs at such facilities had been trained in infection control. Similar results have been shown for Russian HCWs employed at TB hospitals, who exhibited poor knowledge of infection control and understanding of their risk despite having an acceptable level of knowledge of TB disease and its treatment (Woith, Volchenkov & Larson, 2010). Training in infection control is therefore needed to reduce TB risk in HCWs in high burden settings.

### **LTBI prevalence**

The LTBI prevalence as measured by TST, QFT-GIT and TSPOT.TB was 84%, 65% and 60% respectively for the group. Surprisingly it was not significantly different between primary and secondary level staff. The prevalence of LTBI as measured by TST is higher than that reported in a review of LTBI in HCWs from LMICs (33 – 79%), and markedly higher than from a recent studies of South African HCWs in Johannesburg which reported a 57% prevalence (Joshi et al, 2006; van Rie et al., 2013). It is, however, similar to community based general population studies in the Western Cape, which reported LTBI prevalence of 81%-88 (Mahomed et al, 2006; Wood et al., 2010; Middelkoop et al., 2008). Wood et al (2010) showed an increasing prevalence of latent TB infection with increasing age in a population of HIV negative individuals aged 5-40 years drawn from high TB prevalence areas, with 88% of adults 31-35 years of age testing positive on

TST. The prevalence of LTBI among HCWs in this study is therefore not indicative of greater TB infection risk than is found in community participants in the Western Cape.

The lower LTBI prevalence estimated by using IGRAs is in keeping with studies among HCWs from low and intermediate incidence settings which have generally reported lower LTBI prevalence measured by IGRAs, primarily using QFT-GIT, than for TST (Zwerling et al., 2012). This has been ascribed to its greater specificity and less confounding by BCG vaccination. By accurately identifying those who are truly negative for LTBI, lesser numbers of HCWs have to be considered for IPT and follow-up than is the case with TST. This allows for more targeted IPT and has economic benefit as well as decreasing the morbidity associated with adverse effects due to IPT, in such settings. Studies using IGRAs among HCWs from high TB incidence settings have produced varied results. A study of Indian HCWs reported equivalent rates of LTBI using TST and QFT-GIT (41% and 40%, respectively), whilst a Vietnamese study showed a different prevalence of LTBI ( 47% for QFT-GIT and 61% with TST) (Pai et al, 2005;Lien et al., 2009). LTBI estimates using IGRAs in this study, approximate those produced by a community based South African study involving healthy adults, which performed a head-to-head comparison between TST and IGRAs (Mahomed et al., 2006). As with TST, LTBI prevalence as measured by IGRAs was similar in HCWs and the community.

## **TB disease**

Despite a high proportion of individuals testing positive on preliminary screening for TB disease using symptom screen (26%) and chest x-ray (7%), only five new cases of tuberculosis was diagnosed at baseline. All cases detected by screening were diagnosed on sputum culture. Three cases were HIV positive, three had radiographic evidence of TB, three were positive on symptom screen and none were positive on microscopy. The use of only one

clinical test in this setting would have resulted in missing one or more cases of active TB.

The new cases detected together with two co-prevalent cases already on treatment resulted in a TB disease prevalence of 7/505 or 14/1000. This is slightly higher than the national TB prevalence estimated at 4 – 13 cases / 1000 for 2010 (World Health Organization, 2011). Notably all five new cases tested positive on TST, but not on IGRAs. Two HIV positive cases tested negative on QFT-GIT, with one case testing negative on both IGRAs. Using active TB as a proxy gold standard for LTBI, these findings suggest that in this study TST had superior sensitivity to both IGRAs and performed better in immunocompromised individuals at detecting LTBI than did IGRAs.

### **Factors associated with LTBI prevalence stratified by test**

#### *TST*

The lack of an association between BCG vaccination and a positive TST test in both univariate and multivariate analysis confirms that BCG does not confound TST response in this population. This finding has been replicated in HCW studies from high and intermediate incidence settings (India, Georgia and China) and in a local community based study, which showed no association between TST and BCG in subjects vaccinated in childhood (Mahomed et al, 2006; Pai et al, 2005; He et al., 2012; Mirtskhulava et al., 2008). However the high prevalence of BCG vaccination (>90%) due to near universal coverage makes it difficult to adequately test this association.

The stratified analyses to evaluate associations between LTBI and occupational factors revealed interesting differences between primary and secondary level HCWs. The negative association between TST and HIV

status is to be expected because of the known depressive impact of HIV on cellular immunity and resultant energy. Duration of employment in healthcare has been shown to be associated with increased risk of TST positivity and is presumed to be indicative of “cumulative” exposure. This correlation has been previously established and was replicated in a recent South African study of LTBI risk among medical students and established HCWs showing a doubling of TST positivity in HCWs compared to medical students (van Rie et al., 2013; Zwerling et al., 2012). Studies among Russian HCWs have shown similar results, with clinical staff exhibiting a threefold greater risk of LTBI than medical students (Drobniewski et al., 2007). In the current study, these associations were, however, only demonstrated among staff at primary level facilities.

#### *QFT-GIT*

The negative association between BCG vaccination and a positive QFT-GIT test is surprising as most studies have shown no association between BCG and IGRA test outcomes. The lack of an association has in part been explained by the improved specificity of IGRAs when compared to TST in BCG vaccinated populations.

Strong associations with QFT-GIT were shown for exposure to environmental tobacco smoke (ETS) (in primary level staff), which has not been reported previously. Whilst smoking has been shown to be associated with increased risk for TB in a meta-analysis the association was stronger for TB disease than LTBI (Bates et al., 2007; Pai et al., 2007). ETS exposure may be associated with increased TB risk, and is pertinent in countries where high population rates of smoking and TB prevail and with poor implementation of tobacco control policies. Co-exposure to TB contacts and smoking in public spaces may well contribute synergistically to TB risk.

A recent study evaluating the potential TB transmission risk among users of public transport reports an annual risk of 3.5 – 5% of TB infection among users of minibus taxis suggesting that settings characterized by poor ventilation and high respiratory contact rates, may play an important role in sustaining tuberculosis transmission in South Africa (Andrews, Morrow & Wood, 2013; Uys et al, 2011).

In this study, staff that performed sputum collection duties had a threefold increased risk of a positive QFT-GIT test. Sputum collection may result in the aerosolization and spread of tuberculous bacilli and is considered a high risk procedure for transmission of TB. Despite this known risk, a recent study of drug resistant TB facilities in South Africa has shown that of 24 facilities, only two facilities had an appropriate infrastructure for the safe collection of sputum (Farley et al., 2012).

In secondary level health facilities training in infection control procedures were associated with a decreased risk of LTBI as measured by QFT and TST, suggesting a potential protective effect. This is consistent with findings from a South African study of drug resistant TB treatment facilities which has shown higher levels of clinical training to be associated with greater knowledge of infection control ( $P < 0.001$ ), more appropriate attitudes ( $P < 0.001$ ) and less time spent with coughing patients ( $P < 0.001$ ) (Farley et al., 2012).

### *T-SPOT.TB*

Several occupational factors were associated with having a positive T-SPOT.TB test. The threefold increase in LTBI odds (as tested by T-SPOT.TB) associated with home-based care is relevant as high burden settings struggle with providing adequate health care to TB patients. Existing hospital facilities in the Western Cape are overstretched. Many TB patients

are resistant to prolonged hospitalization, e.g. those who are drug-resistant, and removal from their communities, contributing to an increased rate of treatment default. This has resulted in the advocacy for, and establishment of treatment programmes which involve community-based care, even for those with drug resistant TB (Nardell & Dharmadhikari, 2010).

Paradoxically, staff working in facilities that practised cough etiquette were also at increased risk of having a positive T-SPOT.TB test, as were primary level staff who worked at facilities providing disposable masks to infectious patients. The anomalous associations between increased occurrence of LTBI and the practice of infection control measures may arise because facilities with higher caseloads of TB and HIV positive patients are more likely to implement infection control practices. There may also have been a degree of reporting bias from such facilities as actual implementation of control measures were not verified in this study. Farley et al (2012), in a study of infection control, has shown inconsistency between data supplied by key informants and that from direct observation on infection control practices, in drug-resistant TB hospitals in South Africa.

Speaking Xhosa as a home language is considered a proxy measure for indigenous African ethnicity and lower socio-economic status in the Western Cape. This is on account of a racialized society which has skewed employment and educational opportunities in the Western Cape for those of ethnic African origin. The association between African ethnicity and LTBI occurrence (as measured by T-SPOT.TB), was not replicated following stratified analysis, whilst all the other associations remained present but significant only for primary level staff.

## **Predictive models**

Models generated to best predict test outcome shows male gender to be a consistently positive predictor across all test outcomes, whilst increasing age showed a significantly positive, and BCG vaccination a significantly negative association with both IGRA outcomes. The predictive model for TST positivity in addition included being an African language speaker (proxy for lower socio-economic status and indigenous African ethnicity) and exposure to secondary smoke, a known association with LTBI which has been previously described (Bates et al., 2007). In this population male HCWs of indigenous African ethnicity with exposure to environmental tobacco smoke had the highest risk of having a positive TST.

Surprisingly predictive models for IGRA positivity differed for QFT-GIT and T-SPOT.TB. Increasing age and male gender were common positive predictors, whilst BCG vaccination was a negative predictor for testing positive on either IGRA. Other predictors such as the occupational tasks of sputum collection, home-based care, ETS exposure and the presence of infection control measures were variably associated with one or other of the IGRA tests. These differences are suggestive of innate differences in test properties between the IGRAs and cannot be easily explained.

### **7.2.2 AGREEMENT BETWEEN IGRA ASSAYS AND TST AND A COMPARISON OF TEST PERFORMANCE USING LATENT CLASS ANALYSIS**

#### **Concordance**

In this study there was only fair agreement between TST and IGRAs as reflected in  $\kappa$  values of 0.28 for QFT-GIT and 0.25 for TSPOT.TB

respectively, whilst agreement between the two IGRA assays as expected was substantial with  $\kappa=0.65$ . This is consistent with agreement studies in HCWs from low and intermediate incidence settings and that of a community based study from South Africa (Zwerling et al., 2012;Mahomed et al., 2006).

