Preliminary Epidemiological Study of Latent Tuberculosis in Mexican Agricultural Workers In The Niagara Region, Canada

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Faculty of Applied Health Sciences Brock University St. Catharines, ON

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Abstract

It is well documented that the majority of Tuberculosis (TB) cases diagnosed in Canada are related to foreign-born persons from TB high-burden countries. The Canadian seasonal agricultural workers program (SAWP) operating with Mexico allows migrant workers to enter the country with a temporary work permit for up to 8 months. Pre-immigration screening of these workers by both clinical examination and chest X-ray (CXR) reduces the risk of introducing cases of active pulmonary TB to Canada, but screening for latent TB (LTBI) is not routinely done.

Studies carried out in industrialized nations with high immigration from TB-endemic countries provide data of lifetime LTBI reactivation of around 10% but little is known about reactivation rates within TB-endemic countries where new infections (or re-infections) may be impossible to distinguish from reactivation. Migrant populations like the SAWP workers who spend considerable amounts of time in both Canada and TB-endemic rural areas in Mexico are a unique population in terms of TB epidemiology. However, to our knowledge no studies have been undertaken to explore either the existence of LTBI among Mexican workers, the probability of reactivation or the workers' exposure to TB cases while back in their communities before returning the following season. Being aware of their LTBI status may help workers to exercise healthy behaviours to avoid TB reactivation and therefore continue to access the SAWP.

In order to assess the prevalence of LTBI and associated risk factors among Mexican migrant workers a preliminary cross sectional study was designed to involve a convenience sample of the Niagara Region's Mexican workers in 2007. Research ethics clearance was granted by Brock University. Individual questionnaires were administered
to collect socio-demographic and TB-related epidemiological data as well as TB knowledge and awareness levels. Cellular immunity to *M. tuberculosis* was assessed by both an Interferon-γ release assay (IGRA), QuantiFERON-TB Gold In-Tube (QFT™) and by the tuberculin skin test (TST) using Mantoux.

A total of 82 Mexican workers (out of 125 invited) completed the study. Most participants were male (80%) and their age ranged from 22 to 65 years (mean 38.5). The prevalence of LTBI was 34% using TST and 18% using QFT™. As previously reported, TST (using ≥10mm cut-off) showed a sensitivity of 93.3% and a specificity of 79.1%. These findings at the moment cannot predict the probability of progression to active TB; only longitudinal cohort studies of this population can ascertain this outcome. However, based on recent publications, IGRA positive individuals may have up to 14% probability of reactivation within the next two years.

Although according to the SAWP guidelines, all workers undergo TB screening before entering or re-entering Canada, CXR examination requirements showed to be inconsistent for this population: whereas 100% of the workers coming to Canada for the first time reported having the procedure done, only 31% of returning participants reported having had a CXR in the past year. None of the participants reported ever having a CXR compatible with TB which was consistent with the fact that none had ever been diagnosed with active pulmonary TB and with only 3.6% reporting close contact with a person with active TB in their lifetime.

Although Mexico reports that 99% of population is fully immunized against TB within the first year of age, only 85.3% of participants reported receiving BGC vaccine in childhood. Conversely, even when TST is not part of the routine TB screening in
endemic countries, a surprisingly high 25.6% reported receiving a TST in the past. In regards to TB knowledge and awareness, 74% of the studied population had previous knowledge about (active) TB, 42% correctly identified active TB symptomatology, 4.8% identified the correct route of transmission, 4.8% knew about the existence of LTBI, 3.6% knew that latent TB could reactivate and 48% recognized TB as treatable and curable.

Of all variables explored as potential risk factors for LTBI, age was the only one which showed statistical significance. Significant associations could not be proven for other known variables (such as sex, TB contact, history of TB) probably because of the small sample size and the homogeneity of the sample. Screening for LTBI by TST (high sensitivity) followed by confirmation with QFT™ (high specificity) suggests to be a good strategy especially for immigrants from TB high-burden countries. After educational sessions, workers positive for LTBI gained greater knowledge about the signs and symptoms of TB reactivation as well as the risk factors commonly associated with reactivation. Additionally, they were more likely to attend their annual health check up and request a CXR exam to monitor for TB reactivation.
Acknowledgements

I would like to express my gratitude to all who gave me the opportunity to complete this thesis.

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Especially, I would like to give my special thanks to my loving husband who stood beside me and encouraged me constantly and my children whose patient love enabled me to complete this Masters.

I would like to dedicate this work to my mother whose love is boundless.
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<th>Full Form</th>
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<tbody>
<tr>
<td>BCG</td>
<td>Bacille Calmette-Guérin</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CFP-10</td>
<td>Culture filtrate protein-10</td>
</tr>
<tr>
<td>CIC</td>
<td>Citizenship and Immigration Canada</td>
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<tr>
<td>CMA</td>
<td>Canadian Medical Association</td>
</tr>
<tr>
<td>DOT</td>
<td>Directly observed therapy</td>
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<tr>
<td>DOTS</td>
<td>Directly observed therapy short-course</td>
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<tr>
<td>DTH</td>
<td>Delayed-type hypersensitivity</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>ESAT-6</td>
<td>Secretory antigen target-6</td>
</tr>
<tr>
<td>IGRA</td>
<td>Interferon gamma released assay</td>
</tr>
<tr>
<td>INF-γ</td>
<td>Interferon-gamma</td>
</tr>
<tr>
<td>LTBI</td>
<td>Latent tuberculosis infection</td>
</tr>
<tr>
<td>MDR-TB</td>
<td>Multi-drug resistant TB</td>
</tr>
<tr>
<td>MOTT</td>
<td>Mycobacteria other than tuberculosis</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NTM</td>
<td>Non-tuberculous mycobacteria</td>
</tr>
<tr>
<td>PHAC</td>
<td>Public Health Agency of Canada</td>
</tr>
<tr>
<td>PPD</td>
<td>Purified protein derivative</td>
</tr>
<tr>
<td>QFT™</td>
<td>QuantiFERON®-TB Gold</td>
</tr>
<tr>
<td>RNI</td>
<td>Reactive nitrogen intermediates</td>
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<tr>
<td>SAWP</td>
<td>Seasonal agricultural workers program</td>
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</table>
Latent Tuberculosis in Mexican Migrant Workers

TB: Tuberculosis

TNF-α: Tumor necrosis factor alpha

TST: Tuberculin skin test

WHO: World Health Organization

XDR-TB: Extensively drug resistant TB
GLOSSARY

**Acid Fast bacteria**: The ability of bacteria, especially *Mycobacterium tuberculosis* and other members of the genus, to retain certain dyes after treatment with acid-alcohol. In the microscopical examination of sputum and other clinical specimens, *M. tuberculosis* is seen as red rods against a green or blue counterstain background.

**Anergy**: A condition in a person where the ability to exhibit delayed T-cell hypersensitivity reaction to antigens has diminished because of an altered immune function.

**Bacille Calmette-Guérin (BCG)**: A vaccine for tuberculosis named after French scientists Calmette and Guérin. It is used in multiple countries where TB disease is endemic. The vaccine is effective in preventing disseminated and meningeal TB disease in infants and young children.

**Caseous necrosis**: The characteristic central area of tissue necrosis seen in tuberculous lesions, most evident in post-primary lesions. The term caseous necrosis or caseation is in allusion to the cheese-like nature of this necrotic material.

**Cavity**: A necrotic tuberculous lesion which communicates with the airways, enabling tubercle bacilli to enter the sputum and to be coughed out.

**Delayed-type hypersensitivity (DTH)**: Cell-mediated inflammatory reaction that is recognized by the immune system, due to previous exposure to the antigens.

**Directly observed therapy (DOT)**: It is the administration of anti-tuberculosis drugs to TB patients under direct supervision.

**Droplet nuclei**: Small particles (1-5 μm) which contain few TB bacilli and are responsible for transmission of TB. They are generated during coughing, talking or sneezing by an active TB patient, and can remain for long periods of time in the air.

**Induration**: A reaction produced by immune system in response to tuberculin antigen that is introduced into the skin. The firmness or the raised skin (not the redness) is measured and is recorded in millimeters (mm). The measurement is compared to guidelines to determine whether the test result is classified as positive or negative.

**Multi drug resistance TB**: *Mycobacterium tuberculosis* resistant to two or more first-line anti-TB drugs (rifampin or isoniazid)

**Point Prevalence**: The number of cases of a disease in a community at a given point of time.

**Post primary TB**: Tuberculosis occurring after a period of latency.
Purified protein derivative (PPD) tuberculin: It is a collection of mixed proteins from filtered heat-killed cultures of *M. tuberculosis* to be used in diagnostic tests for latent tuberculosis infection.

**Reactivated TB:** Old tuberculosis infection (whether previously known or not) which has become active.

**Tuberculin:** A protein extracted from *M. tuberculosis* that is used in the skin test to determine if a person has been exposed to tuberculosis.

**Tuberculin skin test (TST):** A test used to aid in the diagnosis of *M. tuberculosis* infection. A small dose of tuberculin is injected following the Mantoux method, and the area is examined for induration 48–72 hours after the injection.

**XDR-TB:** *M. tuberculosis* resistant to both first- and second-line drugs

**Ziehl-Nielsen staining:** The name given to the most widely used technique for acid-fast staining of mycobacteria.

**References:**


CHAPTER ONE – INTRODUCTION

“TB Anywhere is TB Everywhere”, 2007’s theme for Tuberculosis Day emphasizes the need for continued concern about tuberculosis (TB) around the world (1). Although the 2007’s World Health Organization (WHO) report states that the worldwide incidence rate of TB has levelled off in the previous year, TB remains a threat to millions of people (2). In 2006, 9.2 million new TB cases were reported worldwide (55% in Asia and 31% in Africa) with an estimated total mortality of 1.6 million people. Furthermore, it is reported that one in ten new cases of TB is resistant to at least one first-line drug of treatment. The spread of drug resistance strains from one person to another increases costs for treatment, reducing the possibility of success of global TB programs (2).

In addition to the active cases (i.e., TB disease), it is widely reported that one third of the world population is infected with the inactive form of TB, known as latent TB infection (LTBI). In developed nations, it is also estimated that a person with LTBI has a lifetime risk for reactivation of 5-10%. In fact, it is assumed that many cases of active TB in these countries are relapsed cases, that is, due to reactivation (3, 4).

In Canada, TB incidence rate is still one of the lowest in the world. According to the Public Health Agency of Canada (PHAC), 1,621 cases of new active and relapsed cases of TB were reported in 2006 which accounted for a rate of 5.0 per 100,000 population (5). The improvement of living standards and the public health interventions, including detection of active cases and treatment could lead the possibility of TB elimination. However, the epidemiology of TB in Canada has changed in the last two decades. Most cases of TB are reported in the foreign-born population and Canadian-born aboriginals. In 2006, approximately 65% of total of TB cases in Canada were diagnosed
in foreign-born individuals and about 20% in Canadian-born Aboriginals, whereas 12% occurred among non-Aboriginal Canadian born population (5, 6). The increment of immigrants from countries with high incidence and prevalence rates of TB has been documented (5, 7). British Columbia, Ontario and Quebec, the provinces with the highest rates of immigration account for approximately 73% of the whole country’s reported cases. Of the foreign-born TB cases reported for 2000, 10% developed active TB (reactivation of LTBI) within the first year of arrival, 17% within 2 years and 35% within 5 years (8).

Immigrants to Canada undergo medical examination before entering the country. All medical conditions that are considered a threat to the Canadian population or represent an extra-demand for the Canadian health care system are treated in their home countries (9). Among immigrants, a permanent group of migrant workers from Mexico and the Caribbean come every year to Canada for up to 8 months to work in the horticultural industry (10). The number of workers who come every year to Canada is significant. In 2006, over 10,000 Mexicans workers were admitted, of which more than 80% came to Ontario as their principal destination. According to FARMS, the Niagara Region alone received 3,000 workers in 2006 (11).

Eligible candidates to the Mexican Seasonal Agricultural Workers Program (SWAP) must present no (or minor) health conditions and not require medical care or follow-up in Canada that could interfere with the demanding job. In this sense, for migrant workers, health is synonymous with job opportunities in Canada. Among the health screening exams these workers undergo, tests to determine active TB consisting of clinical history and chest X-rays are particularly important. Ruling out the possibility of
active TB has a two-fold importance: for the workers, in order to obtain the job as expected, and for Canada, the risk for local transmission is reduced (11). Screening for LTBI is not part of the routine tests for migrant workers, but the probability of harbouring dormant, non-infectious asymptomatic *M. tuberculosis* infection is high considering that the national TB incidence rate in Mexico is 32 cases/100,000 in 2004, around 7 times higher than that of Canada (12). If infected, the potential for TB reactivation in these workers is unknown, as no reports can be found in the literature. However, anecdotes exist of several active TB cases among workers many years ago (McLaughlin, Janet, University of Toronto, personal communication). The stress of working in Canada, the climate and dietary changes, etc., may be important factors in the reactivation of latent TB. In the event a worker experienced TB reactivation while in Canada or shortly before returning the following season, many factors such as limited access to health care and language barriers could interfere with the diagnosis and treatment (13). In summary, it is important for these workers to be screened for LTBI and be cognizant about their status.

The proposed study aimed to develop an understanding of the occurrence of LTBI in a convenience sample of Mexican migrant workers in the Niagara Region. The study determined the prevalence of LTBI in this population using two diagnostic tests, tuberculin skin test and QuantiFERON-TB Gold In-Tube (QFT™). The performance of TST was evaluated using QFT™ as a confirmatory test. In addition, the current knowledge Mexican workers had in respect to active TB and LTBI, (symptoms, routes of transmission, severity, etc) was examined. The presence of known risk factors associated with LTBI as well as the presence of specific characteristics of this population that could be associated with LTBI was explored. As part of this project, educational sessions
focusing on healthy behaviours, prevention and recognition of TB symptoms and a proactive attitude towards one’s health were held with research participants.

This was the first study of this nature in this particular population in the Niagara Region, and was part of a larger investigation on TB in Mexican migrant workers. This study was conceived from discussions with Mexican researchers based in Benemerita U of Puebla, who were very interested in looking at the social determinants of migration to Canada and the role of tuberculosis as a major health determinant for eligibility to work in Canada. Puebla is a state with great cultural and economic social disparities between the industrial urban centres and the rural areas characterized by greater indices of poverty and migration. Puebla is a major contributor of agricultural workers to Canada and such study would contribute to the understanding of the health and economic impact of TB among this migrant population.

Both studies are funded under the “Mexico-Canada Joint Health Research Program in Tuberculosis”. The component in Niagara Region was funded by the Canadian Institutes of Health Research (CIHR) and component in Mexico by the National Council for Science and Technology (Consejo Nacional de Ciencia y Tecnologia) (CONACyT). Both studies will make an important contribution to the knowledge of LTBI in Mexico, in Canada and specifically in this population.
CHAPTER TWO– REVIEW OF LITERATURE

1. Tuberculosis

2.1 History of Tuberculosis

The history of TB is at least as long as human existence or perhaps older. For centuries both humans and the pathogen have been in a dynamic interplay, implementing different strategies to survive. As is true in the present, the mobility of people around the world and the incremental number of people within communities and confined spaces were also important conditions for the effective transmission and spread of TB disease in the past (14).

Before the introduction of antibiotics, sanatoria and public health intervention played a tremendous role in the declining number of cases of active TB. The creation of sanatoria in 1854 notably reduced transmission and contributed to public health control of TB. Sanatoria were places with “fresh air” where diseased people were isolated and forced to rest and have a special diet to help in self-healing (15, 16). Later, treatment of TB with antibiotics nearly eradicated the disease in some areas of the world. Nowadays, however, the emergence of multi-drug- resistant TB strains (MDR-TB) combined with the AIDS pandemic have increased the resurgence of TB, in spite of TB control programs throughout the world. Table 1 depicts major events in the history of tuberculosis (2).