Inter-test agreement at follow-up testing one year after baseline was however improved for QFT-GIT and TST (fair to moderate) but only marginally so for T-SPOT.TB and TST. The improvement in agreement between QFT-GIT and TST is possibly an effect of boosting induced by earlier administration of TST, a phenomenon which has been previously described (van Zyl-Smit et al., 2009a; van Zyl-Smit et al., 2009b).

In this study varying the TST cut off to greater than 10mm did not improve inter-test agreement at baseline. This is similar to findings of HCW studies in other high TB incidence settings (Lien et al., 2009; Pai et al., 2005). Findings from HCW studies in low and intermediate TB settings have, however, shown improvement in agreement when using higher TST cut-point (15 mm) (Zwerling et al., 2012). Varying the IGRA cut-points upwards (QFT-GIT $\geq$ 0.70 IU/L or using an 8 spot increment for T-SPOT.TB) to denote a positive test did not enhance agreement with TST $\geq$ 15 mm, in the current study.

### **Factors associated with discordant LTBI test results**

The predominant discordant response reported from HCW studies has been that of a TST positive/ IGRA negative pattern (Zwerling et al., 2012). In this study such a pattern of discordance (TST+ / IGRA -) ranged from 23% for QFT to 28% for T-SPOT.TB. Whilst a positive association was shown with BCG vaccination and this pattern of discordance for both IGRAs, this was not significant. This confirms the lack of significant BCG effect on TST positivity that has previously been demonstrated in this study.

Those with longer years spent in a healthcare environment were less likely to exhibit a pattern of TST- / QFT + discordance in this study, suggesting that participants with long duration of employment would tend to have concordant results. A TST - / QFT + discordance was the predominant pattern of discordance found in a recent study of Canadian HCWs(Zwerling et al., 2012).The lack of an association between QFT positivity and recent TB exposure in this study is suggestive of a potentially high rate of false positives reflected in this discordant pattern. This raises the possibility that the initial high rate of QFT positives may indicate suboptimal specificity (high rate of false positives) that may lead to unnecessary use of IPT in HCWs who do not really require it.

For T-SPOT.TB, a pattern of TST+/ IGRA - discordance was less likely in older HCWs and those engaged in home-care of TB patients. Risk factors associated with TB such as being HIV positive, having had previous treatment for TB, working in a facility that practiced isolation of TB patients from others as part of infection control and being symptom screen positive were strongly associated with TST-/ T-SPOT.TB + discordance. The markedly increased risk of a discordant test response in HIV-infected participants(OR=4.72) has been replicated in other studies in similar settings and suggests that T-SPOT.TB test performance is relatively unimpaired by moderate HIV infection (as reflected in a median CD4 count of 392/mm<sup>3</sup>)and potentially more sensitive than TST for detecting LTBI in HIV-infected participants (Rangaka et al., 2007a; Rangaka et al., 2007b). Whilst a meta-analysis by Cattamanchi et al (2011) did not show IGRAs to be consistently more sensitive than TST,T-SPOT.TB did exhibit higher sensitivity, and appeared to be less affected by immunosuppression than TST or QFT-GIT.

The association between this pattern of discordance and known TB risk factors such as previous TB treatment or being symptom screen positive remains unexplained. Such unexplained discordance has been described in studies from both low and high TB incidence settings and it is postulated that

both the epidemiological context and the use of standard cut-offs may be contributory factors (Pai et al., 2006; Pai et al., 2007). For example persons with tuberculosis in high-incidence countries often have advanced disease and are likely to be infected with HIV or malnourished. Anergy due to advanced HIV-associated immune suppression may affect test performance of IGRAs and TST leading to discordant test responses. The unexpected association between the implementation of infection control measures (isolation of TB patients from others) and a TST negative T-SPOT.TB positive discordant pattern in this study is similarly unexplained.

### **Latent class analysis modelling**

In the absence of a gold standard for LTBI, and the problems inherent in the use of surrogate measures for LTBI, the use of latent class analysis and a fixed effect model allowed a more direct comparison of test performance in this population (Dendukuri, Hadgu & Wang, 2009; Pai, 2008; Dendukuri & Joseph 2001). This technique showed TST to have excellent sensitivity (93%) in this study. This is superior to both IGRAs for which the sensitivity was shown to be 84% for QFT-GIT and 79% for T-SPOT.TB. These findings suggest that as a first line screening test TST is better at detecting LTBI than IGRAs in this population. IGRAs appear to be more specific at ruling out LTBI than TST, displaying excellent specificity ranging from 93 -96%, compared to TST with 55% specificity.

This suggests that in high burden settings such as this, TST performance as a first line screening test is highly sensitive. The superior specificity of IGRAs and increased sensitivity in HIV positive individuals may potentially make them useful adjunct tests in the diagnosis of LTBI in this population.

### **7.2.3 ANNUAL INCIDENCE OF TB INFECTION IN HEALTH CARE WORKERS AND FACTORS ASSOCIATED WITH INCREASED RISK OF TEST CONVERSION**

#### **Participation and loss to follow-up**

A large number of participants were lost to follow up resulting in a 67% participation rate by the end of the study. The greater proportion (68/91) could not be traced, suggesting a mobile HCW workforce and high staff turnover in health care facilities that formed part of the study. Those staff who participated in follow-up were significantly different from those lost to follow-up. They were generally healthier (less likely to smoke or be HIV infected), of older age and longer duration of employment in healthcare. This healthy worker effect would result in a degree of attrition bias that could affect estimates of LTBI and active TB generated by this study. Such bias would usually have the effect of attenuating estimates of adverse outcomes such as TB disease.

#### **Serial screening with IGRAs better tolerated than with TST**

There was also very low uptake of repeat TST, but excellent uptake of the repeat IGRA assays, suggesting that despite being relatively invasive, the ease of administration (one visit), and absence of side effects or clinical reactions rendered it a better tolerated serial screening tool in this population. A possible reason for this is that TST administration resulted in some discomfort for participants with greater than 80% testing positive at baseline and the median size of the skin reaction being 18mm.

## **Incidence of TB Infection as measured by test conversion**

### *TST*

The annual incidence of infection in this population was extraordinarily high at 38% (95% CI; 22% - 56%) using TST, but needs to be interpreted with caution in view of the relatively wide confidence intervals. A proportion of the conversions may also be attributable to boosting. This figure is much higher than demonstrated in two Indian studies among HCWs (Table 6.7) (Christopher et al., 2011; Pai et al., 2006). Reasons for this difference may be that both Indian studies were conducted on nursing students or a combination of nursing and medical students, who might be less exposed to TB bacilli in the occupational context than HCWs in formal employment. A recent study in South Africa similarly showed a large difference in LTBI prevalence between medical students and HCWs (van Rie et al., 2013). Whilst there are no comparable studies among South African adults, a recent study of infection among adolescents in a high HIV prevalence community in Cape Town, reported an annual incidence of infection of 11% (Middelkoop et al., 2008). The authors argue that social contact is likely to be at its peak during adolescence and that TB risk of infection is more likely to resemble that of adults in the community than that of childhood. Using this estimate as a proxy measure for adult community risk of infection, this suggests that HCWs have a threefold higher risk of becoming infected during a one year period than a community comparison group from a high burden setting.

### *IGRAs*

Serial testing with IGRAs also produced high conversion rates of 22% in this study, for both assays. Two studies of HCWs in India have produced comparable IGRA conversion rates ranging from 11.6 %– 21% using QFT-GIT (Zwerling et al., 2012). Conversion estimates among HCWs in low and

intermediate TB incidence settings have varied from 1.8% -14.4 % for IGRA assays, mainly using QFT-GIT.

In this study, only a small number of reversions (1%) was demonstrated using serial TST. This is in contrast to IGRAs which showed a 7% and 16% reversion rate for QFT-GIT and T-SPOT.TB respectively. Reversion rates of a study conducted by Pai et al (2006) have varied from 7% among baseline concordant positives to 70% in those with TST-/ IGRA + discordance. Joshi et al (2009) as cited by Zwerling et al (2012) have similarly shown a 27% reversion rate after a 6 month interval and a 40% reversion rate after a 12 month interval among Indian HCWs.

Studies of HCWs in low and intermediate TB incidence settings have reported similar high reversion rates ranging from 40%-52.9%. High rates of reversions have recently been shown in a large US-based study of HCWs followed up for 18 months and tested at 6 month intervals. The authors conclude that most conversions among HCWs in low TB incidence settings appear to be false positives, and the frequency of conversion was six to nine times higher with IGRAs than with TST. They propose that repeat testing of apparent converters should be considered (Dorman et al., 2014).

Another US based study by Slater et al (2013) has proposed that given the relative stability of TST conversions when compared to IGRAs, a QFT-GIT cut-off of 5.3 IU/ml or higher (manufacturer's cut-off is  $\geq 0.35$  IU/ml) would yield a conversion rate of 0.4%, which was equal to the known TST conversion rate for the study population . The authors suggest that the current manufacturer's definition of QFT conversion results in an inflated conversion rate that is incompatible with the low risk study setting and that a much higher QFT cut-off value was needed to define a true conversion. The high rate of non-reproducible conversions in a majority of converters suggested a high rate of false positive results (Slater et al., 2013). There is therefore considerable uncertainty as to what the best cut-point for

conversion should be when using IGRAs for serial screening (Zwerling et al., 2013; van Zyl-Smit et al., 2009a). This is true for both high and low TB incidence settings.

In this study, varying the conversion cut-points by using more stringent definitions resulted in a modest decrease in the conversion rates. The role of natural variability in test response and a possible boosting effect on IGRAs by TST are two factors that may play a role in influencing the optimal cut-off that determines whether a conversion truly reflects a new infection or not. This renders the use of IGRAs problematic in the clinical setting. The existence of natural variability in IGRA test response is lent further credibility by the far higher rate of reversion than demonstrated for TST in this study (7-16% vs. 1%).