Tuberculosis was declared a global health emergency by the WHO in 1993 (2). In 2007, the WHO declared that although the global TB epidemic had levelled off in 2005, the number of people with TB increased in Africa, Eastern Mediterranean and South-East Asia regions (17).
Table 1. Major events in the history of tuberculosis.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>EVENT</th>
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<tbody>
<tr>
<td>4000 BC</td>
<td>Evidence of the disease from about 4000 BC in Egyptian mummies (18).</td>
</tr>
<tr>
<td>700 BC</td>
<td>Evidence of the disease in the Americas in Peruvian mummies from the Andean Region in the Americas (14).</td>
</tr>
<tr>
<td>460 BC</td>
<td>The term <em>Pththis</em> was introduced in Greece by Hippocrates who recognized the importance and severity of the disease. The disease was considered hereditary and a consequence of mental and moral weakness (19, 20).</td>
</tr>
<tr>
<td>174 BC</td>
<td>The Greek physician Galen defined TB as an ulceration of the lungs, chest or throat and described the symptomatology of TB as cough, fever and “wasting away of the body”. Also, he recommended “fresh air, milk and sea voyages” as a treatment for TB (19).</td>
</tr>
<tr>
<td>By 1650</td>
<td>The spontaneous generation theory was generally accepted: some life forms arose spontaneously from non-living things (21).</td>
</tr>
<tr>
<td>1679</td>
<td>Franciscus de la Boe described the tubercles found in TB patients as characteristic lesions in the lungs that can progress into caverns and ulcers (20).</td>
</tr>
<tr>
<td>1854</td>
<td>Hermann Brehmer, a German physician, suffered from TB himself but was cured after spending some time in the Himalayas with fresh air and healthy eating habits. He built the first German sanatorium where patients could recuperate under the same healthy conditions (14).</td>
</tr>
<tr>
<td>1882</td>
<td>German scientist Robert Koch demonstrated <em>Mycobacterium tuberculosis</em> as the etiologic agent of TB (21, 22).</td>
</tr>
<tr>
<td>1895</td>
<td>Wilhem von Röntgen discovered X rays and TB diagnoses become more accurate (20).</td>
</tr>
<tr>
<td>1907</td>
<td>Clement von Pirquet developed the tuberculin skin test (TST) and demonstrated latent TB infection (LTBI) in children for the first time (14).</td>
</tr>
<tr>
<td>1921</td>
<td>Albert Calmette and Camille Guérin developed the first TB vaccine from an attenuated virulent strain of <em>M. bovis</em>; BCG (Bacille Calmette-Guérin) (14, 20).</td>
</tr>
<tr>
<td>1943</td>
<td>Streptomycin, discovered by Schatz and Waksman, was the first antibiotic that showed efficacy against <em>M. tuberculosis</em>. Other drugs such as para-aminosalicylic (PAS) and thiosemicarbazone were also used (18).</td>
</tr>
<tr>
<td>1948</td>
<td>The first TB control strategy sponsored by World Health Organization after War World I, involved TST testing followed by BCG vaccination (14, 20).</td>
</tr>
<tr>
<td>1952/57</td>
<td>Isonizid (INH) first oral TB antibiotic and rifamycins started a new era of anti-tuberculosis drugs. Sanatoria were closed (14, 18).</td>
</tr>
<tr>
<td>1980</td>
<td>The association between HIV and <em>M. tuberculosis</em> dramatically increased the risk of developing tuberculosis. The incidence of TB begins to rise (23).</td>
</tr>
<tr>
<td>1990's</td>
<td>TB strains resistant to antimycobacterial drugs emerged, mainly from inadequate therapy: improper prescription of treatment regimens, poor quality drugs and non-adherence by patients (24).</td>
</tr>
<tr>
<td>1991</td>
<td>Multi-drug-resistance strains (MDR-TB) arise as a consequence of sequential accumulation of mutations that conferred resistance to single agents. MDR-TB strains are resistant to second-line drugs (24).</td>
</tr>
<tr>
<td>1991</td>
<td>The WHO, the Centers for Disease Control and Prevention and other authorities recommended directly observed therapy (DOT) to achieve success in adherence with TB drug treatment. Short-course directly observed therapy (DOTS) was developed to combat disease globally (2).</td>
</tr>
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Latent Tuberculosis in Mexican Migrant Workers

<table>
<thead>
<tr>
<th>YEAR</th>
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<tbody>
<tr>
<td>1993</td>
<td>WHO estimated that in this year eight million people were infected with TB, with 95% of the cases in developing countries. They estimated 2 million deaths and declared TB a global emergency (2).</td>
</tr>
<tr>
<td>1998</td>
<td>Efforts were joined to combat TB globally with specific working groups in: DOTs expansion, TB/HIV research, new TB drugs, new vaccines and new diagnoses methods (2).</td>
</tr>
<tr>
<td>2005</td>
<td>A serious public health threat emerged in Africa. A strain of TB called extensively drug resistant TB (XDR-TB) that resists second line of drugs was identified (19).</td>
</tr>
</tbody>
</table>

2.2 The Genus *Mycobacterium*

Microorganisms from the genus *Mycobacterium* includes more than 70 species ranging from pathogenic to environmental and opportunistic bacteria (14, 25, 26). Mycobacterium species are grouped into complexes comprising from the etiologic agents of major diseases such as TB, leprosy and other lung diseases in humans or animals, to a small number (not less important) of opportunistic diseases caused by non-tuberculous mycobacteria (NTM) [also known mycobacteria other than tuberculosis (MOTT)](19, 26).

2.2.1 *Mycobacterium tuberculosis* Complex

The *M. tuberculosis* complex is comprised of the following species: *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti* and *M. canetti* (25, 26). Species of the *M. tuberculosis* complex can cause tuberculosis in humans, however *M. tuberculosis* itself is the major pathogen to humans and humans are its only known reservoir. These species are the etiological agents of TB in humans and animals and share about 80% of similarities in their genome (19). They differ in their epidemiology, biochemistry, host range and importance in human disease, for this reason, further differentiation between species is necessary (25).

*Mycobacterium tuberculosis* is the major pathogen for humans, responsible for infecting one-third of the world population causing more that 2 million deaths annually
worldwide (2). *Mycobacterium bovis* causes TB in animals such as cattle and goats, but it can also infect humans causing mostly gastrointestinal disease, especially by drinking contaminated and unpasteurized milk from infected cattle (27). *Mycobacterium africanum* is the major cause of human TB in Africa; in some regions, more that 60% of reported isolates from patients with active TB are represented by this specie. *M. microti*, also known as “Vole Bacillus”, causes TB in rodents but has been considered to be non-pathogenic for humans. *M. canetti* has been reported to cause pulmonary TB in a few immunosuppressed patients (28). The taxonomy of *M. tuberculosis* complex and other species are detailed in Table 2.

Table 2. Taxonomic position of *Mycobacterium tuberculosis* complex and other species [after Palomino, et al, (2007) (19)]

<table>
<thead>
<tr>
<th>Domain</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Actinobacteria</td>
</tr>
<tr>
<td>Class</td>
<td>Actinobacteria</td>
</tr>
<tr>
<td>Subclass</td>
<td>Actinobacteridae</td>
</tr>
<tr>
<td>Order</td>
<td>Actinomycetales</td>
</tr>
<tr>
<td>Suborder</td>
<td>Corynebacterineae</td>
</tr>
<tr>
<td>Family</td>
<td>Mycobacteriaceae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Mycobacterium</em></td>
</tr>
<tr>
<td>Species:</td>
<td><em>M. tuberculosis</em></td>
</tr>
<tr>
<td></td>
<td><em>M. bovis</em></td>
</tr>
<tr>
<td><strong>MTB COMPLEX</strong></td>
<td><em>M. africanum</em></td>
</tr>
<tr>
<td></td>
<td><em>M. canetti</em></td>
</tr>
<tr>
<td></td>
<td><em>M. microti</em></td>
</tr>
<tr>
<td></td>
<td><em>M. leprae</em></td>
</tr>
<tr>
<td><strong>NTB</strong></td>
<td><em>M. avium</em></td>
</tr>
<tr>
<td></td>
<td><em>M. kansasi</em></td>
</tr>
<tr>
<td></td>
<td><em>M. marinum</em></td>
</tr>
</tbody>
</table>

* Non-tuberculous mycobacteria: all other mycobacteria different from MTB complex and *M. leprae*
The only vaccine for TB that has been used for almost a century is a live, attenuated strain of *M. bovis*, Bacillus Calmette-Guerin (BCG). The effectiveness of BCG vaccine has been debated for many years; however it is indicated in countries where the prevalence of TB is high, protecting particularly children from tuberculous meningitis and miliary TB (19).

### 2.2.2 Non-tuberculous Mycobacteria

Non-tuberculous mycobacteria (NTM or MOTT) include all the remaining species of the genus *Mycobacterium* except *M. leprae*, which causes leprosy. These species are infrequent pathogens for humans and person to person spread has not been demonstrated. Most of these species are free-living organisms ubiquitous in the environment and some representatives are *M. gordonae, M. fortuitum, M. terrae, M. stegmatis*, etc. These bacteria have been recovered in animals and environmental sources, particularly water (20).

Non-tuberculous mycobacteria may cause disease in immunosuppressed people (26). The clinical manifestation could be similar to active TB. Globally, HIV infection has increased the incidence of *M. avium-intracellulare* complex (MAI complex) as an important pathogen, which is responsible for chronic pulmonary disease, often lethal in 25-50% of the patients with AIDS (26). Other species such as *M. kansasii, M. marinum, M. scrofulaceum* may also cause disease in human, but are very rare.

Particularly important to the present research is the fact that people who have been exposed to NTM could be sensitized to antigens that are similar to *M. tuberculosis* and present cross-reactivity with the tuberculin skin test (TST) resulting in false positives (26). Similarly for Interferon-γ release assays, particularly QuantiFERON®-TB Gold In-
Latent Tuberculosis in Mexican Migrant Workers

2.3 General Characteristics of Mycobacterium tuberculosis

*Mycobacterium tuberculosis* is a rod-shaped, obligate aerobe, non-motile and non-spore forming bacterium, measuring 2-4 µm in length and 0.2-0.5 µm in width (19). In addition to peptidoglycan, mycobacteria have in their cell wall a layer composed of over 60% lipids, especially mycolic acid and other free lipids. This wall forms a hydrophobic barrier, which plays an important role in the bacterial pathogenesis, particularly for *M. tuberculosis* (30). Resistance to drugs and dehydration, capacity to survive inside of macrophages and permeability to chemical compounds are the most significant examples (31, 32, 33, 34). Due to the nature of the cell wall, all mycobacteria are variable to Gram staining but have the special characteristic of being acid-fast or resistant to decolorization with acid-alcohol solution during acid-fast staining. Therefore, Ziehl-Nielsen stain is widely used to identify acid-fast bacilli (AFB) (figure 1) in samples suspected of having *M. tuberculosis* (26).

Figure 1. Sputum smear showing acid-fast bacillus, *Mycobacterium tuberculosis*, stained with Ziehl-Nielsen. (University of Washington, 2006)
Mycobacterium tuberculosis is a slow growing bacterium, with a replication time of approximately 20 hours. It could take as long as two months to isolate the bacteria in standard cultures, thus delaying the microbial diagnosis (31). Mycobacterium tuberculosis thrives at moderate temperatures (25-41°C) and is sensitive to heat and UV light so the survival in the environmental is limited (19).

One of the most resourceful strategies that aid M. tuberculosis’ pathogenicity and persistence is its adaptability to different environments; in-vitro culture and inside macrophages in lung cavities, etc. Depending on the nutritional and oxygen availability, the bacillus is able to switch its metabolic pathway from active to hibernation in order to survive under stressful and deprived conditions (19, 35, 36). These characteristics are the basis for the chronic nature of infection and cause delay in microbiological diagnosis.

2.4 Clinical Aspects of Human Tuberculosis

2.4.1 Transmission

Person to person airborne transmission via respiratory droplets is the most efficient and common mode of TB spread (2, 17, 26, 37, 38). Rarely, M. tuberculosis enters the human body through other routes. Some hospital or laboratory procedures, such as sputum induction, bronchoscopy or manipulation of contaminated samples may produce infectious aerosols that could be transmitted to health care workers or other patients (26, 38).

Tuberculosis transmission begins with the inhalation of airborne droplet nuclei containing the tubercle bacilli. These minute droplets (1-5 μm) are generated when a person with active TB (pulmonary or laryngeal disease) coughs, sneezes, or talks and remain suspended in the air for a long period of time which increases the opportunity for
a susceptible individual to inhale them. Once inhaled, droplet nuclei travel through the airways and reach the alveoli, which are considered the primary infection site (17, 25, 26). A successful transmission depends on (14, 26, 38, 39):

- Virulence mechanisms of the specific strain
- Number of bacillus being expelled by an infected person (determined by airway cavitations and frequency of cough)
- Duration and intimacy of the exposure
- Immune system of the exposed person

In addition to the above, some environmental conditions aid in transmission (14, 26, 39):

- Ventilation and space at the site of contact
- Concentration of organisms in the air
- Amount of ultraviolet light available

2.4.2 Immunopathology and Host’s Immune Response

There are three potential outcomes after inhalation of droplets containing bacteria via respiratory route (Figure 2) (39, 40, 41):

1. Immediate clearance of infection by the innate immunity without leaving immunological memory.

2. Latent TB infection (LTBI): control the infection by the immune system ensuing immunological memory, which accounts for around 90-95% of the cases of *M. tuberculosis* infections.

3. Development of primary active disease after inhalation. Only 10% of infected people develop primary TB.
Inhalation of infectious droplet nuclei

1. Clearance of Infection (Spontaneous healing)  
   ?  
   90%  
   10%  

2. Latency
   ——— Dissemination ———  
   Continued latency  
   Reactivation (Active TB)  
   5-10%  

3. Active TB
   Miliary TB

Figure 2. Possible outcomes after inhalation of *M. Tuberculosis* in an immunocompetent person. Based on Parrish et al(1998) (41), Van Crevel (2002X40).

The host immune response against *M. tuberculosis* is still not fully understood. Once in the alveolus, *M. tuberculosis* is engulfed by resident macrophages and tissue dendritic cells, first line of defence of the innate immune system in charge of intracellular killing (42). Various receptors have been identified in macrophages and dendritic cells for phagocytosis of *M. tuberculosis*. Complement receptors (type 1: CR1, type 3: CR3, Type 4: CR4) bind complement-opsonized *M. tuberculosis* and mannose receptors bind non-opsonized bacteria through recognition of receptors in the bacteria surface. Complement receptor 3 (CR3) has been reported the most important phagocyte receptor as it mediates the majority of complement-opsonized phagocytosis. The binding of *M. tuberculosis* to phagocytic cells throughout CR3 activates phagocytes, promoting phagocytosis and further killing of the bacteria (43). Similarly, binding of non-opsonized
*M. tuberculosis* to phagocytes during primary infection is important since complement components are limited in the alveoli. In either case, it has been reported that the type of receptor can influence the further response in the phagocyte (44).

At this point, it has been reported the possibility of spontaneous healing by the innate immune system without leaving immunological memory. This clearance, however, depends on the activation of phagocytic cells, host genetic factors and virulence and concentration of the *M. tuberculosis* strain (35, 45). The proportion of persons that undergo spontaneous healing is unknown, although Manabe & Bishai (2000) stated that it can be as high as 70% (46).

Macrophages unable to clear the infection become chronically infected. In fact, *M. tuberculosis* has evolved strategies for surviving inside the macrophages: while macrophage’s mechanisms to destroy pathogens are activated, mycobacteria overcomes this obstacle by blocking them, establishing a niche inside the macrophage, where it can survive for a long time (37, 42). Normally, these mechanisms are the formation of the phago-lysosome and the production of nitric oxide (NO) and reactive nitrogen intermediates (RNI) (40, 47). *Mycobacterium tuberculosis* blocks phagosome-lysosome fusion (48) and encodes genes that produce proteins that counteract the effects of NO and RNI (49).

*Mycobacterium tuberculosis* then replicates within macrophages and more phagocytes are recruited in response to chemokines released by infected macrophages and dendritic cells (50, 51). In turn, these phagocytic cells migrate to the draining lymph nodes to present epitopes to naïve T cells from the adaptive immune system (37, 50). T helper lymphocytes (CD4) and cytotoxic T lymphocytes (CD8) are activated and
expanded in number to migrate into the lung to control the bacteria and to form a nascent granuloma (35, 45).

*Mycobacterium tuberculosis* is controlled in a granuloma, a spherical cell accumulation composed by macrophages, different T cell populations and other inflammatory cells. It surrounds and contains infected macrophages and limits spread of bacteria from the lungs to other parts of the body. In addition, the granuloma serves to protect the alveolar tissue and control the number of bacteria (35, 51, 52). However, it is not only an “immune microenvironment” to contain the infection but also is the *M. tuberculosis*’ habitat for long time (35, 49). It has been reported that *M. tuberculosis* remains viable, or dormant, for many years, changing its aerobic metabolic pathway to the glyoxylate cycle, which uses acetate or fatty acids as the sole carbon source to survive in that hostile anaerobic environment inside of the granuloma until reactivation or calcification (35, 49). The granuloma is the principal component of LTBI but is invisible in the lung radiograph, therefore continues undetected.

Maintenance of the granuloma is dynamic process of the immune system, sometimes for life. Chemokines and cytokines signalling during TB infection controls the activation, recruitment and migration of leukocytes at sites of infection as well as granuloma formation and maintenance (35, 49, 52). The hostile conditions for *M. tuberculosis* inside the granuloma is provided by macrophages which are constantly activated by an important cytokine, interferon-gamma (IFN-γ), secreted by T helper lymphocytes. At the same time, T helper lymphocytes response is modulated by another cytokine, IL-12, secreted by macrophages and dendritic cells (35). The role of IFN-γ and other cytokines such as tumor necrosis factor alpha (TNF-α) is to maintain granuloma
and to produce sustained protective immunity against *M. tuberculosis* infection (49, 53, 54).

Human granuloma remain viable for an unknown period of time and either undergo calcification (visible on X rays) or reactivation, which is the most common way of TB disease (post-primary TB) development in non-endemic countries (36, 46). People who harbour the latent infection remain at risk for reactivation if their immune system is weakened. When the immune balance that keeps the granuloma is lost, caseous lesions develop inside the granuloma and form an ideal culture medium where the bacteria can multiply and may either cause pulmonary TB or disseminate to other tissue and organs. *Mycobacterium tuberculosis* can spread within the lungs (pulmonary TB) or other tissue via the lymphatic and blood systems (miliary or extrapulmonary) (40). Cavitations are formed with a zone of collagen that often can be seen in a chest x-ray (35, 47, 49).

Reactivation of LTBI can be explained by an underlying immune suppression process (e.g. HIV infection/AIDS, immunosuppressive medication, advanced age, malnutrition, etc) (26, 35, 49, 55). The risk for reactivation for an immunocompetent individual is 5–10% in the first 2 years after infection in areas of low transmission (26). On the contrary, the risk for reactivation for an immunosuppressed individual is 14% every year (26, 56). Tuberculosis is the most common opportunistic disease in HIV infected people. After primary infection or by endogenous reactivation, there is a high risk of progression to active TB (26, 56).

Studies of LTBI have been difficult due to the lack of animal model to replicate accurately the process of granuloma formation, maintenance and dissolution in humans. There are, however, animal models that mimic at least some aspect of human LTBI (50,
Latent Tuberculosis in Mexican Migrant Workers

One of the most famous models for latency was developed by researchers at Cornell University, USA, in the 1950s. This model is based on infection of mice with *M. tuberculosis* and subsequent treatment with antibiotics (isoniazid and pyrazinamide), resulting in non culturable bacteria. Thus, after 90 days of cessation of antibiotics, the infection reactivates either spontaneously or induced by immunosuppression. This model has been used as a tool for testing treatment regimens and for identification of factors involved in the mechanisms of LTBI (35, 49).

2.4.3 Clinical presentations and treatment

2.4.3.1 Active Tuberculosis

As explained in figure 2 (pg. 13), there is a 10% likelihood of developing active disease after primary infection. Tuberculosis generally affects the lungs and respiratory tract, which represent the main port of entry and transmission. Pulmonary TB is the most frequent clinical manifestation of TB (35, 37, 47, 53). Cough could be non-productive at the beginning of the illness but as the cavity is forming, sputum is usually produced as well as hemoptysis, fever, weight loss and night sweating, other specific symptoms and signs of pulmonary TB (26, 38).

Extrapulmonary TB can affect pleura, pericardium, larynx, lymph nodes, bones, genitourinary tract, eyes, and skin (17, 26) and causes both specific-organ related and general nonspecific symptoms such as fever, anorexia, weight loss, malaise and fatigue (57). Disseminated TB (also known as miliary TB) develops as a result of haematogenous dissemination of *M. tuberculosis* through the body, involving multiple organs. Therefore, clinical manifestations of disseminated TB are generally non-specific and depend on the severity of the disease in the organs affected (38).
Tuberculosis can be treated with standard first-line anti-tuberculosis drugs (isoniazid, rifampin, pyrazinamide and ethambutol) for a minimum of 6-9 months. After a completed treatment, the patient is cured and rendered non-infectious, unable to spread the disease to other individuals. Multidrug-resistant TB (MRD-TB), however, can develop due to poor adherence to treatment or improper antibiotic regimen (2). In this case, the patient will need longer treatment with second-line of drugs (rifapentine and rifabutin), which are expensive and produce more side-effects than first-line antibiotics. Similarly, if the treatment for MRD-TB is mismanaged, extensively drug-resistant TB (XDR-TB) can develop which is resistant to first- and second-line of drugs, limiting the possibility of cure and challenging the worldwide TB control programs (26).

Since 1991, directly observed therapy, short-course (DOTS) strategy was implemented to improved access to TB treatment with supervision, availability of drugs and patient support (2). This strategy comprises case detection (strengthening TB laboratories), standardized treatment with supervision and drug supply. In addition, political commitment and sustained financial and monitoring and evaluation of programs, TB cases, budget, etc are key components of this worldwide strategy (58). As a result, DOTS has helped many countries (182 countries = 93% world population) to improve their TB programs, especially those with high burden of TB (2).

2.4.3.2. Latent Tuberculosis Infection

Latent tuberculosis infections are clinically and radiographically imperceptible: the individual is asymptomatic, and not contagious (2, 26, 38). It has been reported, based on TST screening that two billion people worldwide are infected with *M. tuberculosis* (2). Of those, approximately 90% will continue in latency, meaning they have no clinical
disease and possibly are not cognizant of their TB infection but serve as a reservoir of *M. tuberculosis* until reactivation and spread.

Treatment for LTBI can reduce the development of active TB and has been a critical component of the TB-elimination strategy, especially in developed countries and HIV infected people. In developing countries, is also recommended for immunocompetent persons with increase risk of TB disease (co-infected with HIV and children under 5 years of age) (2, 26).

Isoniazid is the antibiotic of preference for nine months and to improve adherence, directly observed preventive therapy (DOPT) has been implemented (39). Risk of hepatitis (liver damage with enzymes exceeding the normal level by five times) has been associated to isoniazid treatment. Liver function testing at baseline and at least monthly is recommended for persons older than 34 years, or with pre-existing liver diseases or history of alcohol abuse. For patients for whom treatment is contraindicated or who refuse or who do not complete it, regular follow-up (chest x-ray) is highly recommended (26).

2.5 Diagnoses of Tuberculosis

2.5.1 Active Disease

*Chest Radiography:*

- Upon clinical suspicion, chest radiography is the first tool used to identify pulmonary TB, although is not the gold standard for diagnosis of active pulmonary disease (26).

*Laboratory:*
1. Acid-fast bacillus staining (AFB): could be done from different samples such as serial sputum, bronchoalveolar lavage, gastric aspirate or tissue biopsy (59). This staining is useful and highly specific but depends on the quality of specimen collected and experience of the technician who examines the smear (26).

2. Mycobacterial culture: is considered the gold standard for diagnosis of \textit{M. tuberculosis} with sensitivity greater than 90%. A series of three cultures is recommended, with further identification of species by biochemical tests, high performance liquid chromatography (HPLC) or DNA tests (26, 59). Culture of \textit{M. tuberculosis} can take up to 8 weeks which delays and complicates the diagnosis and treatment (60).

In addition, clinical symptomatology for pulmonary disease such as chronic cough for more than 3 weeks, hemoptysis, fever, night sweets, and weight lost need to be evaluated. For extrapulmonary disease, specific symptomatology in the site of infection must be assessed (26, 59).

2.5.2 Latent Infection

2.5.2.1 Tuberculin Skin Test (Delayed-type Hypersensitive Test)

Until recently, the standard method to diagnose LTBI was a positive tuberculin skin test (TST). The TST detects the presence of infection by eliciting a person’s cell-mediated delayed-type hypersensitivity reaction in vivo (61). Delayed-type hypersensitivity (DTH) is an antigen specific reaction mediated by the cellular immune system to prior exposure to an antigen. This reaction has been shown to be dependent on the presence of memory T helper lymphocytes (CD4) (42, 62). The general characteristics are a recruitment of immune cells at the site of injection and subsequent
deposition of cell infiltrates (i.e., fibrin) (19). The reaction causes erythema, swelling and induration at the site of antigen injection within 24 to 72 hours (26, 59).

Robert Koch was the first to demonstrate a DTH reaction in 1882. Koch attempted to use culture filtrates of *M. tuberculosis*, called tuberculin, as a prophylactic and therapeutic vaccine, injected intravenously. Instead of protection, it caused the development of pathological symptoms. However, when the antigen was injected intradermally, DTH response could indicate whether or not an asymptomatic person had been exposed to *M. tuberculosis* (21, 22, 63).

The most used technique for TST is Mantoux method, which is an intradermal injection of 5 tuberculosis units of purified protein derivative (PPD) from *M. tuberculosis* in 0.1 mL of solution. The induration is measured within 48-72 hours after administration of PPD. Therefore, it has been established different cut-off values which a TST result is considered positive (i.e., likely LTBI) or negative (i.e., not likely LTBI) (Table 3).

The interpretation of TST reactivity, however, can be complicated by many factors. For example, as it was stated before, previous BCG vaccination or exposure to NTM can result in positive TST reactions. In contrast, factors as HIV infection and recent viral or bacterial infections or vaccination with live virus can reduce response and result a negative TST. In both cases, TST can misclassify individuals regarding infection with *M. tuberculosis* (64).

Based on the size of the induration, a TST is considered positive using the following criteria:
Table 3. Positive tuberculin skin test interpretation based on induration size (26)

<table>
<thead>
<tr>
<th>Induration size</th>
<th>TST is considered positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5 mm</td>
<td>HIV with immunosuppression and the likelihood of TB disease is high</td>
</tr>
<tr>
<td></td>
<td>HIV infection</td>
</tr>
<tr>
<td></td>
<td>Close contact with an active TB case</td>
</tr>
<tr>
<td>5-9 mm</td>
<td>Children suspected of having TB disease</td>
</tr>
<tr>
<td></td>
<td>Abnormal chest X-rays</td>
</tr>
<tr>
<td></td>
<td>Other immune suppression</td>
</tr>
<tr>
<td>≥ 10 mm</td>
<td>Foreign-born individuals from high TB prevalence country</td>
</tr>
<tr>
<td></td>
<td>Individuals who inject illicit drugs</td>
</tr>
<tr>
<td></td>
<td>Individuals with underlying medical condition</td>
</tr>
<tr>
<td></td>
<td>Health care workers and staff or residents of long-term care facilities</td>
</tr>
<tr>
<td></td>
<td>Converters (Individuals who increased their mm by 10 mm or more in 2 years)</td>
</tr>
</tbody>
</table>

Based on Canadian Tuberculosis Standards, 2007 (26)

For some individuals with LTBI, the ability to react to tuberculin over time diminishes (i.e., may have a TST negative). This phenomenon is known as booster reaction. Then, a subsequent TST may be positive. This occurs because the first test stimulated the person’s immune system, therefore, the ability to react. This positive result can suggest two scenarios; a boosted reaction or a recent infection (conversion). To confirm the possibility of booster phenomenon, a second TST is recommended in 1-2 weeks after first TST (two-step TST) (26, 59). In Canada, two-step TST is performed only once when subsequent TSTs will be repeated in regular intervals (health care workers, correctional service workers) or when the first TST is negative (59, 65).
2.5.2.1 Interferon Gamma Release Assays

Interferon-γ release assays (IGRAs) measure IFN-γ production, ex vivo, from previously sensitized lymphocytes in response to the *M. tuberculosis* specific proteins (66). Currently, there are 3 commercial IGRAs: QuantiFERON®-TB Gold and QuantiFERON®-TB Gold In-Tube (Cellestis Ltd, Australia) (29) and T-SPOT.TB (Oxford Immunotec, United Kingdom) (67). QuantiFERON®-TB Gold received approval from the US Food and Drug Administration (FDA) in 2005. Although QuantiFERON®-TB Gold In-Tube (QFT™) is available in Canada (66), the Canadian Tuberculosis Committee Advisory’s Committee Statement (2007) has recommended its use only under specific circumstances (26, 68).

As summarized in section 2.4.2 (the immune response), cellular immunity is induced by infection and is mediated by macrophages, T cells and cytokines such as INF-γ and TNF-α. Interferon-γ release assays determine whether the T cells have been sensitized previously to *M. tuberculosis* antigens in an individual. If so, T cells are stimulated to produce INF-γ in vitro either whole blood (QFT™) (29) or separated peripheral blood mononuclear cells (T-SPOT.TB) (67). The amount of INF-γ released is then measured by an enzyme-linked immunosorbent assay (ELISA) (63, 64, 69, 70, 71). These new tests have better specificity than TST in the diagnosis of LTBI (66).

These assays are not new for the diagnosis of TB. A commercial assay was originally developed for diagnosis of bovine tuberculosis in cattle, sheep and goats using tuberculin from *M. bovis* (bovine gamma interferon test, Bovigam®)(72). An adaptation of this test was developed in 2001 for the diagnosis of TB in humans, using PPD as the first mycobacterial antigen: QuantiFERON test (Cellestis Ltd, Australia)(69). The second
generation of this test used a mixture of synthetic peptides simulating two proteins presents in *M. tuberculosis*: early secretory antigen target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) (69, 70). The sensitivity and specificity of this assay was increased by using more specific stimulatory antigens.

**QuantiFERON®-TB Gold In-Tube**

QuantiFERON®-TB Gold In-Tube is a latest version of the test, which includes a new mycobacterial antigen called TB7.7(p4), in addition to ESAT-6 and CFP-10 (29). This test collects blood directly into specialized collection tubes containing the lyophilized antigens. In addition, a positive control tube (containing phytohemagglutinin) and a negative control tube (containing only saline) are included in the in-tube method. The positive control assesses the adequate respond of a person’s immune system. The negative control measure the background level of INF-γ (29, 70).

The specificity of this test is high since these peptides are restricted to the tuberculosis complex and absent in all BCG strain. Also, they are absent in non-tuberculous mycobacteria with the exception of *M. kansasii, M. marinum, and M. szulgai* (64, 69). In contrast to QFT™, TST has lower specificity because of the cross-reaction with antigens found in non-tuberculous mycobacteria and *M. bovis* (BCG vaccine). A comparison of both tests is shown in Table 4.
Table 4. Comparison of tuberculin skin test and interferon-γ released assays.

<table>
<thead>
<tr>
<th>FEATURES</th>
<th>TST</th>
<th>IGRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application</td>
<td>Invasive</td>
<td>Less invasive</td>
</tr>
<tr>
<td>Patients visit to complete test</td>
<td>2-4 visits</td>
<td>1 visit</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>75-90%</td>
<td>75-95%</td>
</tr>
<tr>
<td>Specificity</td>
<td>70-85%</td>
<td>90-100%</td>
</tr>
<tr>
<td>Cross reactivity with BCG</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cross reaction with NTM*</td>
<td>Yes</td>
<td>Not likely†</td>
</tr>
<tr>
<td>Time required to obtain results</td>
<td>2-days (One step)</td>
<td>1-2 days</td>
</tr>
<tr>
<td>Laboratory infrastructure</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Trained personnel</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Material costs</td>
<td>Low to moderate</td>
<td>Moderate to high</td>
</tr>
</tbody>
</table>

† M. kansas, M marinum and M. szulgai

2.6 Epidemiology

2.6.1 Global

Tuberculosis is the second leading cause of death from an infectious disease worldwide, after HIV, among young people and adults. According to the WHO report (2007), it would be impossible to eliminate TB without addressing the problem of TB at a global scale (26, 39). In 2006, the global incidence of active TB was 9.2 million people, with 95% of the cases in low/middle income countries, especially India, China, South Africa, Indonesia and Nigeria (2). An estimated 1.7 million deaths due to TB occur every year (2). It is also estimated that one out of three people or 2 billion of the world’s population are infected with LTBI, making LTBI the most important reservoir of the disease (2, 39, 73).

Worldwide targets to fight TB have been formulated to meet the Millennium Developing Goals (MDGs): “to halt and reverse TB incidence by 2015 and to halve prevalence and death rates by 2015 compared with a baseline of 1990” (WHO report,
2008). One of the fundamental goals of the global plan the Stop TB Partnership is to have less than one case of TB per million population by 2050 (2).

In developed nations, the TB control efforts have helped to reduced TB incidence rates and mortality that were significantly greater in the past. However, the re-emergence of TB in the 1980’s renewed efforts and to date rates have been declining, except for high-risk groups. Many of the new cases of TB are being reported in foreign-born individuals, especially those from countries with high incidence of TB. The assumption is that they were infected in their home country and then, reactivation of latent infection occurs within the first two years after immigration (3, 6, 35, 74).

In addition, local high-risk groups such as aboriginals, homeless persons, and intravenous drug users are also targeted for TB control programs. Human Immunodeficiency virus (HIV) is the major risk factor for the reactivation of LTBI or the progression to primary active TB shortly after infection. In fact, HIV/AIDS is considered a major risk factor in the increment of TB. Notwithstanding, when compared to low/middle income countries, incidence of TB and its burden is still very low in developed countries (3, 75, 76, 77).

In developing countries, which comprise 76% of world population, the overall trend is towards an increase of TB. Although the world TB campaigns are implemented, more than 95% of TB cases occur in low and low/middle income countries (39, 78). According to WHO (39), TB and poverty are associated: poor communities do not have an adequate health system and the treatment often is lacking or incomplete; in turn, TB reduces capacity for work, rendering the poor even poorer (39). Many patients are never
diagnosed, which contributes to a high number of deaths and more spread, and many cases are not reported, so the burden of disease is underestimated (79).

Tuberculosis cases are particularly high in countries with high HIV prevalence. Indeed, in regions of sub-Saharan Africa, up to 70% of TB cases are diagnosed in people living with AIDS (2). The TB strategies in developing countries have been made possible by the support of several donor countries and other agencies, and include diagnosis of TB cases and contact case finding, expanded universal BCG vaccination in childhood and the DOTS strategy (58). The high impact of TB in endemic countries suggests that the protection of BCG vaccine is not life-long, although, it reduces the risk of severe forms of TB in children, preventing 40-70% of deaths. Despite DOTS and availability of treatment in these countries, adherence to treatment is low, cure rates are less than 50% and the emergence of drug-resistant TB strains is increasing (78).

2.6.2 Canada

In the mid 19th century, TB afflicted one in five Canadians and was the number one killer with an estimated mortality rate of 200 per 100,000 population (16, 65). Poor living conditions in growing cities in Canada and not enough awareness accounted for these high rates (16, 80). The new knowledge about TB and its infectivity at the end of the century led the Canadian Medical Association (CMA) to name it as a national disease and a major public health concern. As social and public health measures improved, the incidence of TB started to decline (76). Improving sanitation and basic living conditions decreased significantly the incidence of TB (39). In fact, in 1901, the TB mortality rate was 180 per 100,000 population and by 1926, it had decreased to 84 per 100,000 population (16).
Starting in 1929, free sanatoria treatment for TB patients and clinical diagnosis services were offered in some provinces and in 1933, BCG vaccination was one of the programs established to fight TB in Canada, primarily to the newborn of families in which a TB case was diagnosed (16). The initiation of antibiotic therapy in the 1950s contributed to a decreasing incidence of the disease by 9% annually when it was used in combination with sanatoria treatment and by 20% when it was mandatory as the only TB treatment (16, 76).

Since the 1980s, the reported incidence of TB has continued to decrease. In 1992, the TB Elimination Strategy was introduced including policy-making, prevention, control, surveillance, health education and research. As a result, the TB incidence rate decreased from 7.4 per 100,000 population to 5.1 per 100,000 population in 10 years (81). Tuberculosis prevention and control in Canada is based on three strategies: first, identify persons with active TB and give them complete treatment. Second, investigate recent contacts with a confirmed case of active TB and third, investigate population at risk of LTBI and progression to active TB (26). Now, a goal to reduce the incidence of TB to 3.6 per 100,000 by 2015, was set by The Canadian Tuberculosis Committee (CTC) in 2006 (26, 56, 82).

The majority of active TB cases in Canada are reported in Ontario, British Columbia, Quebec and Alberta; 60% of all cases are reported in metropolitan areas with more than 500,000 inhabitants and within the lowest socio-economic groups and foreign-born population (65, 80). In Saskatchewan, Manitoba, Yukon, Nunavut and Northwest territories, the majority of the cases reported are among the Aboriginal population, which
includes Status and non-Status Indians, Inuit and Métis. In 2004, these groups accounted for 17% of the Canadian cases, with many cases among the younger population (26).

Although Canada’s TB rate is still one of the lowest in the world (5 per 100,000 population), the epidemiology of TB in this country has changed because of foreign born population. In the last three decades, the migration of people around the world has been increasing with Canada as a frequent destination (74). According to Citizenship and Immigration Canada (CIC), every year, more than 200,000 immigrants entered to the country (83). In 2006, 65% of TB cases were reported among immigrants and 90% of all cases were reported in the same provinces mentioned above, which are the most preferred by immigrants (80, 81).