### **Factors associated with LTBI test conversion**

There were no factors consistently associated with test conversion for both the TST and IGRA assays in this study. There were also no consistent associations with test conversion for the two IGRA assays. The threefold increased risk for QFT-GIT conversion associated with counselling of TB patients was the reverse of the negative association with TST conversion. This anomalous result suggests that the test outcomes are not equivalent and may call into question any suggested substitution of TST with IGRAs in this population.

The association between male gender and QFT-GIT conversion (OR=4.02) in this study has been replicated in a study of serial testing of HCWs in the USA (Belknap, 2010). Male gender was also a consistently positive predictor of LTBI test positivity at baseline in predictive models generated for all three tests (Chapter 4 results) as well as in univariate analysis for conversion (Chapter 6 appendix). Naidoo et al (2006) showed a 45% increased

incidence of TB disease in male HCWs compared to female HCWs in KZN. These findings would suggest that male HCWs were at higher risk of incident LTBI infection and TB disease in South Africa.

The strong association between a baseline positive TST result and a later T-SPOT.TB conversion points to possible suboptimal sensitivity of the T.SPOT.TB in this population. This was similarly reflected in latent class modelling, which showed T-SPOT.TB to be the least sensitive of the three tests for the diagnosis of LTBI in this population. Varying of the T-SPOT.TB cut-point in the analysis by using an eight spot increment (treating 5-7 spots increment as a borderline zone), did not change the associations in multivariate analysis. An alternative explanation of the association between a baseline positive TST and subsequent T-SPOT.TB conversion could be due to boosting of the subsequent T-SPOT. TB test response by TST administration at baseline, which has been previously described (Belknap et al., 2009).

The strong association with employment sector deserves further scrutiny as the provision of TB services has traditionally been a function of local government clinics and staff. This is still the case in combined facilities(run by local and provincial government health department staff). It is therefore plausible that local government staff have greater exposure to infectious TB patients than others. This may explain the strong association shown between TSPOT.TB conversion (OR=14.19) and employment by the local authority, suggesting a causative role for occupational exposure in conversion. However, this estimate lacks precision as reflected in the wide confidence interval (95% CI 1.28 – 157.75).

For most of the occupational and environmental control measures, no demonstrable protective effect was demonstrated against conversion. This is contrary to the known impact of infection control measures on TB infection and disease rates in HCWs. (Baussano et al., 2007; Blumberg et al.,

1995). This lack of a protective effect may be ascribed to a reliance on *reported* implementation of such measures rather than objective measures quantifying the practice and implementation of such control measures, rendering this measure of exposure assessment highly inaccurate.

In conclusion this study demonstrates a high prevalence of LTBI using different testing modalities in HCWs, which mirrors that of the surrounding community. TST has superior sensitivity for the detection of LTBI in this population, but poor specificity when compared to IGRAs. Whilst there was excellent uptake of IGRAs in repeat testing, sequential IGRA test responses were marked by high rates of reversion when compared to TST, possibly as a result of boosting and greater inter-test variability. The markedly higher annual incidence of infection in this study than HCWs and community participants from other high TB incidence settings (Table 6.7), is strongly suggestive of a causative role for occupational exposure. The role of specific occupational and environmental exposures in driving TB infection rates deserves further exploration as effective infection control measures in such settings may reduce this risk.

### **7.3 RESEARCH AND POLICY IMPLICATIONS AND RECOMMENDATIONS**

This is the first prospective HCW study in Africa evaluating TST and both QFT-GIT and T-SPOT.TB in a head-to-head comparison, with a focus on the potential occupational and environmental risk factors associated with LTBI prevalence and incidence. As such, it adds to the body of evidence on the utility of such tests in HCWs employed in a high TB and HIV prevalence setting.

### **7.3.1 POLICY AND PROGRAMME IMPLICATIONS**

#### **Participation**

The low participation rate and high loss to follow-up in this study despite the potential benefit of the study for HCWs and the efforts that went into encouraging participation in this study are limitations on potential screening programmes. Initiatives aimed at surveillance and delivery of occupational health programmes to HCWs need to address this resistance and consult widely on preferred models of service delivery to this population. Service provision models need to address ease of access, confidentiality and the promotion of employee occupational health and safety. This may positively influence the poor uptake and suboptimal participation in such programmes which would hamper efforts to manage the burden of TB infection and disease among HCWs.

#### **Optimal screening test for LTBI**

TST performance was unaffected by BCG vaccination and its sensitivity was superior to IGRAs in the diagnosis of LTBI in this population. TST test response was also very stable with a low rate of reversion when compared to IGRAs, supporting the inference that those who tested LTBI positive at baseline were correctly identified as such. This suggests that where LTBI screening is incorporated into occupational health programmes for HCWs, TST should remain the preferred method of screening in this population. This supports the recommendation in the most recent South African National TB Control Programme which for the first time advocates the regular screening for LTBI in HCWs using the TST (Department of Health, South Africa, 2014). This is aligned to the current WHO recommendation that discourages the use of IGRAs for LTBI screening in HCWs, in low and middle-income countries (World Health Organization, 2011). Specific recommendations are

that data are insufficient and there is low quality evidence on the performance of IGRAs in low- and middle-income countries, typically those with a high TB and/or HIV burden; IGRAs and the TST cannot accurately predict the risk of infected individuals developing active TB disease; Neither IGRAs nor the TST should be used for the diagnosis of active TB disease; IGRAs are more costly and technically complex to do than the TST. Given comparable performance but increased cost, replacing the TST by IGRAs as a public health intervention in resource-constrained settings is not recommended. For TST screening to have value as a programmatic intervention, IPT will have to be provided for those considered at high risk of developing TB disease.

On the other hand, from a logistical point of view, there was greater uptake of repeat IGRAs (99%) than of TST (44%), despite the ostensibly more invasive nature of these assays. This is an important consideration especially in populations where the logistics of TST administration may be too cumbersome to implement.

Ideally the choice of LTBI diagnostic test should be informed by multiple factors inclusive of test performance, available resources such as trained personnel, risk profile of the population and the acceptability and uptake of the test by populations targeted to benefit from such screening.

### **Screening and follow-up of those at risk of TB infection**

Screening for LTBI has been difficult in those most at risk of LTBI such as HIV-infected individuals, young children and silicotics, despite the proven benefit of IPT in such groups. Reasons for the poor uptake are many and will need to be addressed if LTBI screening for HCWs is to be implemented. Such an initiative will have to create awareness of the benefits of IPT and devote additional resources to such programmes.

The current recommendation from the National Tuberculosis Management Guideline advocates six monthly surveillance of HCWs which should include medical history, TB symptom review, TST, physical assessment, chest x-ray and other appropriate tests (Department of Health, South Africa, 2014). Since recent infection is associated with a 5% risk of developing TB disease in the next two years, it would be important to identify those HCWs with recent infection. Additional risk factors such as positive HIV status should also be screened for, as it would greatly increase their risk of progression and should be considered in targeted preventive measures in the form of IPT and workplace infection control.

Whilst the recommendation for medical surveillance is a logical step, it is unclear how this will be implemented given the current paucity of occupational health service provision to HCWs, the lack of occupational health expertise and the chronic underfunding of occupational health services that currently prevail in South Africa (Adams et al., 2012). This strategy if properly implemented could reduce the incidence of active TB in HCWs in South Africa.

### **HIV screening**

Regular HIV screening should be strongly encouraged both at entry into employment and periodically among HCWs. In this study 60% of incident TB cases were HIV positive, whilst 11% of HCWs were HIV positive and therefore at high risk of progression to active TB. HIV screening should be accompanied by the provision of INH prophylaxis for at least 6 months to those who test positive for HIV. Whilst HIV testing is recommended as part of pre-placement screening, it is not included as part of periodic screening in the national guideline. This needs to be addressed as HCWs remain at risk of becoming infected with HIV and TB for the duration of their working lives.

### **Differential risk between staff from primary and secondary level facilities**

The significant differences in the demographic profiles and TB risk profile between staff from primary and secondary level facilities were not reflected in LTBI prevalence, but did reflect in TB disease prevalence as all TB cases were found in the primary care level staff at both the baseline and follow-up phase of the study. At baseline, all new cases of TB disease were community HCWs underscoring the high risk of this category of HCWs, which has been established in a previous study by Kranzer et al (2010).

This suggests that primary care staff are at greater risk of TB disease than secondary level staff in this setting. This is indicative of a high background community exposure to TB and HIV, lower socio-economic status as well as increased occupational exposure to TB. Primary level staff have much greater contact with undiagnosed infectious TB cases and primary level facilities are known to have poor implementation of infection control measures, contributing to their occupational exposure to TB (Naidoo, Seevnarain & Nordstrom, 2012). Greater focus on the protection of primary level staff is therefore recommended. This should include LTBI and HIV screening with targeted implementation of IPT.

### **Potential role of training in infection control**

A significantly protective effect on prevalent TB infection of training was demonstrated in secondary level staff. Training in TB administrative controls would incorporate safe work practices and infection control procedures which may mitigate TB risk if effectively implemented. Training programmes on infection control should therefore be implemented especially in low-resource settings where expensive engineering controls may not be feasible. This is in

keeping with recommendations from the WHO(World Health Organization, 2009).

### **Potential role of exposure factors in LTBI occurrence**

The strong association between certain occupational tasks such as home-based care and sputum collection with LTBI occurrence points to areas of occupational exposure that could be addressed by interventions. Given the shift to home-based care, HCWs and family members engaged in the provision of such care should be trained in the application of infection control measures in such settings. This would have the impact of limiting occupationally acquired TB among HCWs and limiting TB transmission to family members of infectious patients being cared for at home.