Immigration medical screening has been used to identify conditions that could represent a public threat to Canadians such as tuberculosis, syphilis, and more recently HIV/AIDS. The immigration medical screening consists of medical history, physical examination and age-related tests: urinalysis (at >5 years old), chest radiography for TB (at > 11 years old), syphilis serology (at >15 years old) and HIV test (at > 15 years old, children who have received blood or have a known HIV positive mother) (11). Pulmonary TB is screened with CXR and disease-related symptoms and where necessary, laboratory testing. If there is evidence of disease before entering to the country, the individual is treated to minimize the risk of transmission (11, 84). Detection of LTBI is not part of the routine screening. If diagnosis of LTBI should be necessary, the only test available and approved by Canadian TB Standards is TST, which has several limitations (11, 26, 84). The new IGRAs are now recommended by the Canadian Tuberculosis Committee for LTBI diagnosis, but only under some specific circumstances (26, 68).
Latent Tuberculosis in Mexican Migrant Workers

Canadian-born individuals at risk of exposure of active tuberculosis are health care workers, aboriginal populations and alcoholics abusers and injection illicit drug users (26, 65). According to Canadians’ guidelines, risk factors for TB are divided into 2 categories (Table 5): those associated with higher risk of infection or the probability that a person will come in contact with *M. tuberculosis* and those associated with high risk of progression to disease after infection (80):

Table 5. Risk factors for tuberculosis infection and progression to tuberculosis disease

<table>
<thead>
<tr>
<th>Risk of infection with <em>M. tuberculosis</em></th>
<th>Risk of progression from infection to disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immigrants from country with high TB prevalence</td>
<td>HIV infection</td>
</tr>
<tr>
<td>Aboriginal population or background</td>
<td>Other immunosuppressive diseases</td>
</tr>
<tr>
<td>Recent contacts with an active case</td>
<td>Corticosteroid use</td>
</tr>
<tr>
<td>Homeless, substance abusers, prisoners</td>
<td>Diabetes, malnutrition, gastrectomy</td>
</tr>
<tr>
<td>Health care workers, travelers to high prevalence countries</td>
<td>Old TB (healed but untreated or incomplete treatment)</td>
</tr>
</tbody>
</table>

Based on Canadian Tuberculosis Standards, (PHAC, 2007) (26)

The Canadian Tuberculosis Standards recommends TST testing in the following cases (26):

- Contact of a patient with recent diagnosis of active TB
- Immigrants who were born in a TB endemic country (within the first 2 years after arrival)
- Individuals with impaired immunity
- Radiographic evidence of old but healed TB without treatment

Additionally, TST is contraindicated in (26, 59):

- Individuals who had had previous severe reaction
- Individuals with known active TB or history of LTBI
- Individuals with excessive burns or eczema in their arms
- Individuals who received live-virus vaccines in the past month

Tuberculosis control in Canada relies on coordinated efforts among the federal, provincial, territorial, county and municipal governments. The Public Health Agency's Tuberculosis Prevention and Control Division establishes national and provincial legislation and standards for TB control with the help of local health units, which receive notification of cases of active TB and LTBI (11, 76).

2.6.3 Mexico

Mexico as well as other Latin American countries has marked social-economic disparities (ethnic background, social classes, and economics and education opportunities). According to Government of Mexico, socio-economic indicators of 2000-2004 reported 64.1% of Mexican population was aged from 15-64 years old, 70.5% with primary education completed, 89% lived in houses with indoor potable water and indoor flushing toilet; 95% lived in houses with indoor electricity. A summary of indicators for the whole country may not provide an accurate picture of the real situation of the poorest. There are States in Mexico with high levels of welfare and others very poor (85). In general for the entire nation, rural areas have problems of poverty, unemployment and most of the population migrates to urban areas.

In Mexico, TB is the third leading cause of death after pneumonias and gastrointestinal diseases (86). The incidence of all forms of TB disease in 1990 was 64 per 100,000; Mexico was in the list of 14 countries with high incidence of TB worldwide. In 2005, this incidence rate decreased to 23 cases per 100,000 population with around 3,000 deaths per year (2). However, as Garcia (2003) and Villalba (2003) stated, the real number of TB cases are underestimated due to low notification of cases, TB confirmed
cases treated but not reported in private care settings and undiagnosed cases (86, 87). The true number could be as high as threefold the reported cases (86). In the global scale of TB burden in 2006, Mexico was ranked 64 as a medium TB burden country, compared with Canada that was situated in the fifth place as a low TB burden country (88). More than 80% of reported cases are pulmonary TB in adults. In children younger than 5 years old, the most common form is tuberculous meningitis. Approximately, 2-3% of TB patients are HIV positive (89).

All tuberculosis prevention and control action plans are intended to work at a national and state level. The National Fight Against Tuberculosis Committee, founded in 1939, the program Mexico Free of TB (Mexico Libre de Tuberculosis) implemented in 2002 and the creation of the national Stop-TB committee under the umbrella of global Stop TB worldwide campaign, created in 2005 are examples of Mexico’s commitment in the fight against TB (12, 90, 91). In addition, a national evaluation program is in place to measure the impact of the TB campaigns and ensure the states’ compliance (90, 91). Although a reduction in morbidity and mortality rates was achieved due to these interventions (90, 91), there are reports that some States are experiencing difficulties in complying, implementing or following up the directives, which results in a number of undetected active TB cases and increasing number of cases of MDR-TB and XDR-TB (92).

Prevention is a key component in TB national programs. BCG immunization at birth is offered as a measure to prevent severe forms of TB in children (93). According to UNICEF, in 2006, 99% of children younger than 1 year old were vaccinated with BCG vaccine. This measure protects children from TB meningitis and miliary TB, but at the
same time has generated two situations; an erroneous understanding that the TB vaccination would protect the population lifetime and a possible interference with TST reactivity in cases of routine assessment of LTBI in high risk populations (94). Until 1996, the vaccination in infants was complemented with a booster of BCG to children at age of six. However at the present time, particularly in private care level, some physicians follow the North American vaccination scheme, recommended BCG only for those children at high risk of TB (94). Another component in TB programs in Mexico is the detection of active cases and treatment under the DOTS strategy, adopted in Mexico also in 1996 (2). Although the coverage of DOTS is high in Mexico, there is poor treatment adherence when the access to medical care is difficult, and the distribution of drugs is not continuous within the country (87).

In Mexico, regulatory norms require that TB diagnosis should be based on laboratory results (AFB staining and microbial cultures). Tuberculin skin test is not routinely performed in Mexico except in at risk populations such as contact of an active case, HIV positives or persons younger than 15 years of age with no history of BCG vaccination. (93). In contrast, Canada and other developed countries’ essential TB control include screening certain population to detect LTBI, and offer preventive therapy, preventing progression to active TB (26).

Migration within the national territory has been increasing with around 19% of the population residing outside their birth place with a large social and health impact. But the impact on health is greater when the migration is out of the country. Nowadays, the migration has been expanded to women and the indigenous population, with the main reason to migrate being lack of job opportunities and low incomes in Mexico (95). It is
estimated that in the last five years nearly 1.5 million Mexican citizens migrated to the United States (2, 90). This situation has contributed to the establishment of a significant portion of Mexicans in US population (Mexican-American), a group that has become one of the most influential in the country, not only culturally.

In 2001, TB rates on each side of the Mexico-US border were 1.5 times their respective national averages. TB control programs in each side of the border have been difficult to carry out because of limited health care access and an extremely mobile population (95). In 2007, the Department of Health and Human Services in the United States, the Public Health Agency of Canada, and the Ministry of Health of Mexico agreed to coordinate and prepare protocols for surveillance, prevention, and control of infectious diseases on each side of the border for "the protection of the health, well-being, and quality of life of their peoples" (96).

The migration of Mexicans to the US changed with globalization and the North American Free Trade Agreement (NAFTA) set up by United Stated-Mexico-Canada in 1992 (97). NAFTA has made for greater economic integration between these countries, with an expansion and consolidation of the horticultural sector and has initiated ways to improve migration management that favours the Seasonal Agricultural Workers Program in the US and Canada (98, 99).

General Information about Mexico

The United Mexican States is a democratic republic with 31 states and a federal District as seen in Figure 3. In 2006, the total population was 104.2 million with an estimated population growth rate of 1.1% (12, 100). According to the Word Bank Group,
an estimated of 28% of the total Mexican population live in rural areas, around half of Mexicans lived in poverty and 20% in absolute poverty in 2002 (90, 100).

Health care in Mexico is both private and public. Public care is provided by the Secretariat of Health (Secretaria de Salud de Mexico, SS) with the support of institutions such as the Mexican Social Security Institute (Instituto Mexicano del Seguro Social, IMSS) (90).

Figure 3. Political map of United Mexican States
From: www.mapsofworld.com/country-profile/images/mexico-country-map.gif

The National Development Plan 1995-2000 established new health policies oriented toward the reorganization of the Mexican health system to expand its coverage and provide more efficient and effective services (90). Health services are structured into 3 levels of care: the first level includes health promotion, disease prevention and outpatient care. The second level provides specialized care at general or specialized hospitals, and the third level provides specialized care of greater complexity (90).
2.7 Advances in Tuberculosis Research

Linked to the Millennium Development Goals and the Stop TB partnership targets, the main goal of the worldwide TB campaign “Stop TB” is to reduce the global burden of disease by 2015, and to eliminate TB (<1 case per million population) by 2025 (73). As the main foundations and institutes point out, research in diagnosis, control and prevention areas are needed to achieved these goals (2, 78, 101). In diagnosis, improved tests for early diagnosis and the monitoring of infection and disease progression are advancing with new in-vitro assays to measure interferon-γ (70). In control, new or improved medications to combat and prevent multidrug-resistance are being explored. Finally, a better vaccine against TB is urgently needed. The Aeras Global TB foundation, which focuses on developing new vaccines against TB, is exploring vaccine candidates that could promise an induction of protective cellular immunity, which could eventually control the disease (78). For the study of virulence and pathogenicity of drug-sensitive and drug-resistant *M. tuberculosis*, and in the evaluation of candidate anti-TB therapies and vaccines, better animal models are required (2, 78, 101).

II. Seasonal Agricultural Workers Program

2.8 The Program

According to the Commission for Labour Cooperation, a migrant worker is defined as any person who moves from his or her permanent residence to another place temporarily to obtain a job (99). The Mexican Seasonal Agricultural Workers Program started in 1974 when the governments of Mexico and Canada signed a Memorandum of Understanding that allows the legal entrance of Mexican workers to Canada on a temporary basis (10).
This agreement is a model of bi-national cooperation in which both countries gain benefits. For Canadian farmers, the arrival of a regulated and permanent flow of migrant workers every year for up to eight months fulfills their need for agricultural workers. In fact, fewer Canadian residents are available to work in horticulture, and this is a necessary condition for a country hiring foreign agricultural workers. Similarly for Mexicans, the lack of employment in Mexico and job opportunities with Canadian wages and labour conditions are the main reasons for applicants to join the program (102, 103). Workers are allocated to eight provinces throughout Canada: Ontario, Quebec, Alberta, Saskatchewan, Manitoba, New Brunswick, Prince Edward Island and Nova Scotia. The majority of workers (80%) however, are located in Ontario (104).

Mexican workers are hired to work in agriculture. Most of them come to harvest vegetable, fruit and tobacco crops. Among other requisites, applicants must be Mexican, male or female be in good health, have experience working in agriculture and have at least three years of schooling and 22 years old or older (103, 105).

The operation and administration of the program are carried out by both the governments of Canada and Mexico. In Canada, Human Resources and Social Development Canada (HRSDC) regulates the requisition of migrant workers from employers, and communicates the demand of workers to Mexico (102). In 1987, HRSDC made an alliance with representatives of the Canadian agricultural industry in order to privatize the organization of the program in Ontario, forming the Foreign Agricultural Resource Management Services (FARMS). This organization informs employers about the program, performs an administrative role facilitating and coordinating the processing
of successful workers’ applications, compiles statistics, sends reports to HRSDC, etc (106).

Although Mexican SAWP is a federal program, the provincial governments are responsible for the legal labour issues of the migrant workers, such as human rights, employment standards, and health and safety. In Ontario, for example, there are three ministries involved: the Ministry of Labour, the Ministry of Health and Long-Term Care, and the Ministry of Agriculture, Food and Rural Affairs (102).

In Mexico, the government advertises the work opportunities in Canada through the State Employment Service offices. These offices provide information and orientation to the new candidates and also contact workers who are requested by name by a previous farm employer in Canada. According to Maxwell (2006), 70-80% of the migrant workers are re-hired, or “named” by their employer. This process provides the farmer trained personnel and the worker continuity in the program and priority in the immigration process (102).

Canada and Mexico have developed a detailed process to select and screen candidates. In Mexico, the Ministry of Foreign Affairs (Secretaria de Relaciones Exteriores, SRE), the Ministry of Labor (Secretaría de Trabajo y Previsión Social, STPS), and the Ministry of Health (Secretaria de Salud, SS) are responsible for the process of reviewing workers’ applications, recruitment, medical examinations, contracting workers, providing travel documentation and facilitating the legal mobilization of workers to Canada (105). Communication between Citizenship and Immigration Canada (CIC) and the Embassy of Canada in Mexico for the admission of migrant workers in Canada is continual (107).
Medical screening tests are conducted in Mexico and include urinalysis, syphilis serology, HIV and chest X-ray for active TB as mentioned in section 2.6.2 for other immigrants (Canada). After receiving a medical, physical and mental examination, the candidate is evaluated and classified into 5 categories (CIC, nd):

1. Eligible to work in Canada without restrictions. The candidate presents minor health conditions that will not interfere with the demanding job in Canada. This category does not require medical follow-up.

2. Eligible to work in Canada, but the candidate may require medical care and follow-up in Canada.

3. Eligible to work in Canada, but medical care and follow-up are required for the candidate in Canada (old TB or treated syphilis).

4. Not eligible to work in Canada under the SAWP. As a result of the medical examination the candidate requires an extensive investigation of his/her condition that is incompatible with the job. Conditions such as active TB, positive serology for syphilis, mental disorders or conditions may cause an extra demand in the Canadian health care system or impede expected job performance while in Canada.

5. Other conditions or disorders difficult to classify or not enough medical information.

Once in Canada, workers are assisted by the Consulate General of Mexico in Toronto, Montreal or Vancouver.
2.9 The Situation of Migrant Workers in the Niagara Region

As workers, Mexicans are paid according to the legal provincial minimum wage and there is no agreement to raise the rates for skilled workers with extensive experience at the same farm. However, there is a special bonus for those workers who fulfill certain expectations (102, 108). A normal work day is eight hours with two 10-minute breaks and 30 minute for lunch. There is one day-rest for 6 consecutive days worked. Mexicans have the possibility to work overtime with previous mutual consent from the farm owner. However, they are not entitled to overtime pay and only some receive vacation pay (102, 109, 110). Their accommodations are inspected by public health inspectors to confirm that the farm owners follow the Ontario Ministry of Health guidelines, especially regarding overcrowding, indoor plumbing and washing and toilet facilities (13, 102, 110).

As of 2006, agricultural workers were excluded from the Ontario Employment Relations Act and the Occupational Health and Safety Act (OHSA) which implies that they do not have the right to form a union or refuse unsafe work (13, 99, 108). Like other Ontario workers, Mexicans are required to pay Canadian income tax, employment insurance and contribute to the Canadian pension plan. While working in Ontario, Mexican workers are entitled to have health coverage through the Ontario Health Insurance Plan (OHIP) and are covered under Worker’s Compensation (WSIB) as well as private health insurance to which they make mandatory contributions (99, 102, 108).

Working with pesticides and other agro-chemicals, operating machinery or working under extreme weather conditions may cause some risks for Mexicans’ health. Also, the change in adapting to new living conditions, a new society, and being away from their families may generate a disturbance to their health (103, 109). Facing a
different language and culture, the often long distance between farms to the nearest town, no access to public transportation and long work hours all present barriers to Mexican workers being able to seek medical assistance when they have a health concern. Also, they hardly participate in local community activities or interact with the Canadian population around them (102, 103). Mexicans are largely limited to interacting with other compatriots during the season with little local cultural exchange (111).

2.10 The Mexican Migrant Worker As A Research Subject For Tuberculosis

Mexicans migrant workers as a population have been widely studied in the US, especially with respect to migration and health issues. In Canada, Mexican workers are of interest to social scientific and social activism groups. There are reports, analyses and even movies describing workers' social justice and wellbeing, in particular, highlighting the lack of access to health care among this population. To our knowledge, however, no biomedical investigations of any specific infectious diseases issue has been conducted among this group (103, 111).

In 1992, the US Centers for Disease Control (CDC) estimated that farm workers are about six times more likely to develop TB than other adults (112). The first population-based study on TB in migrant workers was done in 1988 in North Carolina. In several US studies conducted in the 1980s for LTBI, a range of positivity rate between 29 to 46% in migrant farm workers using TST was demonstrated (112).

According to the Public Health Agency of Canada and the Tuberculosis Prevention and Control, in Canada, as per 2007, Mexican agricultural workers have not been screened for LTBI. Other foreign-born populations such as refugee claimants and
new immigrants have been included in several studies assessing latent infection using TST. Prevalence of LTBI in such populations is estimated from 15-72% (113).

Through the SAWP, more than 20,000 workers (from both Mexico and the Caribbean) arrive every year to Canada with secure employment to support family needs and increase their living standards in their countries. Pre-immigration screening of these workers includes TB and other infectious diseases that lead to a rejection of candidates if present. There are no available reports indicating the proportion of candidates that are rejected to work in Canada due to active TB, but it is reasonable to suspect that some are (Dr. Carpio, personal communication). From a prevention point of view, it is important for migrant workers to know whether or not they have LTBI so they can take actions to minimize the risk for reactivation and if this happened, to recognize the symptoms of TB disease and seek prompt medical care to protect both their health and their employment.

As mentioned before, this project has a collaborative component in Puebla, Mexico, where our co-investigators will attempt to gain some insight into TB cases among migrant workers. While it is true that TB examination done in Mexico prevent active TB cases from entering to Canada, it is widely recognized that latent TB escapes this screening. Little is known about either the probability of undetected latent infection among these agricultural workers, or its potential reactivation while in Canada, perhaps by stressful working and living conditions, or about the workers' exposure to TB cases while back in their home communities before returning the following season. The present study will be the first to determine LTBI status in Mexican agricultural workers and will make an important contribution to the body of knowledge regarding LTBI in Mexico and in Canada.
CHAPTER THREE: METHODOLOGY

This study was an international cooperation project in which one component conducted in Canada served as the primary basis of Angela Duarte’s M.Sc thesis under the supervision of Dr. Ana L. Sanchez, PhD at Brock University. The complementary component of the project was conducted at the Benemérita Universidad Autónoma de Puebla in the state of Puebla, Mexico and will be published elsewhere. Both components were funded by the Mexico-Canada Joint Health Research Program in Tuberculosis, managed and funded by the National Council for Science and Technology (Consejo Nacional de Ciencia y Tecnologia) (CONACyT) and the Canadian Institutes of Health Research (CIHR).