Training in safe work procedures and the provision of appropriate infrastructure for sputum collection are additional interventions that are needed. The lack of infrastructure for the safe collection of sputum in facilities treating drug-resistant TB cases is cause for concern(Farley et al., 2012). Whilst not objectively evaluated in this study, infrastructural challenges are likely to be worse at primary level facilities which perform a greater volume of diagnostic tests and service larger numbers of infectious patients.

### **TB disease screening in HCWs**

Whilst screening for TB disease was not a primary focus of this study, the results point to a suboptimal yield if only one screening tool is utilized such as sputum microscopy. This is especially relevant in high TB incidence settings where WHO criteria (cough and smear positive for acid fast bacilli) have been shown to have suboptimal sensitivity (56%) in diagnosing TB disease in those at highest risk (HIV positive individuals) (Bassett et al.,

2010). Implementation of screening programmes for the detection of TB disease in HCWs needs to take this into consideration and will have to consider a combination of screening tests to improve case detection rates. In this study all of the individuals diagnosed with TB at baseline were smear-negative, with all five cases testing positive on culture. This makes it imperative that all HCWs suspected of having TB have a sputum culture as part of their initial investigation, in accordance with the South African Department of Health's National TB guideline recommendation (Department of Health, South Africa, 2014).

### **Factors associated with conversion**

The positive association between employment category and conversion for both IGRAs supports an effect of occupational exposure. Whilst the association was only significant for T-SPOT.TB, it does suggest that individuals employed in the provision of TB diagnostic and treatment services (local government staff) were at greater risk of T.SPOT.TB conversion than those in other employment sectors.

The strong association between a baseline TST and a sequential positive IGRA test response (conversion), which was significant for T- SPOT.TB only, points to a probable boosting effect of antecedent TST on subsequent IGRA testing. Screening programmes which utilize both TST and IGRAs need to consider the impact of boosting of IGRA responses by antecedent TST in the interpretation of serial test outcomes.

There were contrary associations between occupational tasks such as "counselling of TB patients" and test outcomes as it was a positive predictor of QFT conversion (OR=3.04) and a negative predictor of TST conversion (OR=0.12). This points to the need to arrive at a better understanding of incident infection using more appropriate cut-points to denote conversion. It

also points to the need for appropriate tools to evaluate occupational exposures of HCWs, which have been validated in high TB incidence settings.

There is global consensus that HCWs need to be better protected against the risk of occupational tuberculosis (WHO, UNAIDS & ILO, 2010). Several strategies have been proposed to address the problem of TB in HCWs. These include: the generation of reliable data on HCW TB burden and infection control practices; ongoing review of policies dealing with the management of TB in HCWs and the rapid identification of new TB cases both among HCWs and the patients they serve (Chai, Mattingly & Varma, 2013). For such strategies to be effective, policy formulation and review should be informed by research and a strong evidence base.

### **7.3.2 RESEARCH IMPLICATIONS**

#### **Burden of disease among HCWs**

Findings generated from the study suggests that LTBI and TB prevalence is very high among HCWs and remarkably similar to that of the surrounding community, making it difficult to clearly differentiate between the role of occupational and community exposure in this setting. It could be argued though that HWCs would be expected to have a lower LTBI and TB disease prevalence than the general population as they are likely to have a higher average socio-economic status. The equivalent LTBI and TB rates may therefore point to occupational exposure as playing a role in the occurrence of LTBI and TB in HCWs. The generation of more accurate estimates of the incidence of LTBI and TB disease among HCWs will require universal surveillance programmes targeting all HCWs, with uniform implementation across the country. Regular medical surveillance of HCWs for LTBI and TB disease has been incorporated into the most recent National TB control

programme for South Africa in support of such a strategy (Department of Health, South Africa, 2014).

### **Tools for infection control measurement**

Studies evaluating infection control practices have shown poor implementation of such measures at both primary and secondary level facilities in South Africa (Farley et al., 2012; Naidoo, Seevnrain & Nordstrom, 2012; Claassens et al., 2013). In this study, paradoxical associations were also found between the practice of certain infection control measures and incident TB infection in HCWs. Claassens et al (2013) have similarly shown paradoxical associations between infection control measures and TB disease rates in HCWs. This has highlighted the need for the development of tools have been validated and tested locally for the monitoring and measurement of infection control practices.

### **Role of TSPOT.TB in diagnosing LTBI in immunosuppressed individuals**

The greater sensitivity of T-SPOT.TB than the other tests in HIV positive individuals deserves further exploration. This is reflected in a strong association between TST-/ TSPOT.TB + discordance and HIV positive status in this study.

The current South African Antiretroviral Treatment Guidelines requires LTBI screening by way of TST only for the initiation of IPT (Department of Health, South Africa, 2013). The WHO currently considers the evidence base for the use of TSPOT.TB in HIV positive individuals too limited to recommend its use for LTBI screening. Whilst a recent meta-analysis by Cattamanchi et al (2011) did not show IGRAs to be consistently more sensitive than TST, the T-SPOT.TB did exhibit higher sensitivity, and appeared to be less affected by

immunosuppression than TST or QFT-GIT. Whilst the overall differences were considered to be too small and inconclusive, in advocating for the use of T-SPOT-TB in place of TST it is suggested that the choice of diagnostic test for LTBI screening should take into consideration resources and logistic demands as well as country-specific guidelines. Arriving at a more accurate diagnosis of LTBI in high risk individuals with HIV positive status, who may face additional risk from occupational exposure to TB, would be an important strategy in the targeted implementation of IPT (Cattamanchi et al., 2011).

### **Reproducibility of assay results in serial testing**

IGRA assays in this study were characterized by high rates of conversion and reversion. Whilst TST had a higher conversion rate than IGRAs, much higher reversion rates were demonstrated for IGRAs than for TST. Since similar findings have been generated in studies from low and intermediate TB incidence settings, the degree of variability must in part be “intrinsic to these assays, and independent of exposure” (Pai, 2012: 1367). Pai suggests that some of this variability may be related to “pre-analytic delays, procedures such as test-tube shaking and test re-test variations.” The use of a binary definition would also invariably result in conversion and reversion rates that are not in keeping with the risk profile of the population. Pai(2012: 1367) therefore advocates the consideration of additional factors such as the “risk profile of the HCW, TST results when available and history of contact, before making decisions about preventative therapy”. In addition he proposes a re-evaluation of the strategy of annual screening of all HCWs in low and intermediate incidence settings, calling for a more targeted approach to LTBI screening.

In summary, risk profiling of high risk individuals, taking into consideration clinical risk factors and exposures, should inform targeted screening for LTBI and IPT. This strategy requires an improved understanding of host and

occupational risk factors associated with LTBI and TB disease risk, in high incidence TB settings.

## **7.5 LIMITATIONS**

A limitation of the study is that of selection bias on account of a reliance on voluntary participation at both the baseline and follow-up phase of the study. Clinical staff at potentially greater risk of occupational TB infection (nurses and physicians) was underrepresented, probably as a result of high workloads and limited time to participate in the study during work hours. Whilst care was taken to not discourage participation on account of HIV testing being included as part of the protocol, there is still considerable stigma associated with the diagnosis and disclosure of HIV status that may have resulted in non-participation, by those most at risk of TB disease.

Loss to follow up was a significant problem with only 67% of participants from the original cohort being available for follow-up testing. The decreased participation reflects in part the mobility and high staff turnover especially amongst the non-governmental sector (NGO) sector. Since all cases of TB disease at the baseline phase of the study emanated from staff working in this sector, this is suggestive of greater susceptibility of this group. Kranzer has similarly shown much higher rates of TB among community health care workers than the general population (Kranzer et al., 2010). TB programmes rely heavily on the use of community health care workers to support large treatment and adherence programmes, to achieve success in managing the epidemic. Surveillance programmes aimed at HCWs need to take account of the increased risk of TB faced by community health care workers, the mobility of this population and promote their inclusion in such programmes.

Other reasons for non-participation may have been a perceived lack of benefit from participating especially amongst those who tested LTBI negative

at baseline as well as time constraints on attending the screening programme which occurred during work hours. Whilst the impact of attrition bias is not known, those who participated in the follow-up phase of the study were older, less likely to be HIV positive, and had longer years of service, pointing to a possible 'healthy worker effect'. This may have had the effect of underestimating adverse outcomes such as incident TB disease which is greatly influenced by HIV status.

Since the study setting was one of the highest TB incidence areas in the country, the findings may not be generalizable to all HCWs in South African or occupational settings where the community incidence of TB may be lower and occupational exposure less than is the case in this study. A recent study of HCWs conducted in Johannesburg reported considerably lower LTBI prevalence among HCWs as measured by both TST and IGRA assay than this study (van Rie et al., 2013).

The reliance on self-reported measures of exposure and infection control measures also rendered it difficult to arrive at accurate measures of such exposure and their relationship with LTBI. Ideally these exposures should be verified by more objective assessment using tools that are validated for use in high burden settings. Such tools are, however, lacking and area problem highlighted in a recent study that evaluated the relationship between infection control and TB risk of staff working in health care facilities in South Africa (Farley et al., 2012).

The cross-sectional study design used to evaluate factors associated with LTBI prevalence makes it difficult to arrive at a true understanding of potential causes underlying LTBI prevalence in this population, as both exposure and outcomes are measured concurrently. Longitudinal studies that evaluate risk factors associated with new TB infection in LTBI negative individuals would provide clearer answers. In assessing occupational

exposures, tools will have to be developed which are adapted to and validated for use in local settings.

Conventional binary cut-points were used in the agreement analysis between IGRA and TST outcomes. Emerging evidence from large serial screening activity in low and intermediate incidence settings, suggests that these cut-points may be inappropriate for reflecting true IGRA conversion (Slater et al., 2013; Fong et al., 2012). A recent study conducted among HCWs in India suggests this is likely to be true for high incidence settings as well (Zwerling et al., 2013).

In this study, the use of cut-points for test positivity was based on the manufacturer's instructions and software provided for assay analysis at the time that the study was conducted. For T-SPOT.TB this represented a 6 spot increment. Later recommendations, based on emerging evidence from studies performed, advised that a 5-7 spot increments be treated as a grey zone, and that an 8 spot or greater increment be considered a positive test. Whilst the original criterion of a 6 spot increment was used in our study, the revised cut point was taken into consideration in the multivariate analysis, but did not lead to a significant difference in results. Current evidence suggests that further revision of cut points is required which may be considerably higher than those currently recommended (Slater et al., 2013).

## **7.6 CONCLUSION**

The high LTBI prevalence and incidence rates among HCWS in this study, coupled with high HIV infection prevalence and occupational exposure, places them at high risk of progression to TB disease. The high rate of TB disease and HIV infection among primary level staff, specifically in the NGO sector, highlights their increased risk in comparison to other HCWs.

Strategies are needed from a program and policy perspective to address this risk. These should focus on the establishment of occupational health programmes aimed at protecting HCWs from contracting TB and implementation of workplace infection control. The regular surveillance of HCWs for TB infection and disease, as currently advocated by the Department of Health, presents an opportunity to start dealing with the problem of TB in HCWs. Whilst more research is needed to inform on the optimal screening strategies for LTBI and TB disease, this should not delay the implementation to programmes to address and manage TB risk in HCWs.

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## APPENDIX 1

**UCT STUDY FOR THE EVALUATION OF IMMUNODIAGNOSTIC TESTS  
FOR TB INFECTION AND DETERMINANTS OF TB INFECTION IN  
HEALTH CARE WORKERS OF THE W.CAPE PROVINCE OF SOUTH AFRICA**

**ENGLISH QUESTIONNAIRE**

Survey Number \_\_\_\_\_

Card 1  
    1-4

**A. IDENTIFICATION DATA**

1. Surname \_\_\_\_\_

2. First name/s \_\_\_\_\_

3. Address  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

4. Residential area \_\_\_\_\_

5-7

5. Contact Telephone numbers  
 home \_\_\_\_\_  
 work \_\_\_\_\_  
 mobile \_\_\_\_\_

6. Contact e-mail \_\_\_\_\_

7. Staff number \_\_\_\_\_

8. Date of birth: Day\_\_\_\_ Month\_\_\_\_ Year\_\_\_\_

8-13

9. Where were you born?  
 Town/District \_\_\_\_\_

14-16

10. Gender: Male (1)  
 Female (2)

17

11. Home Language: English (1)  
 Afrikaans (2)  
 Xhosa (3)  
 Other (4)

18

12. Did your parents work when you were a child?

Yes (1)  
 No (2)  
 Do not know (3)

19

12.1 What type of work did they do?

Mother's Occupation \_\_\_\_\_

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	20-22
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Father's occupation \_\_\_\_\_

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	23-25
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13. Interviewer's initials \_\_\_\_\_

<input type="checkbox"/>	<input type="checkbox"/>	26-27
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14. Date of interview:

Day\_\_\_\_Month\_\_\_\_\_Year\_\_\_\_\_

<input type="checkbox"/>	28-33					
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15 Health Facility: \_\_\_\_\_

<input type="checkbox"/>	<input type="checkbox"/>	34-35
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16. Type of facility

- Primary Care clinic (1)
- Community Health Centre (2)
- Secondary hospital (3)
- TB hospital (4)

<input type="checkbox"/>	36
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## B.MEDICAL HISTORY

### BCG vaccination

1. Have you ever received a BCG vaccination against TB which is an injection in the left arm or shoulder which may leave a scar (usually given at birth)

- Yes (1)
- No (2)
- Do not know (3)

<input type="checkbox"/>	37
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1.1 Do you have a vaccination scar to show? (examine L deltoid area for scar).

- Consistent with BCG scar (1)
- Inconsistent with BCG scar (2)
- No scar visible (3)

<input type="checkbox"/>	38
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### Other illness

2. Do you have any other condition or disease which requires medical treatment?

- Yes (1)
- No (2)

<input type="checkbox"/>	39
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2.1 If yes, could you please specify what the

condition/s is/are?

\_\_\_\_\_

\_\_\_\_\_

40-41

42-43

3. Have you ever been diagnosed with Diabetes?

Yes (1)

No (2)

44

3.1 Are you on any treatment for diabetes?

Yes (1)

No (2)

45

### Crowding

4.1 How many people are in the household?

\_\_\_\_\_

46-47

4.2 How many bedrooms are in the home?

\_\_\_\_\_

48-49

### HIV infection

5. Have you ever been tested for HIV?

Yes (1)

No (2)

Do not know (3)

50

If YES, go on to Question 5.1

If NO, skip to Question 6

5.1 Would you be willing to share the result with us?

Yes (1)

No (2)

51

If YES, go on to Question 5.2

If NO, skip to Question 6

5.2 What was the result of your HIV test?

Positive (1)

Negative (2)

52

6. Would you be willing to have an HIV test now as part of a confidential voluntary, testing programme which

form part of this study?

Yes (1)  
No (2)

53

If YES, go on to Question 6.1

If NO, skip to Question 7

6.1 Do you wish to be given the result of your HIV test?

Yes (1)  
No (2)

54

### Previous screening for TB

7. Have you ever been tested for TB by a health professional as part of routine screening i.e. without having symptoms of TB

Yes (1)  
No (2)  
Do not know (3)

55

8.1. Was this done by means of a Tuberculin skin test (such as a Mantoux/ Heaf / Tine / PPD test)

Yes (1)  
No (2)

56

If YES, go on to Question 8.1.1

If NO, skip to Question 8.2

8.1.1. When was this skin test done?

Month \_\_\_\_\_ Year \_\_\_\_\_

57-60

8.1.2 Were you advised against having any future skin tests for TB because of a bad reaction to the test ?

Yes (1)  
No (2)

61

8.2 Were you ever examined for TB by having a chest x-ray

Yes (1)  
No (2)

62

8.2.1. When was this CXR done?

Month \_\_\_\_\_ Year \_\_\_\_\_

63-66

8.3. Were you ever tested for TB by means of a symptom questionnaire as part of routine screening?

Yes (1)  
No (2)

67

8.3.1. When was this questionnaire done?

Month \_\_\_\_\_ Year \_\_\_\_\_

68-71

8.4 Was your sputum ever tested for TB?

Yes (1)  
No (2)

72

8.4.1 When was this done?

Month \_\_\_\_\_ Year \_\_\_\_\_

73-76

**C. SMOKING HISTORY**

1. Have you ever smoked cigarettes or tobacco?

Yes (1)  
No (2)

Card 2  
 1

If YES, go to question 1.1  
If NO, skip to question 3

1.1. Have you ever smoked tobacco (cigarettes or pipe) for as long as a year?

'YES' means at least 20 packs of cigarettes or 360 grams of tobacco in a lifetime or at least one cigarette per day for one year

Yes (1)  
No (2)

2

If YES, go on to Question 1.2  
If NO, skip to Question 1.3

1.2 How old were you when you started smoking?

\_\_\_\_\_ years old

3-4 5

1.3 Do you now smoke?

'YES' means smoking tobacco in the last month or more

Yes (1)

No (2)

5

If YES, go on to Question 1.4.1

If NO, skip to Question 2

1.4 1-2. How much do you now smoke on average?

1.4.1 Number of cigarettes per day \_\_\_\_\_

6-7

1.4.2 Pipe tobacco in grams/week \_\_\_\_\_

8-10

2. Have you stopped smoking completely?

Yes (1)

No (2)

11

If YES, go on to Question 2.1

If NO, skip to Question 2.2

2.1 How old were you when you stopped smoking completely?

\_\_\_\_\_ years old

12-13

2.2 How many years in total did you smoke cigarettes? (Do not include the years you stopped before you started again)

\_\_\_\_\_ years

14-15

2.3 On average of the entire time you smoked, how much did you smoke?

2.3.1 Number of cigarettes per day \_\_\_\_\_

16-17

2.3.2 Pipe tobacco in grams/week \_\_\_\_\_

18-20

2.4 Do you or did you inhale the smoke?

Yes (1)

No (2)

21

3. Have you been regularly exposed to tobacco smoke from other people smoking cigarettes or pipe in the last

12 months?

'Regularly' means on most days or nights

Yes (1)  
No (2)

22

#### D. ALCOHOLISM SCREEN

1. Have you ever drunk alcohol?

Yes (1)  
No (2)

23

2 Do you drink alcohol now?

Yes (1)  
No (2)

24

If yes, go to question 2.1  
If no, go to section E

2.1 Have you felt the need to cut down your drinking?

Yes (1)  
No (2)

25

2.2 Have you felt annoyed by criticism of your drinking?

Yes (1)  
No (2)

26

2.3 Have you had guilty feelings about drinking?

Yes (1)  
No (2)

27

2.4 Have you taken a drink as a morning eye opener?

Yes (1)  
No (2)

28

#### E. TB SYMPTOM SCREEN

**Current TB symptoms**

1. Have you ever received treatment for TB?

Yes (1)

No (2)

29

If yes, go to question 1.2

If no, go to question 2

1.2 When did you receive treatment for TB?

Month \_\_\_\_\_ Year \_\_\_\_\_

30-33

1.3 For how long did you receive treatment for TB?

Months \_\_\_\_\_

34-35

2. Are you currently receiving treatment for TB?

Yes (1)

No (2)

36

3. Do you have a cough at the moment ?

Yes (1)

No (2)

37

If YES, go on to Question 3.1

If NO, skip to Question 4

3.1 Have you been coughing for more than 2 weeks?

Yes (1)

No (2)

38

4. Have you coughed up blood in the last month?

Yes (1)

No (2)

39

5. Have you suffered from weight loss without trying to lose weight?

Yes (1)

40 8

No (2)

6. Do you experience night sweats currently i.e excessive sweating that drenches your nightclothes / bedding ?

Yes (1)

No (2)

 41

7. Have you had a fever lasting more than two weeks recently?

Yes (1)

No (2)