One of the most relevant areas of research of this Joint Research Program is the prevalence and/or impact of TB in specific populations (children, elderly, aboriginal/indigenous populations, migrants and refugees). Accordingly, the specific population involved in the Canadian component was migrant workers.

3.1 Research Objectives

3.1.1 General

To develop an understanding of the occurrence of latent tuberculosis infection in Mexican migrant workers in the Niagara Region, Canada, and factors associated with risk of infection.

3.1.2 Specific Objectives

1. To determine the prevalence of latent tuberculosis infection using tuberculin skin test and QuantiFERON-TB Gold In-Tube.
2. To evaluate the performance of tuberculin skin test using QuantiFERON-TB Gold In-Tube as a confirmatory test.

3. To explore the presence of known risks factor associated with latent infection and explore specific characteristics of this population that could be associated with TB infection.

4. To assess the current knowledge of migrant workers with respect to both active and latent tuberculosis.

3.2 Research Design

The study was a cross-sectional study conducted with volunteer, healthy Mexican agricultural workers present in Niagara Region in 2007 season to measure the prevalence of latent TB infection.

Sample Size: since there were no previous studies involving this particular population, there were no prior experiences to draw from in regards to the best practices and mechanisms of approaching potential participants or the processes of enrolment, feedback and counselling after the tests were done. Exploratory inquiries and exercises demonstrated that the study implementation was going to be intensive and lengthy and the relatively short duration of the worker’s residence in Niagara became a major obstacle to overcome. Moreover, access to the workers was restricted to non-working hours so the time constraints became even more severe. Therefore, although obtaining a representative sample was the initial aim, it was decided that a convenience sample would be a more realistic approach for this preliminary study.

Participants answered a questionnaire assessing knowledge about TB and TB history. Additionally, LTBI was determined by the tuberculin skin test (TST, Mantoux
method) and an Interferon-γ release assay (QuantiFERON®-TB Gold In-Tube, Cellestis Inc, Australia).

3.2.1 Study Population Selection

Participants were enrolled from the Niagara Region’s farms and wineries employing Mexican workers under the SAWP. A directory of farms that employ Mexican workers in The Niagara Region is not in the public domain; therefore a complete list was requested under the Municipal Freedom of Information and Protection of Privacy Act from the Regional Municipality of Niagara (114). Once the list was acquired, farms in the vicinity of Brock University were selected for establishing initial contact. A letter of invitation and information about the study were personally delivered to the farm owners (Appendix A). Only workers from farms granting permission were approached for three reasons: 1) consent from farm owners was needed to enter their property; 2) the study required follow-up visits that needed to be done in a private place to ensure confidentiality; and 3) direct communication between farm owners and employees needed to be maintained to ensure the good reputation of the study.

3.2.2 Enrolment of Study Participants

Workers were contacted through their supervisors and were invited to attend to an information meeting where the study was explained. Emphasis was made of the fact that despite the owner’s or supervisor’s consent, no worker would be under obligation to participate in the study. In addition, workers were assured that no individual information from any worker would be shared with the owner or supervisor at any stage of the study.
3.2.2.1 Inclusion – Exclusion Criteria

To be eligible to participate in the study, individuals had to be Mexicans, enrolled with the SAWP program, be 18 years old or older (although minimum age for SAWP is 22), understand the study’s risks and benefits and give free and informed written consent to participate in all aspects of the study. Exclusion criteria included: pregnancy (to avoid unnecessary stress to the participant) and those exclusion criteria valid for TST administration: a previous TST in the past 2 months, prior over-reaction to a TST, vaccination against live viruses (e.g., MMR, varicella) in the past 2 months, skin problems (infectious or otherwise) or extensive scarring or burns in both of the forearms so the TST could not be applied.

3.3 Research Ethics and Biosafety

3.3.1 Research Ethics Approval

Research ethics clearance to work with human participants and human tissues was granted by Brock University Research Ethics Board (REB) File # 06-268 DUARTE et al. (Appendix B). The application submitted provided a detailed explanation about the risks and benefits of the study and every procedure necessary for its completion. Such procedures include invasive techniques such as phlebotomy (blood sample collection) and TST administration. The social and psychological risks of being tested for TB were also addressed.

Both confidentiality of the participant and anonymity of information were safeguarded extensively. To ensure confidentiality, special care of meeting with each participant in a private location (e.g. workers’ bedroom) was conducted by the researchers, especially in the questionnaire administration process and while delivering
results. All personal information was handled only by the researchers and was kept in the supervisor’s laboratory.

For the questionnaire administration process, a group of three volunteers (Spanish speakers and one CPR/first aid trained) were added to the research team following due procedure of submitting the appropriate notification form and a general statement of confidentiality to the REB.

3.3.2 Biosafety

Since this investigation used procedures and blood samples that could involve possible risks for the researchers, biosafety precautions were put in place at all times in both the field and in the laboratory. Manuals were available describing procedures for waste decontamination and emergency responses. Level II requirements and other biosafety measures were implemented with appropriate approval to the Office of Environment, Health and Safety (EH&S) of Brock University.

3.3.2.1 Protocol for Working in the Field

Brock University’s Policy on Safety and Liability for Field Research (115) was followed and a Field Trip Risk Assessment and Planning Record was filed to the OEH&S with all researchers and volunteers’ personal information. The researchers used Brock University distinctive clothing and name tags for identification when working off campus.

3.3.2.2 Biosafety Protocol

A complete description for standard precautions for drawing blood from human subjects in the field was written in a Biosafety Protocol. This protocol included a list of materials for blood collection off campus, the technique for blood collection, and the
protocols for blood spills, accidental needle stick and disposal of biohazard and non-biohazard waste. Blood samples and untreated biohazard and non-biohazard waste used in the field were transported in a safe manner to the laboratory situated in MC C307A at Brock University by the researcher (Angela Duarte) who received Transport Dangerous Goods (TDG) training.

3.3.2.3 Biosafety Level II Laboratory

Dr. Sanchez’s laboratory is certified as a Biosafety Level II laboratory (BSL II) (Appendix C). Universal precautions and proper conduct for BSL II were followed for all personnel working in the laboratory. These include equipment handling procedures, personal protective equipment, management of biohazard waste, protocols for spills and management and use of a spill kit.

3.4 Meetings with Research Participants

The research participants had different encounters with the group of researchers; an information meeting, a data collection meeting, a TST administration meeting, a TST reading meeting and a results meeting. Refreshments were offered to participants in every meeting.

Information Meetings

With the proper permission of the farm owners, the researchers contacted the field manager, supervisor or group leader of the Mexicans workers in the farm to ask for a suitable day, time and place for holding an information meeting. An informal flyer (Appendix D) to announce the researchers visit was sent to the supervisor to circulate among the workers. Once the day and hour was confirmed, the researchers prepared the field material and mobilized to the workers’ house or any other meeting place the
supervisor suggested. The objective of this meeting was to provide detailed information about the study, explained the meaning of voluntary participation, confidentiality and anonymity, risks and benefits. This information was given orally, and a written invitation and information letter was also provided (Appendix E). For all oral and written communication, plain Spanish language was used.

After the explanations in the information meeting, the consent form was read and explained out loud to all workers willing to participate. After this, potential participants were approached individually to confirm their decision, upon which they were offered two written consent forms for their signature (Appendix F). The participant retained one copy and the researchers kept the other one for their files.

Data Collection Meeting

During this meeting, a questionnaire was administered verbally to the participant in a private place. At the end of the questionnaire and with the research participants’ permission, a blood sample was drawn for QFT™. The procedures of questionnaire administration and QFT™ processing are explained in the following sections (3.5 and 3.7.1).

Tuberculin Skin Test Administration Meeting

The TST was scheduled in a special meeting with the participants. The procedure required authorized nurses to administer and read the TST under the medical directive of the Medical Officer of Health, Public Health Department of Niagara Region as explained in section 3.6.2.

Tuberculin Skin Test Reading
This meeting was set 48 hours after TST administration; the procedure followed will be explained later in section 3.7.2.

Results Meeting

In this short and private session, designed to convey results of TST and QFT™, a personal results card (Appendix G) was delivered to each research participant providing them with more information or counselling depending on the results as explained in section 3.8.

3.5 Questionnaire and Questionnaire Administration Process

3.5.1 Description of the Questionnaire

The questionnaire (Appendix H) was designed by the Angela Duarte and Dr. Ana L. Sanchez, for purposes of this study and was based on published studies with Mexican migrant workers in other countries (65, 77, 103, 107, 116, 117, 118). The questionnaire was composed of various sections with several questions each, as explained in the following:

1. Demographics: name, age, date of birth, sex, address and telephone number in Canada were asked. This personal information was obtained in order to deliver laboratory tests results at the end of the study.

2. Seasonal agricultural workers program participation: length of participation in the program as well as constancy in the Province, Region and at the same farm.

3. Living conditions while in Canada: type of housing and number of persons per house.
4. Geographic residence and socio-demographic data in Mexico: State and city of birth and place of residence before coming to Canada, type of housing and number of people per household, occupation while in Mexico and highest level of education.

5. TB knowledge: knowledge about symptoms and signs of active TB and LTBI, TB transmission, perceptions of disease severity and potential for cure.

6. History of TB infection and exposures: past TB-history and/or any possible contact with TB-infected persons in their place of origin, BCG vaccination and previous TST administration, recent history (past two weeks and up to two months) of symptoms associated with active TB.

7. Other health issues: participant’s self-reported health status, underlying health conditions, current medications and cigarette and alcohol use.

The questionnaire was piloted with a group of seventeen Mexican workers to determine if it was appropriated for this population and if it was comprehensive enough to collect the information according to the purposes of the study. Minor modifications were made to the questionnaire after this initial test.

Before collecting personal identification data, a research code number was given to be used to ensure anonymity of information. This six-digit number was recorded in the questionnaire (upper-right corner) and meant the town (the first two digits), the specific farm (two middle numbers) and the participant (the final two digits). For example,

```
<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>0</th>
<th>1</th>
<th>1</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Town</td>
<td>Farm</td>
<td>Participant</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
```

3.5.2 Questionnaire Administration Process

The interviewers (researchers and volunteers) met each individual in private and administered the questionnaire orally, face-to-face. The questionnaire administration
process started with the interviewers discussing confidentiality and privacy of the information that would be collected. The questions were asked in Spanish since the majority of participants did not speak English and were recorded in English and Spanish (the interviewers’ mother tongue was Spanish, which facilitated the interview process and allowed for a comfortable exchange of information). The interviewers asked question by question to confirm comprehension and to resolve any doubts. At the end, the interviewers expressed appreciation for sharing their information and with the participant’s permission, the certified phlebotomist took the blood samples. The research number was assigned to each individual to keep their anonymity in later stages of the data processing. This code was used to identify the subsequent samples and the participant in the future for delivering his/her results.

3.5.3 Blood Collection

A member of the research team (Angela Duarte) certified to draw human blood samples performed the venipuncture from the forearm vein (the larger median cubital, basilic or cephalic veins) preferably of the right arm. The skin at the puncture site was disinfected with 70% ethanol and a tourniquet was applied 3-4 inches above the puncture site. Once the sterile needle was inserted, three Vacutainer™ tubes of 1 mL each from QFT™ were collected. Once the needle was withdrawn and the tourniquet released, a sterile pad was applied firmly in the puncture site and the participant was asked to bend their elbow to keep the pad in place for at least two minutes to allow the puncture to coagulate. When bleeding stopped, a band-aid was applied if the participant wanted one. The participant was advised about expected reactions before the procedure was performed.
3.6 Diagnostic Methodology for Delayed-Type Immune Response for Tuberculosis

3.6.1 Interferon-gamma Release Assay

QuantiFERON®-TB Gold In-Tube was used in this study due to its proven superior performance compared to the TST (62, 68, 71, 119) and its availability in Canada.

3.6.1.1 QuantiFERON®-TB Gold In-Tube Principle

The test is based on the quantification of INF-γ released form sensitized lymphocytes in whole heparinized blood after incubation with specific M. tuberculosis (MTB) antigens (ESAT-6, CFP-10 and TB7.7) and controls. The QFT™ is performed in two stages. First, whole blood is collected into each of the blood collection tubes (Nil Control, TB Antigen, and a Mitogen Control) and incubated at 37°C for lymphocyte stimulation. Second, following the incubation period, the tubes are centrifuged, the plasma is removed and the amount of IFN-gamma is measured by an ELISA. Following is a description of the technique according to the manufacturer’s instructions (29).

3.6.1.2 QuantiFERON®-TB Gold In-tube Technique

Stage One: Blood sample, handling and incubation:

QuantiFERON®-TB Gold In-tube uses three collection tubes per person: Nil Control (saline), TB Antigen and Mitogen Control (phytohemaglutinin). Each tube collects 1 mL of blood by the venipuncture procedure described previously (the correct volume can be verified against the black mark on the side of the tubes). To ensure a consistent procedure, blood is drawn in the following order: gray cap tube (Nil Control), red cap tube (TB antigens) and purple cap tube (Mitogen Control). Because the tubes contain antigens and other substances dried in their inner wall, it is essential to mix by
shaking vigorously each tube for 5 seconds ensuring the entire inner surface has been coated with blood. Immediately after collection, the tubes are placed in a portable incubator and kept at 37°C until transferred to the laboratory. Once in the laboratory, the tubes are mixed again and placed in an incubator at upright position at 37°C for 16-24 hours. After incubation, capped tubes are centrifuged for 15 minutes at 2000 to 3000 RFC (g). The gel plug separates the plasma from the cells for easy harvesting. The plasma of each tube are harvested separately into labelled microtubes inside of the Biosafety cabinet class II, following protocols for BSL II. All plasma samples will be stored at -20°C until further analysis with an ELISA.

Stage Two: Human IFN-γ ELISA:

The QFT™ ELISA kit provides the following reagents and components:

1. Microplates strips coated with anti-human INF-γ murine monoclonal antibody.
2. Human INF- γ standard is a recombinant human INF-γ, bovine casein and 0.01% w/v Thimerosal.
3. Green diluent is used to dilute the standard, samples and to prepare the working strength conjugate. It contains bovine casein, normal mouse serum and 0.01% w/v Thimerosal.
4. Conjugate 100X concentrate is anti-human INF-γ HRP which will be reconstituted with deionised or distilled water and will be use to prepare the working strength conjugate.
5. Wash Buffer 20X concentrate contains 0.01% w/v Thimerosal.
6. Enzyme Substrate Solution contains 3,3',5,5' Tetramethylbenzidine
7. Enzyme Stopping Solution contains H₂SO₄

The ELISA procedure is as follows:

All plasma samples and all regents are equilibrated at room temperature (22°C ± 5°C) for at least 60 minutes. The reagents that are freeze dried (i.e., standard and
conjugate) are properly reconstituted and the working strength conjugate and the washing solution are prepared. To generate a standard curve, a series of dilutions of INF-\( \gamma \) standard are done using green diluent. The plasma samples are mixed in a vortex to ensure an even distribution of INF-\( \gamma \) in the sample.

Once all reagents and samples are ready, the procedure is as follows:

ELISA plates layout is arranged for the correct addition of standards and samples; 50 \( \mu \)L of fresh working strength conjugate is added to necessary number of wells and then 50 \( \mu \)L of plasma sample (from the Nil, TB antigen and Mitogen tubes) or 50 \( \mu \)L of standards (S1, S2, S3, S4) is added to the ELISA wells, as appropriate. Immediately and using a microplate shaker, conjugate and plasma samples/standards are mixed for 1 minute, the plate covered with a lid and incubated at room temperature (22\(^{\circ}\)C \( \pm \) 5\(^{\circ}\)C) in the dark for 120 \( \pm \) 5 minutes.

After incubation, ELISA plates are washed 6 cycles with working strength wash buffer using a previously programmed ELISA washer, and subsequently tapped face down to remove any residual wash buffer before adding 100 \( \mu \)L of Enzyme substrate solution. Following 30 minutes incubation at room temperature, 50 \( \mu \)L of Enzyme stopping solution is added to each well in the same order and speed as the substrate.

Optical density of each well is then measured within 5 minutes after stopping the reaction using a microplate ELISA reader fitted with a 450nm filter and with a 620nm reference filter. QuantiFERON®-TB Gold In-tube uses software available from Cellestis to assess the validity of the test, calculate INF-\( \gamma \) concentrations and provide a result for the patient or research participant and a report for the laboratory records.
3.6.1.3 QuantiFERON®-TB Gold In-tube Interpretation

A test is considered positive (*M. tuberculosis* infection likely) when the amount of INF-γ released in TB antigen tube is greater than that in the Nil tube (IU/mL). A test is considered indeterminate when there is low response in the mitogen tube (<0.5 IU/ml), and low response in the TB antigen tube. A negative test is when the amount of INF-γ released in the TB antigen tube is low with adequate response in the mitogen tube (Figure 4) (Appendix I).

![QuantiFERON®-TB Gold In-tube assay interpretation diagram](image)

*Figure 4. QuantiFERON®-TB Gold In-tube assay interpretation (29)*

3.6.2 Tuberculin Skin Test TST-Mantoux Technique

3.6.2.1 Tuberculin Skin Test- Mantoux Technique Principle

The TST measures the delayed-type hypersensitivity response after 0.1 mL intradermal injection of purified protein derivate (PPD) 5 TU (tuberculin units).
3.6.2.2 Tuberculin Skin Test Administration Procedure and Technique

An information sheet with the procedure and possible reactions, and special consent form for the TST in Spanish (Appendix J) were handed at the beginning of the meeting. Participants were advised about expected reactions before the procedure was performed. Researchers and interviewers volunteered for the procedure as a demonstration for the participants.

The procedure was performed by experienced TB nurses as follows: using a tuberculin syringe, 5 TU of purified protein derivative (PPD) [Tubersol®, Avantis Pasteur Limited (61)] or tuberculin, using the Mantoux technique, (i.e., 0.1 mL) was injected into the participant’s inner side of the forearm, aiming for the superficial layer of the skin (i.e., intradermal) (59, 65). To avoid extra discomfort, the TST was usually applied into the left forearm if the participant was right-handed or vice versa. After each TST, the nurses monitored the person for 15 minutes to take care of any anaphylactic reaction. The nurse filled a record of the procedure (dose, manufacturer, lot number, expiration date, and site of injection) in standard format from the Public Health Department of Niagara Region that was translated into Spanish (Appendix J).