 42

8. Have you had chest pain lasting more than 2 weeks recently?

Yes (1)

No (2)

 43

## F. EXPOSURE FACTORS

### General occupational factors

1. What is your current job title?

\_\_\_\_\_

 44-45

2. How long have you been employed in this position?

Years \_\_\_\_\_

months \_\_\_\_\_

 46-47  
 48-49

3. Which department/ section are you working in?

\_\_\_\_\_

 50-51

4. How long have you been employed in a healthcare environment?

Years \_\_\_\_\_

months \_\_\_\_\_

 52-53  
 54-55

5. Do you have a professional health qualification?

Yes (1)

No (2)

 56

If YES, go on to Question 5.1

If NO, skip to Question 6

5.1 If yes , when did you obtain your qualification?

Month \_\_\_\_\_ Year \_\_\_\_\_

57-60

5.2 Could you please specify what your qualification is?

\_\_\_\_\_

61-62

6. In your judgement how often do you come into contact with suspected TB patients during the course of your work?

- a) Every day \_\_\_\_\_
- b) Less than 2 times a week \_\_\_\_\_
- c) At least once a month \_\_\_\_\_
- d) Less than once a month \_\_\_\_\_
- e) Never \_\_\_\_\_

63

7. Are you during the course of your work involved in any of the following procedures?

7.1 Clerking or interviewing of suspected / known TB patients?

- Yes (1)
- No (2)

64

7.2 Counselling of suspected/known TB patients

- Yes (1)
- No (2)

65

7.3 Examination of suspected /known TB patients?

- Yes (1)
- No (2)

66

7.4 Taking of sputum from suspected/known TB patients

- Yes (1)
- No (2)

67

7.5 General nursing care of suspected/known TB patients?

- Yes (1)
- No (2)

68

10

7.6 Home-based care of TB patients?

Yes (1)  
No (2)

69

7.7 Bronchoscopic procedures on suspected /known TB patients

Yes (1)  
No (2)

70

7.8 Other exposure not mentioned above

Please specify \_\_\_\_\_

71-72

**Facility exposure factors**

8. To your knowledge does the facility have an infection control policy to decrease the risk of TB infection?

Yes (1)  
No (2)  
Do not know (3)

73

9. To your knowledge are control measures in place within the facility to decrease TB risk to the staff such as:

9.1 Ventilation measures (natural or mechanical)?

Yes (1)  
No (2)

74

Natural ventilation refers to the opening of windows and doors to allow for adequate circulation of air

Mechanical ventilation refers to the use of (1) propeller fans and / or (2) exhaust ventilation to allow for adequate circulation of air

9.2 Ultra-violet lights?

Yes (1)  
No (2)

75

9.3 Teaching of cough etiquette to patients?

Yes (1)  
No (2)

76

Cough etiquette/ hygiene means to instruct coughing patients to cover their nose and mouth when coughing or sneezing and if possible to use facemasks or tissues to assist them in covering their mouths

9.4 Early triage of potentially infectious patients through early separating of TB patients from others in the facility?

Yes (1)  
No (2)

77

9.5 Separating TB patients from other patients in the different clinic areas?

Yes (1)  
No (2)

78

9.6 On site TB diagnostic services such as sputum microscopy and/or x-rays

Yes (1)  
No (2)

79

9.7 Provision of disposable surgical masks to infectious patients to wear?

Yes (1)  
No (2)

80

9.8 Supply of special (N95) (non-surgical) masks for staff to wear to protect against TB? (show picture of mask)

Yes (1)  
No (2)

Card 3  
 1

**Recent exposure and contact score for TB**

10. To your knowledge have you recently (in the last 3 months) had contact with a newly-diagnosed person with TB?

- Yes (1)
- No (2)

 2

11. Did the contact occur at work , home or elsewhere?

- Home (1)
- Work (2)
- Other (3)

 3

11.1 If elsewhere, please specify where

\_\_\_\_\_

  4-5

If home /other, go on to Question 12  
If work, skip to Question 13

**Household/other contact exposure score**

12.1 What is your relationship to the TB case

\_\_\_\_\_

 6

12.2 Infectivity of TB case

- a) Sputum test result not known 0
- b) Sputum-negative TB 2
- c) Sputum positive TB 4

 7

12.3 Type of exposure to TB case (proximity)

- a) No known exposure 0
- b) Lives and sleeps in different house 1
- c) Lives and sleeps in same house 2
- d) Sleeps in same room 3

 8

12.4 Duration (hours) per day contact with TB case

- a) No known contact 0
- b) 0 - 3 hours 1
- c) 4 - 7 hours 2
- d) 8 - 11 hours 3
- e) ≥ 12 hours 4

 9

## Occupational contact exposure score

13.1 Which of the following best describes your relationship with the TB patients?

- |                                     |   |
|-------------------------------------|---|
| a) Provider of non-clinical service | 1 |
| b) Provider direct clinical care    | 2 |
| c) Both                             | 3 |

10

Non-clinical service includes administrative duties, clerking of patients, transport of patients, cleaning of facility.  
Clinical care includes examination of patients, general nursing care, radiography, taking of sputum, blood and urine specimens for investigations and dispensing of TB medication

13.2 Type of exposure to TB patients (proximity)

- |  |   |
|--|---|
| a) No direct exposure but works in facility treating TB patients     | 1 |
| b) No direct exposure but works in a department treating TB patients | 2 |
| c) Direct exposure in same room providing non-clinical service       | 3 |
| d) Direct exposure in same room providing clinical care              | 4 |

11

13.3 Average duration (hours) per day contact with TB cases

- |                 |   |
|-----------------|---|
| a) 0 - 3 hours  | 1 |
| b) 4 - 7 hours  | 2 |
| c) 8 - 11 hours | 3 |
| d) ≥12 hours    | 4 |

12

## G. HEALTH AND SAFETY EDUCATION AND TRAINING

14. In your view the probability of getting infected by TB patients in your workplace is?

- |             |   |
|-------------|---|
| a) low      | 1 |
| b) moderate | 2 |
| c) high     | 3 |

13

15. How much training have you had on how to protect yourself when working with TB patients?

- a) no training 1
- b) some training 2
- c) extensive training 3

 14

16. How much training have you received in infection control practices to minimize the risk of transmission of infection from a TB case to other patients?

- a) no training 1
- b) some training 2
- c) extensive training 3

 15

17. Are you provided with personal protective equipment e.g. masks during high risk procedures?

- Yes (1)
- No (2)

 16

18. Do you have any concerns and/or recommendations regarding your risk of acquiring TB as a result of your work?

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 17-18 19-20 21-22 23-24

## APPENDIX 2

**FOLLOW-UP PHASE OF UCT STUDY FOR THE EVALUATION OF  
 IMMUNODIAGNOSTIC TESTS FOR TB INFECTION AND DETERMINANTS OF  
 TB INFECTION IN HEALTH CARE WORKERS OF THE W.CAPE PROVINCE  
 OF SOUTH AFRICA**

**ENGLISH QUESTIONNAIRE**

Survey Number \_\_\_\_\_

Card 1

1-4

**A. IDENTIFICATION DATA**

1. Surname \_\_\_\_\_

2. First name/s \_\_\_\_\_

3. Address \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

4. Residential area \_\_\_\_\_

5-7

5. Contact Telephone numbers  
 home \_\_\_\_\_  
 work \_\_\_\_\_  
 mobile \_\_\_\_\_

6. Contact e-mail \_\_\_\_\_

7. Staff number \_\_\_\_\_

8. Date of birth: Day\_\_\_\_Month\_\_\_\_Year\_\_\_\_

8-13

9. Interviewer's initials \_\_\_\_\_

14-1

10. Date of interview:  
 Day\_\_\_\_Month\_\_\_\_Year\_\_\_\_

16-2

11 Health Facility: \_\_\_\_\_

22-2

**B. SMOKING HISTORY**

1. Have you ever smoked cigarettes or tobacco?

Yes (1)  
No (2)

24

If YES, go to question 1.1  
If NO, skip to Section C

1.1 Do you now smoke?

Yes (1)  
No (2)

25

If YES, go on to Question 1. 2  
If NO, skip to Question 2

1.2 1-2. How much do you now smoke on average?  
(Answer one or the other option provided)

1.2.1 Number of cigarettes per day \_\_\_\_\_

26-2

1.2.2 Pipe tobacco in grams/week \_\_\_\_\_

28-3

2. How many years in total did you smoke or have you smoked cigarettes? (Do not include any years you stopped before you started again)

\_\_\_\_\_ years

31-3

**C. EXPOSURE FACTORS**

**General occupational factors**

1. What is your current job title?  
\_\_\_\_\_

33-3

2. How long have you been employed in this position?

Years \_\_\_\_\_  
months \_\_\_\_\_

35-3  
 37-3

3. Which department/ section are you working in?  
\_\_\_\_\_

39-4

4. Who is your employer ? currently?

- Provincial Health Dept 1
  - City of Cape Town 2
  - NGO (Specify which) 3
  - Other (Specify which) 4
- 

41

**D. TB SYMPTOM SCREEN**

**Current TB symptoms**

1. Have you been diagnosed with TB since we last screened you?

- Yes (1)
- No (2)

42

If yes, go to question 1.2  
If no, go to question 2

1.2 Have you received treatment for TB since we last screened you?

Month \_\_\_\_\_ Year \_\_\_\_\_

43-4

1.3 For how long have you received treatment for TB?

Months \_\_\_\_\_

47-4

2. Are you currently receiving treatment for TB?

- Yes (1)
- No (2)

49

3. Do you have a cough at the moment ?

- Yes (1)
- No (2)

50

If YES, go on to Question 3.1  
If NO, skip to Question 4

3.1 Have you been coughing for more than 2 weeks?

- Yes (1)
- No (2)

51

4. Have you coughed up blood in the last month?

Yes (1)  
No (2)

52

5. Have you suffered from weight loss without trying to lose weight?

Yes (1)  
No (2)

53

6. Do you experience night sweats currently i.e excessive sweating that drenches your nightclothes / bedding ?

Yes (1)  
No (2)

54

7. Have you had a fever lasting more than two weeks recently?

Yes (1)  
No (2)

55

8. Have you had chest pain lasting more than 2 weeks recently?

Yes (1)  
No (2)

56

**E. HIV infection**

1. Have you ever been tested for HIV?

Yes (1)  
No (2)  
Do not know (3)

57

If YES, go on to Question 2.1  
If NO, skip to Question 3

2.1 Would you be willing to share the result with us?

Yes (1)  
No (2)

58

If YES, go on to Question 2.2  
If NO, skip to Question 3

2.2 What was the result of your HIV test?

Positive (1)

Negative (2)

59

3. Would you be willing to have an HIV test now as part of a confidential voluntary, testing programme which form part of this study?