The immune reaction (induration) was measured in millimetres 48-72 hours later by a trained nurse; this was done in a private meeting with the participant, as it will be explained later (59, 65, 120).

3.6.2.3 Tuberculin Skin Test - Reading

A special meeting was scheduled to perform the TST reading. To observe consistency of the reading technique and follow guidelines for regulated medical acts (59, 65, 120), nurses from Public Health department performed the reading in a private setting.
with each individual and conveyed the result to the participant. Researchers were present at the meetings to interpret for the nurses and answer questions the participants had. The reading technique the PH nurses followed is called the “ball-point method” (26, 65) and was done 48-72 hours following the PPD administration.

The “ball-point method” uses the tip of a pen to mark the border of the induration in two opposite edges at the site of administration. The tip is moved in a transversal manner towards the site of injection until it stops, showing the edge of the induration. The distance between these two points is measured with a calliper and recorded in millimetres. The lack of induration is recorded as “0 mm”. Redness or rash reactions without induration are not considered positive. (26).

Nurses recorded the reading in the standard forms as mentioned above and provided one copy to the participant and one copy to the researchers. After the reading session, the researchers provided an explanation to those participants with a positive TST and clarified that their results would be confirmed with the QFT™ test, upon which a definitive result would be obtained and informed to them.

3.6.2.4 Tuberculin Skin Test Interpretation

According to the Canadian TB standards (59, 65) a TST reaction of $\geq 10$ mm is considered positive and probably can be attributed to \textit{M. tuberculosis} infection; a TST reaction $<10$ mm is considered negative.

3.7 Results, Feedback and Counselling

3.7.1 Interpretation of Combined Results

As mentioned before and with previous agreement with the PH Department, QFT™ served as a confirmatory test for TST results for LTBI in this study. The standard
procedure for diagnosis of LTBI in Canada is 2-step skin testing if the first TST is negative (65, 120). Two-step TST uses a booster of PPD in the following two weeks after the first TST, at it was explained in section 2.5.2.1. In this study, however, one-step TST was done. According to the Public Health Agency of Canada (2007), IGRA$s$ are recommended as a confirmatory test for a positive TST for immigrants having no clinical conditions and a normal chest x-ray (68).

The interpretation of the combined TST and QFT™ was done as follows:

1. TST negative and QFT™ negative: LTBI not likely
2. TST positive and QFT™ positive: LTBI likely
3. TST negative and QFT™ positive: LTBI likely. TST could be false negative due to incorrect administration or some biological conditions (immunosuppression, malnutrition, severe illnesses, certain treatments; etc)
4. TST positive and QFT™ negative: LTBI not likely. TST could be false positive due to BCG vaccination (<10 years) or exposure to NTM.

3.7.2 Feedback and Counselling to Participants in Regards to their Results

The researchers met each individual in private meeting scheduled especially to inform them of their QFT™ results. To provide an accurate assessment of the likelihood of LTBI, the results were discussed in combination with the TST results, as explained above. When the algorithm indicated “LTBI: likely”, the researcher offered the participant a handout depicting the two choices to consider (i.e., take no immediate action but be observant of their health or seek medical counselling to verify the LTBI status) (Appendix K).
The three most important goals addressed in the counselling session were: a) the recognition of symptomatology in the event of active TB; b) the importance of the annual health check-up, especially the chest x-rays; and c) the assurance that LTBI is asymptomatic and not transmissible.

A small token of appreciation ($10) was offered the day they of the TST reading to all participants that completed the study.

3.7.3 Educational Sessions

Health education was a crosscutting theme throughout the study. Every meeting had an educational component, in which researchers explained concepts, procedures, and results as well as shared anecdotes and stories related to TB and other infectious diseases. Special written materials in Spanish were created, delivered and explained in group sessions (Appendix L).

3.8 Data Analysis

3.8.1 Data Management

Data collected in the questionnaire and the laboratory results were aggregated into a spreadsheet using Microsoft Office Excel 2003. All information was verified with the original questionnaire. In order to maintain confidentiality, code numbers were assigned to each participant. Data were then entered and analyzed using STATA version 9.0, (StataCorp. 2005, USA).

3.8.2 Statistical Analyses

Descriptive analyses were reported to depict characteristics of the subjects in the sample, specifically demographics and living conditions in Mexico and Canada as well as health and employment conditions.
Prevalence of LTBI was estimated using TST and QFT\textsuperscript{TM}. An evaluation of TST performance was done by calculating sensitivity and specificity of the TST with QFT\textsuperscript{TM} as gold standard. In order to compare levels of sensitivity and specificity of TST compared with QFT\textsuperscript{TM}, they were recalculated using three different cut-offs values (i.e., 5 mm, 10 mm and 15 mm). The optimal cut-off level was estimated from a receiver operator characteristic (ROC) curve using the continuous values of TST induration measures and QFT\textsuperscript{TM}. Agreement between the tests was assessed using kappa statistic. Confidence intervals (CI) of 95% were reported for sensitivities, specificities and kappa values.

Univariate and multivariate logistic regression analysis were performed to identify known risk factors and explore other specific characteristics of this population that could be associated with the positive LTBI results. The odds ratios (OR) of the associations and their respective 95% confidence intervals (CI) were reported. Two-tailed \( p \)-values were considered at a significant level of 5%. Variables that were taken into consideration to evaluate the known risk factors of having LTBI for both tests include: sex, age, TB contact, TB history and previous TST. Similarly, other specific characteristics of this sample such as education, occupation in Mexico, type of housing in Mexico, smoking, alcohol drinking, TB knowledge, Mexican state of origin and number of seasons working in Canada with SAWP were evaluated. Non-significant variables were excluded (i.e., \( p \geq 0.05 \)) to fit a model as predictor of having LTBI.

To assess the current knowledge in respect to active and latent tuberculosis, descriptive analysis of the questionnaire component’s answers “Tuberculosis- Knowledge
and awareness” was used: TB knowledge, symptoms, route of transmission, treatment and cure.
CHAPTER FOUR: RESULTS

The study was conducted in the Niagara Region with Mexican agricultural workers in the 2007 season. A farm registry was obtained from the Municipality of Niagara Region which provided an inventory listing with all housing facilities (378) registered to 229 farms employing migrant workers. Farms were located in the cities of Beamsville, Fenwick, Fonhill, Grimsby, Jordan, Jordan Station, Lincoln, Niagara Falls, Niagara-on-the-lake, Pelham, Ridgeville, Smithville, St. Anns, St. Catharines, Vineland and Wainfleet.

Due to proximity to Brock University, only farms located in cities of Jordan Station, Niagara-on-the-lake, St. Catharines and Vineland (239 households belonging to 132 farms) were selected for delivering a letter of invitation and information asking the farm owner to collaborate in the study and for the workers to attend the information meeting. Letters were prepared for each farm and field trips were made to deliver them and explain the study to the farm owner personally. Due to time restrictions, farms were visited just once.

The letters could not be delivered in 78 out of the 132 farms due to invalid address, empty houses or houses with no principal office to approach (the addresses actually corresponded to living facilities for the workers as opposed to the farms’ administration main offices). In the remaining 54 farms, the owner or staff was available to receive the letter. Twelve farms agreed at the first visit (farm response rate 22.2%) and the rest requested more time to make a decision but did not contact the researchers again. Those twelve farms ranged from 2-80 migrant workers’ employees. Out of 12 farms, five were selected to commence the study on the basis of larger size and proximity. Four
farms agreed that researchers approached all their workers and one with more than 80 migrant workers, accepted with the condition of selecting the workers themselves (11 workers). Of those five farms, a total of 125 Mexican workers were invited to the study, of whom 93 agreed to enrol (74.4% participants’ response rate) and provide data for the questionnaire, blood samples for QFT™ and accepted TST administration. Eleven participants did not complete the study, four dropped out and seven were lost to follow-up after the second meeting. A total of 82 workers (65.5%) completed the study.

The encounters with research participants were initially planned in four sessions: the “information meeting”, the “data collection meeting”, the “reading meeting”, and the “result meeting”. After the first day of field work when the questionnaire was validated, however, the information and data collection meeting as well as reading and results meeting were joined for convenience of the research participants. The TST administration that was included in the data collection meeting in the initial plan was moved for an exclusive day since the procedure would be done by hired nurses from the Niagara Region Public Health Department. As a result the encounter with research participants was set for questionnaire administration and blood sample collection for QFT™ in the first meeting, the TST administration in a second meeting, the TST reading in another meeting and results delivery and counselling in a final meeting.

4.1 Characteristics of Research Participants

A total of 82 persons (65.5%) completed the study. Most participants were male (80%) and their age ranged from 22 to 65 years (mean male 38.5±9.72; female 34.4±7.9). Overall, workers came from 17 Mexican states, most commonly from Mexico State (30.5%), Tlaxcala (11%), Puebla (8.5%) and Guanajuato (7.3%). More than half of
participants (68.7%) had completed primary school and (62.2%) worked in Mexico as farmers. Other characteristics are depicted in table 6.

Table 6. Demographic characteristics of research participants (n=82)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n=82 (% of sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
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<tr>
<td>&lt; 29</td>
<td>15 (18.2)</td>
</tr>
<tr>
<td>30-39</td>
<td>35 (42.5)</td>
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<tr>
<td>40-49</td>
<td>21 (25.6)</td>
</tr>
<tr>
<td>≥ 50</td>
<td>11 (13.4)</td>
</tr>
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<td>Sex</td>
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</tr>
<tr>
<td>Male</td>
<td>66 (80)</td>
</tr>
<tr>
<td>Female</td>
<td>16 (20)</td>
</tr>
<tr>
<td>Mexican State of origin</td>
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</tr>
<tr>
<td>Mexico</td>
<td>25 (30.4)</td>
</tr>
<tr>
<td>Tlaxcala</td>
<td>9 (10.9)</td>
</tr>
<tr>
<td>Puebla</td>
<td>7 (8.5)</td>
</tr>
<tr>
<td>Guanajuato</td>
<td>6 (7.3)</td>
</tr>
<tr>
<td>Oaxaca</td>
<td>4 (4.8)</td>
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<td>Queretaro</td>
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<tr>
<td>Veracruz</td>
<td>4 (4.8)</td>
</tr>
<tr>
<td>Yucatan</td>
<td>4 (4.8)</td>
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<tr>
<td>Education</td>
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<tr>
<td>Primary no completed</td>
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<tr>
<td>Primary (completed)</td>
<td>32 (39.0)</td>
</tr>
<tr>
<td>Secondary (completed)</td>
<td>33 (40.2)</td>
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<td>Occupation in Mexico</td>
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<td>Farmer</td>
<td>51 (62.2)</td>
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<tr>
<td>Constructor</td>
<td>7 (8.5)</td>
</tr>
<tr>
<td>Merchant</td>
<td>4 (4.8)</td>
</tr>
<tr>
<td>Other</td>
<td>18 (24.3)</td>
</tr>
<tr>
<td>Type of Housing in Mexico*</td>
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</tr>
<tr>
<td>Type I</td>
<td>54 (68.3)</td>
</tr>
<tr>
<td>Type II</td>
<td>20 (25.3)</td>
</tr>
<tr>
<td>Type III</td>
<td>4 (5.0)</td>
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<tr>
<td>Type IV</td>
<td>1 (1.2)</td>
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<tr>
<td>Number of people per house hold in Mexico</td>
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</tr>
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<td>3</td>
<td>10 (12.2)</td>
</tr>
<tr>
<td>4</td>
<td>14 (17.0)</td>
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<tr>
<td>5</td>
<td>20 (24.3)</td>
</tr>
<tr>
<td>6</td>
<td>14 (17.0)</td>
</tr>
<tr>
<td>7</td>
<td>10 (12.2)</td>
</tr>
<tr>
<td>8 or more</td>
<td>14 (17.0)</td>
</tr>
</tbody>
</table>

*Type of Housing is classified by the presence of indoor potable water, indoor flushing toilet, indoor electricity and floor made of cement or tiles in whole house. Type I: include all four characteristic; Type II: any three characteristics; Type III: any two characteristics; type IV: only one characteristic; Type V: none.
Living Conditions of Research participants in Mexico and Canada

To characterize living conditions in Mexico and in Canada, study participants provided information about household services and characteristics and number of family members. According to the Government of Mexico, access to public services is a standard measurement of living conditions in Mexico. For purposes of this study and the one conducted in Mexico, housing conditions were classified from Type I to V according to the presence or absence of basic services (i.e., indoor potable water, indoor flushing toilet, indoor electricity) and one construction characteristic (i.e., floor made of cement or tiles) in the entire house. Type I housing included all for characteristics, whereas Type II-V included three, two, one or none, respectively. The majority (93.6%) of workers indicated they lived in Mexico in Type I and II houses. As shown in table 6, the number of persons per house in Mexico ranged from 1-17 (mean 5.8) with 56.9% adults (including the Mexican worker who was interviewed), 16.39% adolescents (13-19 years old) and 26.6% children (0-12 years old). Almost 70% (59/82) had lived in the same address in Mexico for their whole life.

While in Canada, 96.3% participants lived in Type I houses and only three workers lived in a trailer with all basic services. A range of 4-16 people per household (mean 10.5) and 1-6 people per dormitory (mean 3.1) was reported.

Self-reported Health of Research Participants

Overall, good health and no underlying medical conditions that could affect immunity (diabetes, arthritis, etc) were self-reported by the workers. Around 80% participants reported excellent/good health status at interview. The other 20% reported their health was fair. None of them ranked their health as poor.
In general 29.2% (24/82) participants reported smoking cigarettes (range of 1-5 cigarettes per day) and 62.2% (58/82) reported drinking alcohol (range, 1-3 servings at least once a week).

Participants' History of Tuberculosis and Tuberculosis Screening

None of the participants reported a past diagnosis of active TB and 4/82 (4.8%) reported having been in contact with someone with TB in the last five to 30 years, none of them in the last 12 months. Seventy participants (85.3%) reported receiving the BCG vaccine in childhood. In addition, 21/82 (25.6%) recalled being administered the TST however they could not remember their test results. None of the participants reported having ever been rejected into the program due to TB, having presented symptoms compatible with TB disease in the last 12 months or receiving anti-TB treatment in their lifetimes. Similarly, none of the participants reported having had a chest X-ray exam suggestive of TB. As part of the SAWP screening process, all workers reported having had a chest X-ray when they first signed up for the program. However, not all reported having this exam done on a regular basis. In fact, recurrent workers reported that chest X-ray was not usually prescribed if their permanence in Canada was longer than 8 months. Only 37 participants (45.1%) declared undergoing chest X-ray as a requisite for the 2007 season, 17 of whom were in Canada for the first time.

Permanency in the Seasonal Agricultural Workers Program

Permanency in the SWAP program was high: 79.3% of participants had been coming to work in Canada for a range of 2-22 seasons (mean 6.7). A total of 17 workers (20.73%) came for the first time in 2007. Almost 80% of the participants involved in the study had been enrolled in the program to work in Ontario. The remaining (22.2%) were
placed in other provinces before, Quebec being the most reported (66.6%). Within Ontario, 39.51% had worked in other cities with Leamington (50%) and Simcoe (21.8%) being the most reported.

4.2 Current Knowledge about Tuberculosis

A total of 61/82 (74.3%) participants had heard about TB before the study. Of those, only one participant knew TB with another name and 33/61 (54.0%) correctly identified at least one symptom of TB, cough being the most reported 29/33 (87.7%), followed by fever 8/33 (24.2%), presence of sputum 5/33 (15.1%), weight loss 4/33 (12.1%) and hemoptysis 3/33 (9.0%). Regarding route of transmission, 18 participants out of 61 (29.5%) correctly identified air as route for TB transmission, 7/61 (11.4%) also answered air but also incorrectly reported other vehicles such as water, food or mosquitoes. More than half of participants that knew about TB (65.5%) recognized TB as curable and treatable with adequate treatment.

Out of those participants that had heard about TB before, only 5/61 (8.1%) reported having heard of LTBI before the study. Three of those five answered that LTBI could reactivate and two answered it is a dormant form of TB.

4.3 Prevalence of Latent Tuberculosis Infection

Tuberculin skin tests using the Mantoux method – one step (0.1 mL of 5 TU of PPD) were administered in compliance with the Canadian TB Standards. In Canada, persons from medium-high TB burden countries are assumed at high risk and are classified positives for LTBI if the TST is ≥ 10 mm of induration (26). Overall, 28 participants (34.1%, 2 women and 26 men) were TST positive. The range of induration measurements among TST positive was from 10 to 42 mm (mean 15.9).
QuantiFERON-TB Gold In Tube was carried out and interpreted according to the manufacturer’s instructions as described in Methodology (chapter 3). Briefly QFT™ is deemed ‘positive’ when the amount of INF-γ released in the TB antigen tube is greater than the negative control (or background level of INF-γ); ‘negative’ when the amount of INF-γ released in TB antigen tube is low with adequate response in the mitogen tube (or control positive); and ‘indeterminate’ when there is low response in the mitogen and TB antigen tube. Calculations and interpretation were done using the software available from Cellestis (29). Of the 82 participants who completed the study, 15 (18%, all men) had positive QFT™ results.

The prevalence of LTBI using the two different tests differed considerably: 34% prevalence using TST and 18% prevalence using QFT™. A t-test was conducted to determine whether differences in LTBI prevalence between TST and QFT™ were statistically significant. Significant difference was observed between both LTBI tests with a \( p = 0.02 \) as seen in table 7.

Table 7. Statistical analysis between the prevalence of latent tuberculosis infection using tuberculin skin test \((x)\) and QuantiFERON-TB Gold In-Tube \((y)\)

| Variable | Mean  | Std. Err. | z     | P>|z|   | [95% Conf. Interval] |
|----------|-------|-----------|-------|-------|---------------------|
| x        | .3414634 | .0523667  | .2388266 | .4441002 |
| y        | .1829268 | .0426935  | .099249  | .2666046 |
| diff     | .1585366 | .0675649  | .0261119 | .2909613 |

| under Ho: 0.0686896 2.31 0.021 |

| diff = prop(x) - prop(y) | z = 2.3080 |

Ho: diff = 0
Ha: diff < 0  Ha: diff != 0  Ha: diff > 0
Pr(Z < z) = 0.9895  Pr(|Z| < |z|) = 0.0210  Pr(Z > z) = 0.0105
This finding contributes to the increasing body of knowledge supporting that IGRAs may become the test of choice for LTBI at least in developed countries where existing resources facilitate implementation. However, IGRAs for human diagnosis have been around and studied for less than a decade and more research is needed in some specific populations such as children, the elderly and HIV positives as well as determining important factors linked with the test performance, such as cross reactions with NTM, conversion after exposure and their value in the prognosis in disease progression. Perhaps in the following years, when longitudinally studies provide more data in those aspects, IGRAs will be recognized as a better alternative than TST in the diagnosis of LTBI.

Table 8 presents a comparison of findings with each test and the sex and age of participants.

Table 8. Comparison by gender and age of tuberculin skin test and QuantiFERON-TB Gold In-Tube (n=82)

<table>
<thead>
<tr>
<th>Sex</th>
<th>TST</th>
<th>QFT&lt;sup&gt;TM&lt;/sup&gt;</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥10 mm</td>
<td>&lt; 9 mm</td>
<td>Positive</td>
<td>Negative</td>
<td>Indeterminate</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Male</td>
<td>66 (80)</td>
<td>26 (39.4)</td>
<td>40 (60.6)</td>
<td>15 (22.7)</td>
<td>50 (75.7)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Female</td>
<td>16 (20)</td>
<td>2 (12.5)</td>
<td>14 (87.5)</td>
<td>0 (0)</td>
<td>16 (100)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22-30</td>
<td>17 (21)</td>
<td>5 (29.4)</td>
<td>12 (70.6)</td>
<td>3 (17.6)</td>
<td>14 (82.4)</td>
<td></td>
</tr>
<tr>
<td>31-40</td>
<td>35 (42)</td>
<td>10 (28.6)</td>
<td>25 (71.4)</td>
<td>3 (8.6)</td>
<td>31 (88.6)</td>
<td>1 (2.8)</td>
</tr>
<tr>
<td>41-50</td>
<td>22 (27)</td>
<td>7 (31.8)</td>
<td>15 (68.2)</td>
<td>4 (18.2)</td>
<td>18 (81.8)</td>
<td></td>
</tr>
<tr>
<td>&gt;51</td>
<td>8 (10)</td>
<td>6 (75)</td>
<td>2 (25)</td>
<td>5 (62.5)</td>
<td>3 (37.5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>82 (100)</td>
<td>28 (34)</td>
<td>54 (66)</td>
<td>15 (18)</td>
<td>66 (81)</td>
<td>1 (1.2)</td>
</tr>
</tbody>
</table>
As shown in Table 8, one participant had an indeterminate QFT™ result (i.e., results from mitogen TB and antigen tubes were low indicating inadequate basal immune response). Conversely, this participant was TST positive (16 mm). This person was a 37 year old male who had a serious underlying medical condition (i.e., extensive cirrhosis of the liver) before entering the program but did not report this at interview. It was not possible to repeat the IGRA due to a long hospitalization period while in Canada at the end of the season.

A comparison between the measurements of TST in millimetres and positive, negative and indeterminate results of QFT™ are shown in table 9. In general, all QFT™ positive tests were also positive by the TST, but not all TST positive results were confirmed positives by the QFT™. There was one case TST negative but QFT™ positive. Since repeated testing with QFT™ showed reproducible positive results, to rule this case as a TST false negative result it would have been necessary to repeat the TST at a later date. Unfortunately due to logistic constrains this was not possible so the question remains whether this was a case of anergy, technique error in the TST administration or a case of QFT™ false positive.

Table 9. Comparison between tuberculin skin test measurements and results of QuantiFERON-TB Gold In-Tube (n = 82)

<table>
<thead>
<tr>
<th>TST</th>
<th>Positive n (%)</th>
<th>Negative n (%)</th>
<th>Indeterminate n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;9 mm</td>
<td>1 (1.2)</td>
<td>53 (64.4)</td>
<td></td>
</tr>
<tr>
<td>10-14 mm</td>
<td>6 (7.3)</td>
<td>9 (11)</td>
<td></td>
</tr>
<tr>
<td>&gt;15 mm</td>
<td>8 (9.8)</td>
<td>4 (4.9)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>15 (18)</td>
<td>66 (81)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>
Performance of Tuberculin Skin Test compared to QuantiFERON-TB Gold In-Tube

Sensitivity and Specificity

To examine the performance of TST, its sensitivity and specificity were calculated. The lack of a gold standard test for LTBI has been a limitation for many studies to compare the performance of new techniques such as interferon-γ release assays (and also old TST). In this particular study, QFT™ was used as a confirmatory test since IGRAs offer important advantages over TST, as discussed in chapter two.

In Canada, persons are classified positives for LTBI if the TST is ≥ 10 mm of induration. Using QFT™ as a confirmatory test, when 10 mm of induration was used as a cut-off, the sensitivity of TST was 93.3% (95% CI, 68%-99%) and the specificity was 79.1% (95% CI, 67%-88%). In addition, the sensitivity and specificity of TST were calculated using different cut-offs against the QFT™. The results based on three different cut-off values are shown in Table 10.

Table 10. Number of positive subjects with tuberculin skin test using three different cut-offs compared to gold standard (QuantiFERON).

<table>
<thead>
<tr>
<th>QFT™ Positive</th>
<th>TST Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥ 5 mm</td>
</tr>
<tr>
<td>QFT™ Positive</td>
<td>26 (32%)</td>
</tr>
<tr>
<td>QFT™ Negative</td>
<td>14 (17%)</td>
</tr>
<tr>
<td>Total</td>
<td>40 (49%)</td>
</tr>
</tbody>
</table>

A total of 40 (48.7%) participants had TST induration responses ≥ 5 mm. Tuberculin skin test showed high sensitivity using a 5 mm (93.3%; 95%CI, 68%-99%) but low level of specificity (62.1%; 95%CI, 49%-73%). When 15 mm was used as a cut-off, the sensitivity of TST was low (53.3%; CI, 26%-78%) and the specificity was higher compared to other cut-offs (92.5%; CI, 83%-97%) (Table 11).
Table 11. Sensitivity and specificity of TST using different three different cut-offs.

<table>
<thead>
<tr>
<th>TST Cut-offs</th>
<th>5 mm</th>
<th>10 mm</th>
<th>15 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>93.3% (68-99%)</td>
<td>93.3% (68-99%)</td>
<td>53.3% (26-78%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>62.1% (49-73%)</td>
<td>79.1% (67-88%)</td>
<td>92.5% (83-97%)</td>
</tr>
</tbody>
</table>

As seen in Table 11, sensitivity and specificity of TST compared to QFT\textsuperscript{TM}, change at each cut-off value. To determine the optimal or ideal cut-off level of TST, a receiver operator characteristic (ROC) curve was plotted (Figure 5). Receiver operating characteristic curve is a graphical technique to establish the optimal cut-point. In the x-axis, the false positive rate (1-specificity) is plotted and in the y-axis, sensitivity. Ideally, the best cut-off for TST would be the one that has higher sensitivity and higher specificity or that is located in the ROC curve in the upper left corner.

![Figure 5](image_url)

Figure 5. Receiver Operating Characteristic curve for the performance of TST.

Sensitivity and specificity of TST for each cut-off point value (in this case, each measurement in millimetres) were calculated (Table 12).

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0.00</td>
</tr>
<tr>
<td>1</td>
<td>93.33</td>
<td>41.79</td>
</tr>
<tr>
<td>2</td>
<td>93.33</td>
<td>44.78</td>
</tr>
<tr>
<td>3</td>
<td>93.33</td>
<td>50.75</td>
</tr>
<tr>
<td>4</td>
<td>93.33</td>
<td>53.73</td>
</tr>
<tr>
<td>5</td>
<td>93.33</td>
<td>61.19</td>
</tr>
<tr>
<td>6</td>
<td>93.33</td>
<td>65.67</td>
</tr>
<tr>
<td>7</td>
<td>93.33</td>
<td>68.66</td>
</tr>
<tr>
<td>9</td>
<td>93.33</td>
<td>77.61</td>
</tr>
<tr>
<td>10</td>
<td>93.33</td>
<td>79.10</td>
</tr>
<tr>
<td>11</td>
<td>73.33</td>
<td>83.58</td>
</tr>
<tr>
<td>12</td>
<td>66.67</td>
<td>86.57</td>
</tr>
<tr>
<td>13</td>
<td>60.00</td>
<td>88.06</td>
</tr>
<tr>
<td>14</td>
<td>53.33</td>
<td>91.04</td>
</tr>
<tr>
<td>15</td>
<td>53.33</td>
<td>92.54</td>
</tr>
<tr>
<td>16</td>
<td>46.67</td>
<td>94.03</td>
</tr>
<tr>
<td>17</td>
<td>40.00</td>
<td>95.52</td>
</tr>
<tr>
<td>22</td>
<td>33.33</td>
<td>97.01</td>
</tr>
<tr>
<td>23</td>
<td>13.33</td>
<td>97.01</td>
</tr>
<tr>
<td>25</td>
<td>13.33</td>
<td>98.51</td>
</tr>
<tr>
<td>42</td>
<td>6.67</td>
<td>100</td>
</tr>
</tbody>
</table>

According to the figure 5 and table 12, TST sensitivity remains unchanged whether at 1 mm or at 10 mm; above that cut-off, the sensitivity decreases as specificity increases. That is, an increase in TST cut-off will carry a high probability of false negative. In this case, a cut-off of 10 mm has high sensitivity, which is the expected for a screening test, although the specificity still remains low.

The area under the ROC curve (AUC) is a summary measure of test performance. It can take values for 0 to 1. The maximum value, 1 (100% sensitivity and 100% specificity), indicates a perfect test in theory. This value is interpreted as the probability that a test will rank a randomly chosen positive case higher than a randomly chosen negative case. The AUC for TST was 0.87 (95% CI, 0.75 – 0.97). That implies that there is 87% likelihood that a person with LTBI will be correctly classified as positive.
Agreement between Tuberculin Skin Test and QuantiFERON-TB Gold In-Tube

The strength of agreement between TST and QFT™ was evaluated using kappa statistic. A kappa value >0.80 represents excellent agreement beyond chance, a kappa value between 0.40-0.80 represents fair to moderate agreement and a kappa <0.4 represent slight agreement (121). As shown in Table 13, the concordance between TST (10 mm cut-off) and QFT™ was 81.71% (kappa=0.54).

Table 13. Agreement between Tuberculin skin test (cut-off = 10mm) and QuantiFERON-TB Gold In-Tube.

<table>
<thead>
<tr>
<th></th>
<th>TST positive</th>
<th>TST negative</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>QFT™ Positive</td>
<td>14 (17.0%)</td>
<td>1 (1.2%)</td>
<td>15 (18%)</td>
</tr>
<tr>
<td>QFT™ Negative</td>
<td>13 (15.8%)</td>
<td>53 (64.6%)</td>
<td>66 (81%)</td>
</tr>
<tr>
<td>QFT™ Indeterminate</td>
<td>1 (1.2%)</td>
<td>0</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>28 (34%)</td>
<td>54 (66%)</td>
<td>82 (100%)</td>
</tr>
<tr>
<td>Agreement %</td>
<td>81.71%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K coefficient</td>
<td>0.54</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.4 Presence of known risk factors associated with latent tuberculosis infection

Variables that were taken into consideration to evaluate the known risk factors of having LTBI for both tests include: age, sex, TB contact, previous TST, and TB history.

Even though for the present study an IGRA was used as a confirmatory test for TST results, as an exercise statistical analyses were done using TST positivity as an indicator of LTBI. In univariate logistic regression analysis, no statistical association could be proven for TST positivity and sex, TB contact, previous TST, or TB history (p ≥ 0.05) (Table 14). Age was the only variable with a statistically significant association: for every one year increment in age, there were 1.05 higher odds (95% CI, 1.0-1.1; p =
0.048) of having a positive TST (Table 14). For example, a 50 year-old person will be 10 times more likely of having a positive TST result than a 40 year-old person.

Table 14. Univariate logistic regression analysis of tuberculin skin test and known risks factors present in Mexican workers

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (one year)</td>
<td>1.05 (1.0-1.1)</td>
<td>0.04</td>
</tr>
<tr>
<td>Sex (male vs. female)</td>
<td>4.55 (0.9-21.6)</td>
<td>0.05</td>
</tr>
<tr>
<td>TB contact (yes/no)</td>
<td>1.07 (0.7-1.4)</td>
<td>0.06</td>
</tr>
<tr>
<td>Previous TST (yes/no)</td>
<td>2.17 (0.7-6.0)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Univariate logistic regression analysis using QFT™ positivity as an indicator of LTBI, showed age was the only variable significantly associated with a positive result. The odds of being QFT™ positive were higher among older workers (OR, 1.07; 95%CI, 1.0-1.1; p = 0.015) as seen in Table 15.

Table 15. Univariate logistic regression analysis of QuantiFERON-TB Gold In-Tube and known risks factors present in Mexican workers

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (one year)</td>
<td>1.07 (1.0-1.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Sex (male vs. female)</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>TB contact (yes/no)</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Previous TST (yes/no)</td>
<td>1.59 (0.4-5.3)</td>
<td>0.45</td>
</tr>
<tr>
<td>History of TB</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

* Undefined because a zero participants had QFT positive in that category.

Since there were not statistical significant variables except age, a multivariate analysis could not be done. For purposes of the thesis, IGRA’s results (prevalence) will used in the following section and further discussion.
4.5 Presence of specific characteristic of the sample associated with latent tuberculosis infection

To explore the probability that other variables could play a role for LTBI in this population, the following specific characteristics of this sample were analyzed: education, occupation in Mexico, type of housing in Mexico, cigarette smoking, alcohol use, TB knowledge, Mexican state of origin and number of seasons working in Canada with the SAWP. As shown in table 16, it could not be demonstrated that any of these characteristics were statistically associated with LTBI (p ≥ 0.05), however, and unlikely trend was observed between smoking and LTBI, indicating for a p = 0.06, suggesting that cigarette smoking could have a protective effect. Including this variable in a multivariable logistic regression model with age, the protective effect disappears.

Table 16. Sample’s specific characteristics explored associated to positive latent tuberculosis infection using QuantiFERON-TB Gold In-Tube.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education (higher education)</td>
<td>0.91 (0.1-1.6)</td>
<td>0.8</td>
</tr>
<tr>
<td>Occupation Mexico (Farmer vs. others)</td>
<td>0.89 (0.2-2.8)</td>
<td>0.84</td>
</tr>
<tr>
<td>Type of housing</td>
<td>1.22 (0.5-2.7)</td>
<td>0.62</td>
</tr>
<tr>
<td>Number of seasons (one year)</td>
<td>0.97 (0.8-1.1)</td>
<td>0.76</td>
</tr>
<tr>
<td>TB knowledge (yes/no)</td>
<td>0.93 (0.2-3.3)</td>
<td>0.91</td>
</tr>
<tr>
<td>Smoking (yes/no)</td>
<td>0.13 (0.01-1.1)</td>
<td>0.06</td>
</tr>
<tr>
<td>Alcohol (yes/no)</td>
<td>0.89 (0.2-2.8)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

* Adjusted odds ratio. Number of seasons was controlled with age

Since there were not statistical significant variables except age, a multivariate analysis could yield to any change in the significance of any variable tested.
CHAPTER FIVE – DISCUSSION

Tuberculosis is recognized as a life-threatening disease that kills nearly 2 million people every year. In the 20\textsuperscript{th} century, most developed nations experienced a considerable decline of the disease due to public health intervention and the introduction of antibiotics. But in the mid 1980s, TB re-emerged causing an epidemic that has caused alarm in both developed and developing countries. The global incidence of active TB was 9.2 million in 2006 with an estimate of 1.7 million deaths every year. Almost 95\% of TB cases occurred in low/middle income countries, with Africa having the higher TB rates. Tuberculosis is associated with HIV and poverty and the emergence of new strains resistant to TB treatment place this disease as principal target of agencies that coordinate international public health efforts. Most of the private and public foundations are currently devoted to researching the physiology of \textit{M. tuberculosis}, the human immune response, possible vaccine candidates and new diagnostic test that could be applied globally to fight this persistent disease.

One third of the world population (2 billion people) is infected with the latent form of TB. In fact, after inhalation of \textit{M. tuberculosis} from an active pulmonary case, there is 90\% possibility that the susceptible person will harbour the bacteria in asymptomatic and non-contagious state. People with LTBI are usually unaware of their status. Latency is a successful strategy of \textit{M. tuberculosis} to persist for long periods of time until reactivation. Later in life, in about 10\% of those LTBI positive the infection could progress to disease, causing post-primary active TB. Worldwide TB control programs are primarily concerned with detection of active cases, their early diagnosis and appropriated treatment. Nevertheless, because latent infection is main reservoir for active
cases, LTBI also merits detection and treatment. This would have a positive impact in the overall fight against TB by preventing future disease and reducing public health costs.

In developed countries screening for LTBI -mainly with TST- is recommended for populations at risk of having the infection (e. g., contact of an active case, immigrants from high TB burden countries, immunosuppressed persons). For example, in the United States, “targeted TST testing for LTBI is a strategic component of TB control that identify persons at high risk for developing TB who would benefit for treatment of LTBI, if detected” (CDC, 2008) (122). In developing countries where TB is prevalent, however, screening for latent infection is not practical and TST administration is restricted to specific circumstances in which active TB diagnosis cannot be confirmed by the first line diagnostic tool (AFS and culture) or in children younger than 15 years of age and HVI positives.

Until recently, TST was the only test available to detect LTBI. This test often gives false-positives and treatment of TST reactors has been a long standing dilemma. Nowadays, highly specific novel IGRAs can detect LTBI more accurately, thereby reducing unnecessary treatments. Because of this high specificity, IGRAs could be used in a variety of circumstances and populations in which TST administration is not practical and/or its interpretation is not reliable. One such population is the migrant agricultural workers in Canada. The vast majority of these workers are originally from high- or medium-TB burden countries and is likely that a proportion will harbour latent TB. In this population mitigating the controllable risks for reactivation is crucial to their health and livelihood. In order to decrease the risk of reactivation, however, it is first necessary to determine their LTBI status. No studies have been done to assess the
prevalence of LTBI in migrant workers in Canada and since they are highly mobile and of difficult access IGRA s may be a better choice than TST.