Yes (1)

No (2)

60

If YES, go on to Question 3.1

If NO, skip to Section F

3.1 Do you wish to be given the result of your HIV test?

Yes (1)

No (2)

61

#### **F. Health Services for TB and HIV**

1. Did the researchers or study staff refer you for TB and /or HIV health care services after your initial screening ?

Yes (1)

No (2)

62

If YES, go on to Question 1.1

If NO, skip to Question 2

1.1 Where did you attend for TB / HIV care ?

1. Place of employment

2. Other clinic/ healthcare facility

3. Private Care

4. Did not attend

63

1.2 Did you find it easy to access such services?

Yes (1)

No (2)

64

**We are interested in your opinion:**

2. How do you think TB and HIV health services should be provided to staff ?

1. At public sector clinics as for patients
2. Occupational health clinics specially provided for staff (and funded by employers)
3. Referred to private medical care
4. Other: Specify \_\_\_\_\_

65

3. Would you use Occupational Health services provided at your site of employment for TB and HIV care for yourself?

- Yes (1)  
No (2)

66

If NO, go on to Question 3.1

3.1 If No, please provide reason

\_\_\_\_\_

67

## APPENDIX 3

**UCT STUDY FOR THE EVALUATION OF IMMUNODIAGNOSTIC TESTS  
FOR TB INFECTION AND THE DETERMINANTS OF TB INFECTION IN  
HEALTH CARE WORKERS OF THE WESTERN CAPE PROVINCE  
OF SOUTH AFRICA – 2009**

**ENGLISH CONSENT FORM**

**1. Title of research project**

An evaluation of immunodiagnostic tests for tuberculosis infection and determinants of TB infection in healthcare workers of the Western Cape of South Africa

**2. Purpose of the research**

The University of Cape Town is conducting this important study on TB infection and its determinants in health care workers. This study is going to be done by researchers who are independent of the Provincial Dept of Health and the City of Cape Town. We will be studying healthcare workers in public sector health facilities that serve communities with a high prevalence of TB and HIV infection. It is hoped that this study will provide greater insight into the utility of newer immunodiagnostic test for detecting latent TB infection (LTBI), the burden of LTBI and TB amongst health care workers and the exposure factors that increases TB risk in this population. Study findings may also be used to inform the need for improved risk control measures and surveillance systems to be implemented in order to reduce the incidence of tuberculosis among health care workers.

**3. Description of the research project**

If you agree to participate you will be asked to complete the following tests during working time:

**a) Complete a questionnaire**

A member of our study team will interview you in privacy to complete the questionnaire. You will be asked questions about TB symptoms, current and previous employment history, risk factors related to potential TB exposure ( questions will cover environmental, occupational and personal factors).

**b) Examination**

If BCG vaccinated or you are uncertain of having received such vaccination you will be required to show the interviewer the site of vaccination (usually upper left arm)

**c) Chest radiograph**

A postero-anterior chest x-ray will be taken to evaluate your lungs for signs of TB disease. The X-ray will be read by doctors from UCT.

**d) Tuberculin skin test**

A tuberculin skin test will be done to see whether you react to TB antigen. A nurse will inject a small amount (0.1ml) of tuberculin into the skin of your forearm. The reaction will be read and measured 48-72 hours later using a ball point pen to mark the diameter of the induration (reaction) and a ruler to measure it with.

**e) Blood tests**

A 3ml volume of venous blood will be collected from all subjects by a nurse into the three tubes (1ml per tube) and will be sent to a laboratory to check for an immune response to TB infection (Quantiferon Gold in tube test). The blood samples will be stored and may be tested to look for the presence of other biological substances (biomarkers) associated with TB at a later stage. Two ten ml blood will also be collected in heparinized tubes for the T SPOT TB test which will be sent to the laboratory for analysis.

f) **HIV test**

You will be asked to provide blood for an HIV test. This will be obtained from a fingerprick sample taken by a trained nurse and member of the research team. You will be asked whether you wish to be given your result. If you do wish to have your result, you will be provided with counseling and appropriate referral for further care depending on your test result. Your test result will be kept confidential and disclosed only to you. You have the right to refuse the HIV test but still enroll into the study.

g) **Confidentiality of information collected**

Your name will not appear in any reports on this study. The records of skin tests, blood tests, and x-rays will be kept completely confidential and will be seen only by members of the study team.

5. **Risks and discomforts of the research**

a) **From the blood tests.** You will feel a single needle stick when the blood is taken. Sometimes a small bruise may occur from the needle stick, but this is minor and will heal quickly. The total amount of blood taken is quite small and your body will quickly replace it.

b) **Tuberculin skin test**

You may experience redness, swelling and/or ulceration at the site of the tuberculin skin. Very rarely an allergic reactions to the tuberculin skin test may occur. In the event of such a reaction a trained nurse will be present to provide you with treatment for the allergic reaction. It will also be arranged with the facility to have a doctor located nearby and ready to help if necessary.

c) **From the chest x-ray**

You will be exposed to a small amount of radiation during taking of the x-ray. However this represents a negligible exposure and has not been associated with increased risk of illness due to radiation.

d) **From the questionnaire**

There are no risks associated with the questionnaire and all information will be treated as confidential.

6. **Expected benefits to you and to others**

You will be given a written copy of all your test results along with an explanation of what they mean, unless you tell us that you do not wish to receive this. You may wish to show these to your doctor if you are having any problems. These tests will help determine if you have latent TB or active TB. What we learn from this study will help us to know whether the new TB test may be used for detecting latent TB among healthcare workers and what are the environmental and occupational factors associated with increased TB risk. Preventative programme can then address these factors to reduce health care workers' risk of contracting TB.

7. **Costs to you resulting from participation in the study**

The study is offered at no cost to you. In the event of an abnormal test result requiring further investigation or management you will be referred to a facility of your choice for further investigation and care

8. **Contact person.**

You may contact one of the following persons for answers to further questions about the research, your rights, or any injury you may feel is related to the study.

**University of Cape Town Researchers:**

Dr. Shahieda Adams, Telephone No. (021) 406-6665  
Mobile 083 2857665

**University of Cape Town Research Ethics Committee:**

Contact person (ethics administrator) 406-6492

**UCT STUDY FOR THE EVALUATION OF IMMUNODIAGNOSTIC TESTS  
FOR TB INFECTION AND THE DETERMINANTS OF TB INFECTION IN  
HEALTH CARE WORKERS OF THE WESTERN CAPE PROVINCE  
OF SOUTH AFRICA – 2009**

**ENGLISH CONSENT FORM**

**STUDY NO.** \_\_\_\_\_

**9. Consent of the participant**

I have read the information given above, or it has been read to me. I understand the meaning of this information, Dr./Mr./Ms. \_\_\_\_\_ has offered to answer any questions concerning the study. By signing this form, I hereby consent to participate in the study. I also understand that I am free to withdraw from the study at any time without penalty.

**10. Documentation of the consent**

One copy of this signed document will be kept together with our research records for this study. A copy of the information sheet about the study will be given to you to keep.

\_\_\_\_\_  
Printed name of participant

\_\_\_\_\_  
Signature, Mark, or Thumb Print

\_\_\_\_\_  
Interviewer's name (Print)

\_\_\_\_\_  
Signature

**DATE:** \_\_\_\_\_

## APPENDIX 4

**UCT STUDY FOR THE EVALUATION OF IMMUNODIAGNOSTIC TESTS  
FOR TB INFECTION AND THE DETERMINANTS OF TB INFECTION IN  
HEALTH CARE WORKERS OF THE WESTERN CAPE PROVINCE  
OF SOUTH AFRICA – 2010[FOLLOW-UP]**

**ENGLISH CONSENT FORM**

**1. Title of research project**

An evaluation of immunodiagnostic tests for tuberculosis infection and determinants of TB infection in healthcare workers of the Western Cape of South Africa

**2. Purpose of the research**

The University of Cape Town is conducting this important study on TB infection and its determinants in health care workers. This study is going to be done by researchers who are independent of the Provincial Dept of Health and the City of Cape Town. We will be studying healthcare workers in public sector health facilities that serve communities with a high prevalence of TB and HIV infection. It is hoped that this study will provide greater insight into the utility of newer immunodiagnostic test for detecting latent TB infection (LTBI), the burden of LTBI and TB amongst health care workers and the exposure factors that increases TB risk in this population. Study findings may also be used to inform the need for improved risk control measures and surveillance systems to be implemented in order to reduce the incidence of tuberculosis among health care workers.

**3. Description of the research project**

If you agree to participate you will be asked to complete the following tests during working time:

**a) Complete a questionnaire**

A member of our study team will interview you in privacy to complete the questionnaire. You will be asked questions about TB symptoms, employment history, risk factors related to potential TB exposure and questions related to health service access.

**b) Chest radiograph**

A postero-anterior chest x-ray will be taken to evaluate your lungs for signs of TB disease. The X-ray will be read by doctors from UCT.

**c) Tuberculin skin test**

A tuberculin skin test will be done to see whether you react to TB antigen. A nurse will inject a small amount (0.1ml) of tuberculin into the skin of your forearm. The reaction will be read and measured 48-72 hours later using a ball point pen to mark the diameter of the induration (reaction) and a ruler to measure it with.

**d) Blood tests**

A 3ml volume of venous blood will be collected from all subjects by a nurse into the three tubes (1ml per tube) and will be sent to a laboratory to check for an immune response to TB infection (Quantiferon Gold in tube test). The blood samples will be stored and may be tested to look for the presence of other biological substances (biomarkers) associated with TB at a later stage. Two ten ml blood samples will also be collected in heparinized tubes for the T SPOT TB test which will be sent to the laboratory for analysis.

e) **HIV test**

You will be asked to provide blood for an HIV test. This will be obtained from a fingerprick sample taken by a trained nurse and member of the research team. You will be asked whether you wish to be given your result. If you do wish to have your result, you will be provided with counseling and appropriate referral for further care depending on your test result. Your test result will be kept confidential and disclosed only to you. You have the right to refuse the HIV test but still enroll into the study.

f) **Confidentiality of information collected**

Your name will not appear in any reports on this study. The records of skin tests, blood tests, and x-rays will be kept completely confidential and will be seen only by members of the study team.

5. **Risks and discomforts of the research**

a) **From the blood tests.** You will feel a single needle stick when the blood is taken. Sometimes a small bruise may occur from the needle stick, but this is minor and will heal quickly. The total amount of blood taken is quite small and your body will quickly replace it.

b) **Tuberculin skin test**

You may experience redness, swelling and/or ulceration at the site of the tuberculin skin. Very rarely an allergic reactions to the tuberculin skin test may occur. In the event of such a reaction a trained nurse will be present to provide you with treatment for the allergic reaction. It will also be arranged with the facility to have a doctor located nearby and ready to help if necessary.

c) **From the chest x-ray**

You will be exposed to a small amount of radiation during taking of the x-ray. However this represents a negligible exposure and has not been associated with increased risk of illness due to radiation.

d) **From the questionnaire**

There are no risks associated with the questionnaire and all information will be treated as confidential.

6. **Expected benefits to you and to others**

You will be given a written copy of all your test results along with an explanation of what they mean, unless you tell us that you do not wish to receive this. You may wish to show these to your doctor if you are having any problems. These tests will help determine if you have latent TB or active TB. What we learn from this study will help us to know whether the new TB test may be used for detecting latent TB among healthcare workers and what are the factors associated with increased TB risk. Preventive programme can then address these factors to reduce health care workers' risk of contracting TB.

7. **Costs to you resulting from participation in the study**

The study is offered at no cost to you. In the event of an abnormal test result requiring further investigation or management you will be referred to a facility of your choice for further investigation and care

8. **Contact person.**

You may contact one of the following persons for answers to further questions about the research, your rights, or any injury you may feel is related to the study.

**University of Cape Town Researchers:**

Dr. Shahieda Adams, Telephone No. (021) 406-6665  
Mobile 083 2857665

**University of Cape Town Research Ethics Committee:**

Contact person (ethics administrator) 406-6492

**UCT STUDY FOR THE EVALUATION OF IMMUNODIAGNOSTIC TESTS  
FOR TB INFECTION AND THE DETERMINANTS OF TB INFECTION IN  
HEALTH CARE WORKERS OF THE WESTERN CAPE PROVINCE  
OF SOUTH AFRICA – 2010 FOLLOW-UP**

**ENGLISH CONSENT FORM**

**STUDY NO.** \_\_\_\_\_

**9. Consent of the participant**

I have read the information given above, or it has been read to me. I understand the meaning of this information, Dr./Mr./Ms. \_\_\_\_\_ has offered to answer any questions concerning the study. By signing this form, I hereby consent to participate in the study. I also understand that I am free to withdraw from the study at any time without penalty.

**10. Documentation of the consent**

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\_\_\_\_\_  
Printed name of participant

\_\_\_\_\_  
Signature, Mark, or Thumb Print

\_\_\_\_\_  
Interviewer's name (Print)

\_\_\_\_\_  
Signature

**DATE:** \_\_\_\_\_

## APPENDIX 5

# UNIVERSITY OF CAPE TOWN



OCCUPATIONAL AND ENVIRONMENTAL HEALTH RESEARCH UNIT

## School of Public Health & Family Medicine

Faculty of Health Sciences  
Anzio Road  
Observatory 7925  
SOUTH AFRICA  
Telephone: (27 21) 406 6665  
Fax: (27 21) 406 6163  
e-mail: shahieda.adams@uct.ac.za

### UCT STUDY FOR THE EVALUATION OF IMMUNODIAGNOSTIC TESTS FOR TB INFECTION AND THE DETERMINANTS OF TB INFECTION IN HEALTH CARE WORKERS OF THE WESTERN CAPE PROVINCE OF SOUTH AFRICA - 2009/2010

#### MEDICAL REPORT

Date : \_\_\_\_\_

Name : \_\_\_\_\_

This is the report of the medical examination and tests conducted on you by medical staff from the University of Cape Town, as part of the research on TB infection in healthcare workers

#### 1. Questionnaire result

Questionnaire completed

Questionnaire not completed

Symptom screen suggestive of Active TB:

Yes

No

#### 2. Chest radiograph result

Test not done

Normal

Abnormal

If Abnormal specify:

- Signs of inactive TB
- Signs of active TB
- Signs of other pathology

3. Tuberculin skin test results

- Test not done                       Negative                       Positive

- If Positive specify:
- Mildly positive ( $\leq 5$ mm induration)
  - Moderately positive ( $\leq 10$ mm induration)
  - Markedly positive ( $\leq 15$ mm induration)
  - Ulcerating

3. OVERALL COMMENTS

**(Please note that the tick in the box applies to you)**

- The results indicate that you have no evidence of latent or active TB infection
- The results indicate that you may have latent TB infection. The current policy is not to offer treatment for TB infection to health care workers. However you are advised to seek medical care should you suffer from any symptoms suggestive of active TB such as cough  $>2$  weeks, night sweats, loss of weight or haemoptysis.
- The results indicate that you may have active tuberculosis. You will be referred for sputum microscopy and culture.
- The results indicate that you are at high risk for TB disease and will require referral for further care and management

Please do not hesitate to contact Dr S Adams (Telephone: 083 2857665) should you have any queries or require more detailed results of the investigations done on you.

Yours faithfully,

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Dr S Adams

## APPENDIX 6

# UNIVERSITY OF CAPE TOWN



## UCT STUDY FOR THE EVALUATION OF IMMUNODIAGNOSTIC TESTS FOR TB INFECTION AND THE DETERMINANTS OF TB INFECTION IN HEALTH CARE WORKERS OF THE WESTERN CAPE PROVINCE OF SOUTH AFRICA – 2010/2011

### PERSONAL MEDICAL REPORT

Date : \_\_\_\_\_

Name : \_\_\_\_\_

This is the report of the medical examination and tests conducted on you by medical staff from the University of Cape Town, as part of the research on TB infection in healthcare workers

#### 1. Questionnaire result

Questionnaire completed                       Questionnaire not completed

Symptom screen suggestive of Active TB:

Yes

No

#### 2. Chest radiograph result

Test not done       Normal       Abnormal

If Abnormal specify:

Signs of inactive TB

Signs of active TB

Signs of other pathology

#### 3. HIV test

Negative                       Reported as negative       Test not done  
 Positive                       Reported as positive

#### 4. Tuberculin skin test results

Test not done                       Negative                       Positive

If Positive specify:  **Mildly positive ( $\leq 5$ mm induration) [only if HIV positive/immunocompromised]**  
 Positive ( $\leq 10$ mm induration)  
 Markedly positive ( $\leq 15$ mm induration)  
 Ulcerating

#### 5. Sputum test results

Test not done

Microscopy  Positive                       Negative

Culture  Positive                       Negative

#### 6. OVERALL COMMENTS

**(Please note that the tick in the box applies to you)**

- The results indicate that you have no evidence of latent or active TB infection
- The results indicate that you may have latent TB infection. The current policy is not to offer treatment for TB infection to health care workers who are HIV negative. However you are advised to seek medical care should you suffer from any symptoms suggestive of active TB such as cough  $>2$  weeks, night sweats, loss of weight or haemoptysis.
- The results indicate that you may have active tuberculosis. You will be referred for sputum microscopy and culture to exclude active TB.
- The results indicate that you are at high risk for TB disease and will require referral for further care and management

Please do not hesitate to contact Dr S Adams (Telephone: 083 2857665) should you have any queries or require more detailed results of the investigations done on you.

Yours faithfully,

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Dr S Adams

## APPENDIX 7

**UCT STUDY FOR THE EVALUATION OF IMMUNODIAGNOSTIC TESTS FOR TB INFECTION AND THE DETERMINANTS OF TB INFECTION IN HEALTH CARE WORKERS OF THE WESTERN CAPE PROVINCE OF SOUTH AFRICA - 2009/2010**

**REFERRAL LETTER**

Dear : \_\_\_\_\_

Name : \_\_\_\_\_

Was a participant in a research project evaluating immunodiagnostic tests for Latent TB infection.

1. Questionnaire result

Questionnaire completed

Questionnaire not completed

Symptom screen suggestive of Active TB:

Yes

No

2. Chest radiograph result

Test not done

Normal

Abnormal

If Abnormal specify:

Signs of inactive TB

Signs of active TB

Signs of other pathology

**3. HIV test**

- Negative  Reported as negative  
 Positive  Reported as positive  
 Test not done

**4. Tuberculin skin test results**

- Test not done  Negative  Positive

- If Positive specify:  Mildly positive ( $\leq 5$ mm induration) [only if HIV positive/immunocompromised]  
 Positive ( $\leq 10$ mm induration)  
 Markedly positive ( $\leq 15$ mm induration)  
 Ulcerating

**5. Sputum test results**

- Test not done   
Microscopy  Positive  Negative  
Culture  Positive  Negative

6. Reason for referral

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Yours faithfully,

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Principal investigator  
Dr S Adams  
Tel 083 285 7665