In keeping with the scope of the research program to which the present project was proposed, the study was set to undertake a LTBI prevalence assessment in a population of migrant Mexican migrant workers in the Niagara Region using both the traditional TST and a new IGRA.

Following is a detailed discussion of the different aspects of the study.

Study Population

Carrying out a research project involving TB and Mexican migrant workers proved to be a complex and challenging endeavour. First, both active and latent TB are notifiable diseases in Canada. Therefore, the study needed to involve the Public Health Department of the Niagara Region to comply with the procedures and standards of the Region. Second, not only is TB still a disease associated with social stigma, but the disease is a primary concern when the workers apply to the program. Third, migrant workers are considered a vulnerable population. For this reason an extensive research ethics application was elaborated to address not only the physical risks associated with the study, but other less tangible risks as well as the social, psychological and work related risks. Fourth, there were no previous scientific studies done in this population in the Niagara Region that could have informed the study design and the methodology of the present study. Deciphering location and mechanisms for invitation of potential participants, subsequent enrolment, data and sample collection and TST application were subject to numerous brainstorming meetings with different community and academic stakeholders who could advise the researchers the best approach to this population. In the
end, it was impossible to resort to any other way of invitation and recruitment of participants other than in their private dwelling after work hours. This substantially reduced the number of farms that would agree to have their workers approached and effectively slowed down the enrolment-to-feedback process. As it will be explained later, even with the small number of research participants and other limitations, the present study is considered a successful first attempt to conduct biomedical research in a population of Mexican Migrant workers in the Niagara Region.

Data Collection Process

A detailed application for ethical review of research involving human participants was submitted, where the description of invasive techniques, such as blood sample collection and TST administration as well as risks and benefits for the participants were addressed extensively. The REB approved the application “as is” and gave the study clearance to proceed. Similarly, since studies involving blood samples may pose risk for the researchers, it was necessary to procure biosafety clearance from the office of OEH&S describing in detail all the biosafety protocols for working in the field, blood spills, accidental needle stick and disposal of biohazard. For this study, the laboratory was certified as biosafety level II to ensure the protection of researchers.

One of the greatest challenges of this study was the recruitment of participants. As it was mentioned, Mexican workers work and live in farms, often live far away from the nearest town and have little, if any, access to public transportation. It is difficult to have them mobilize from their work places to a specific place, for example, shopping stores. Therefore, the recruitment was set to be done in their place of employment or in their private houses. Due to the privacy of information, addresses of Niagara Region’s farms
that hire migrant workers could only be obtained through an inventory listing provided by The Municipality of Niagara Region. They are not in the public domain.

Five farms were selected for the study due to the higher number of Mexican workers they hired for the season, for a total of 125 workers. The information meetings were a key component in the study. The meetings were held in Spanish so there were no difficulties for the participants to understand or clarify aspects of the study. The researchers were of Hispanic heritage and with some experience working in rural communities in developing countries. This permitted a smooth level of communication and a relaxed atmosphere for the participants. Mexican workers are familiar with medical tests as part of the screening process for the SAWP application in Mexico. However, the mention of "tuberculosis" was worrisome to them for several reasons. For example, the perception may affect their future permanency in the program, the stigma of having TB, or merely for the health concern of being found positives for LTBI. The information meeting addressed widely the matter of the study, and the benefits and risks associated to it. Emphasis that this study would not jeopardize their permanence in the SAWP was made. Researchers reassured participants that their results were confidential and that they would not be shared with outsiders (e.g., employers, peers, Mexican Consulate, Public Health Department, etc). In addition, since employers were fully aware of their workers' participation, research participants did not feel they were doing something unauthorized by their employers. The term "volunteer participation" was discussed at length so workers could fully understand the right to drop-out from the study whenever they wanted to do so. This and the following meetings during data collection were always after work hours, and despite being exhausted after a long day at work, participants were
always receptive and willing to participate. A positive aspect of visiting the workers in their private dwellings was that they could continue with their normal activities such as cooking, watching TV, studying or resting while researchers were interviewing their peers.

All Mexicans who voluntarily agreed to participate met the inclusion criteria and were eligible to participate in the study. The response rate was high (74.4%). Along the study, seven participants were lost to follow-up because of an unexpected early return to Mexico and four dropped-out the study, mostly because of they did not fell comfortable with having the TST administered.

5.1 Characteristics of Research Participants

As mentioned earlier, applicants to the SAWP need to meet certain socio-demographic characteristics (e.g., some experience in agriculture, live in rural areas and have a minimum education of third grade primary school) (105) which make the Mexican migrant worker population fairly homogeneous. Data describing detailed demographic characteristics of Mexican migrant workers in Canada is of difficult access and at the time of writing this thesis, this information was yet to be obtained. In an effort to determine if the population of the present study may represent the Mexican migrant workers in Canada in general, an extensively search of the published and gray literature was performed and only one paper [by Verduzco & Lozano (2004)] that contained such information was found.

Table 17 depicts a comparison of the demographics of the Mexican migrant workers found by Verduzco & Lozano and the population of the present study. It can be
seen that the predominant sex, state of origin, main job in Mexico and permanence in the SAWP are similar, reflecting this homogeneity.

Table 17. Comparison of similar demographics of the sample in this study versus population of Mexican migrant workers described by Veduzco & Lozano.

<table>
<thead>
<tr>
<th></th>
<th><strong>Verduzco &amp; Lozano</strong> (n=364)</th>
<th><strong>Present Study</strong> (n=82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predominant sex</td>
<td>Male</td>
<td>Male (80%)</td>
</tr>
<tr>
<td>Mean Age</td>
<td>No data</td>
<td>30-39 years old (42.5%)</td>
</tr>
<tr>
<td>Average education</td>
<td>7.7 years</td>
<td>Primary complete (5 years) (39.0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secundary completed (40.2%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Technical (6.1%)</td>
</tr>
<tr>
<td>Predominant state of origin</td>
<td>Mexico State</td>
<td>Mexico State (30.5%)</td>
</tr>
<tr>
<td>Main job in Mexico</td>
<td>Agriculture</td>
<td>Agriculture (62.2%)</td>
</tr>
<tr>
<td>Permanence in the SAWP</td>
<td>1-25 seasons</td>
<td>1-22 seasons</td>
</tr>
</tbody>
</table>


According to other reports, women participation in the SAWP is around 3% (103) but in the studied sample for this thesis, women represented 20% of all research participants.

This high percentage could be explained by the fact that two of the five farms selected for the study were fruit farms, which are more likely to hire women. In contrast, the remaining three farms were wineries, which traditionally hire only men.

The SAWP does not require a high level of formal education but at least third grade of primary school. In this sample, only 14.6% of research participants reported incomplete primary school with 85.4% exceeding the SAWP requirements. In fact, 46.3% research participants reported completion of secondary or technical education which makes half of the sample with higher than that reported by Verduzco & Lozano (2004).
In regards to place of origin, study participants were originally from 17 different Mexican States and in 70% of the cases the place of birth and origin were similar, showing a low level of migration among this group within Mexico. Geographic areas in Mexico, as was mentioned before, present disparities in socio-economic context. Therefore, there are areas in Mexico with higher TB rates. In the present study, as it will be seen later, no association between place of origin and LTBI was found. Most of participants in the present study were originally from Mexico, Tlaxcala, Puebla and Guanajuato, states that for 2002 reported TB incidence rates of 4.95, 4.97, 7.24, and 3.56 respectively.

As for the living conditions in Canada, the majority of research participants reported that they lived in a house, including a separate household for men and women. The number of people per bedroom in each household was 1-6 (mean 3.1) persons per bedroom. However, despite the number of people per bedroom is acceptable, the average number of people per household was 12.5. A dozen people living in close proximity to each other sharing common spaces all the time would have increased chances for transmission of respiratory diseases. If there were a case of active pulmonary TB among these workers, the prolonged exposure to the infectious source (a condition for successful transmission) will likely result in further TB cases or LTBI.

Research Participants’ Health Status

Around 80% of research participants self-reported excellent or good health status at interview. Two reasons could explain this finding. First, all workers were screened in Mexico to be admitted into the SAWP. Second, it is possible that only persons who feel very healthy consider applying to the program or returning subsequent seasons, thus
resulting in self-selection of healthy participants. There is no data available of how many workers have been discharged from the program due to a medical condition. For those who stop coming for whatever reason, the complementary study being done in Puebla, Mexico may detect some causes, especially TB.

None of the research participants reported being rejected ever for having TB or having experienced any symptom compatible with active TB, despite that some come from rural areas where TB incidence is higher than the national average.

It is well-known that applicants to SAWP undergo full medical screening at first time but it is not known what kind of medical examination or criteria are applied in subsequent seasons. From the findings of this study, it is evident that CXR is not mandatory every season. There was, for example, a case in which the last CXR was done 9 years ago.

This is worrisome from TB epidemiology point of view. Migrant workers are a mobile population, alternating their lives between Canada and an endemic TB country, Mexico. Moreover, workers are from rural areas where TB rates could be higher than the national average. In addition, as “TB knowledge and awareness” findings of the study, as explained in the following section, the majority of participants did not identify the correct route of TB transmission nor recognized the symptoms of active pulmonary TB. The probability that either the Mexican or Canadian public health departments missing a TB case among returning workers appears very high and perhaps a revision of SAWP guidelines regarding TB is appropriate.

Research participants who resulted positive for LTBI were recommended to visit their family doctor in Mexico for a thorough evaluation. Workers with no risk factors for
progression to active TB may not need preventive treatment but an annual check-up including CXR could be highly beneficial for monitoring LTBI reactivation.

5.2 Current Knowledge about Tuberculosis

Despite screening tests done in Mexico required to be admitted to the SAWP, some research participants (25%) claimed they did not hear about TB before the study. This finding is very hard to explain and likely reflects a deficiency in the design of the questionnaire. For example, two participants of this group declared that they were tested with TST before and only one did not have the BCG vaccine. These incongruent data could have been avoided by constructing the questions better and including questions that check the validity of others.

Of those research participants that responded knowing about TB prior the study, 29% were aware that TB’s route of transmission is breathing in infected droplets suspended in the air. The remaining research participants expressed that TB could be transmitted by mosquito bites, drinking water or eating contaminated food (although it was not asked or clarified about possible TB transmission by drinking contaminated cow’s milk). Almost 65% of research participants who knew about TB recognized that the disease is curable and treatable, so they were aware of the importance of seeking medical attention to get treatment. Half of research participants who knew about TB identified at least one symptom of TB, cough being the most frequently reported, followed by fever, sputum, weight loss and hemoptysis.

Only five participants knew about LTBI and identified with accuracy that LTBI is caused by dormant bacteria in the body and that it could reactivate. As previously explained, in Mexico, TST administration is only done in specific circumstances. For that
reason, it was surprising to find 21/82 (25.6%) research participants having had a TST prior the present study. Unfortunately, the questionnaire did not contain questions to probe for the reasons for having the test done. The only questions asked were “when and how many millimetres” (see page 134, Appendix H) which did not yield useful information.

5.3 Prevalence of Latent Tuberculosis Infection

A prevalence of 34% and 18% for LTBI was found in the study population using TST and an IGRA respectively. Both tests detect present or past infection with *M. tuberculosis*. Tuberculin skin test is a measure of delayed-type hypersensitivity in response to purified protein derivative (PPD) (26) whereas QFT™ measures interferon-γ released in-vivo in response to synthetic peptides present specifically in *M. tuberculosis* but also found in 3 species (29).

To our knowledge, no other studies to date have been done to assess prevalence of LTBI in Mexican migrant workers in Canada. Since there are no similar studies to compare these findings, an extensive literature review was performed to find populations to which compare our findings. Some studies in Mexican migrant workers in the US and immigrants (refugee claimants) in Canada were analyzed for this purposes. In a study done by Sterling *et al*, (2006) about evaluation of treatment of LTBI in the United States, Mexican-born accounted for more than 25% of individuals with LTBI (117). Similarly, Levesque *et al*, (2004) evaluated the proportion of patients accepting TST in a medical clinic for refugee claimants in Montreal and found a prevalence of 25% of LTBI using TST (77). It is important to note that in those studies, LTBI was assessed with TST alone (one step) so we can only compare with the 34% LTBI prevalence assessed by TST in the
present study. Although a comparison is not entirely appropriate, it can be said that immigrants from developing countries have high rates of LTBI. This is in agreement with the WHO statement that one third of the world’s population harbours the \textit{M. tuberculosis}. It will be interesting to follow in the coming years if the advent of IGRAs and their use for LTBI determination will result in a different estimate for LTBI worldwide, as it is known that much of the TST positivity is due to NTM or BCG immunization.

In the diagnosis of LTBI, there are two documented advantages of IGRAs versus TST. Firstly, the specificity of IGRAs are high since they measure the amount of INF-\(\gamma\) that is released by T-cells in response to stimulation to specific \textit{M. tuberculosis} antigens, implying better specificity than TST for detecting LTBI. There is not cross reaction with antigens found in NTM (except \textit{M. kansasii}, \textit{M. marinum} and \textit{M. szulgai}) or BCG vaccine. Therefore, IGRAs could be used to determine LTBI in the same circumstances where TST is used but have better performance when applied in certain populations, such as immigrants, where the probabilities of false positives could be increased by NTM exposure and/or BCG vaccine. Secondly, IGRAs usually require one visit of the patient and are less invasive than TST. In this study, phlebotomy for QFT\textsuperscript{TM} testing was well accepted as opposed to many concerns (and even drop-outs) caused by the TST administration.

Since IGRAs are relatively new, little is known about their ability to correlate with future disease development (123). Only prospective studies with extensive follow-up in different populations will provide insight to this question. In a recent publication, however, positive individuals for QFT had 14% progression rate to active TB as opposed
Latent Tuberculosis in Mexican Migrant Workers

Latent Tuberculosis in Mexican Migrant Workers

The low specificity of TST is a likely the explanation for the discrepancy of these results (123).

Performance of Tuberculin Skin Test compared to QuantiFERON-TB Gold In-Tube

The sensitivity and specificity of TST were calculated using QFT™ as confirmatory test. The lack of a gold standard test for LTBI has been a limitation for many studies to compare the performance of new techniques such as interferon-γ release assays. In Canada, an induration of ≥ 10 mm in the TST is considered positive in foreign-born individuals from high TB prevalence countries and other populations as discussed in chapter three. With the findings of the present study, it was calculated that TST at ≥ 10 mm has a sensitivity of 93.3% (95% CI, 68%-99%) and a specificity of 79.1% (95% CI, 67%-88%). It is widely accepted that TST is highly sensitive. For example, Tsiouris, et al, (2006) compared patients with positive cultures for M. tuberculosis and positive TST for a sensitivity of 90% (95% CI, 84-94%) (124).

To further explore TST performance, sensitivities and specificities of TST based on three different cut-offs were calculated. The cut-off has been standardized in many countries as >5 mm, ≥10mm and >15mm according to population at risk (26). In this study, a ROC curve was created to calculate the optimal or ideal cut-off level of TST with QFT™ as a confirmatory test. According to Table 11 (pg. 71), the standard pre-establish cut-off of ≥10 mm used in many countries as indicative of LTBI infection was confirmed as the best cut-off in this sample. Therefore as a screening test, the TST performs accordingly: high sensitivity and low specificity.

The agreement between TST and QFT™ was 81.7% (k = 0.57). According to Franken, et al (2007), the agreement between these two tests depends on prevalence of
TB, BCG vaccination and on the clinical-epidemiological setting (125). In Franken’s study, the percent agreement between tests was 82% very similar with the present study; however Franken’s report a kappa statistic of 0.19, meaning that the agreement due by chance alone was low.

In a meta-analysis done by Menzies, *et al* (2007), a comparison between TST and IGRAs for diagnosis of LTBI was done in healthy and immunosuppressed adults (71). Because of lack of gold standard for assessing performance of tests, the authors estimated sensitivity from studies of patients with active TB (microbiologically confirmed) and contacts with TB patients. In addition, Menzies, *et al*. (2007) estimated specificity using studies of healthy and younger (< 40 years old) people with low probability of TB exposure, residents of low TB incidence countries with no occupational or travel exposure. Sensitivity and specificity of QFT™ averaged 76% (range 70-83%) and 97% (range, 95-99%), respectively (71).

The high specificity of QFT™ makes this test very convenient as confirmatory test for TST positive persons. In countries with BCG vaccination scheme, QFT™ could be advantageous because of its specificity. In countries with low transmission of TB, the scheme of TST followed by QFT™ as a confirmatory test will target true LTBI positives, reducing the chances of unnecessary treatment for those false-positives, therefore reducing the possibility of emergence of drug-resistant strains.

5.4 Presence of known risk factors and other specific characteristics of the sample associated with latent tuberculosis infection

According to Canadian TB Standards, there are certain known factors that increase the risk of having LTBI in a population. Those factors include; immigration
from countries with high TB prevalence, recent contact with a TB case, homelessness, being aboriginal and occupational risks such as health care workers (26). For immigrants, the known risk factors for having LTBI include age, prevalence of TB in the country of origin and how long they lived in the endemic area (75). The study population of the present work presented an inherent risk factor for LTBI, that is, being natives of and residing in Mexico. As shown in the Results chapter, the known variables investigated for association with LTBI in the participants were sex, age, TB contact and history of TB. In a univariate logistic regression analysis, age was the only variable associated with LTBI, an expected association since the longer the exposure, the more probability of infection. As for the other three variables, the regression analysis could not be performed because none of the workers with those characteristics tested positive for QFT™, the dependent variable used in the analysis.

Creatore *et al*, (2005) investigated variables associated with the risk of developing TB in recent immigrants that arrived in Ontario between 1990-1997 (75). They analyzed age, sex and region of birth and found that sub-Saharan African immigrants have a very high risk of developing TB within eight years of arrival to Canada. Age at arrival and time in Canada was also significant; however, sex was not significant. Conversely, a study done by Jimenez-Corona *et al*, (2006) based on gender differentials in patients with pulmonary TB in endemic area in Mexico found that pulmonary TB and reactivation cases of LTBI were higher in men than in women (126). The authors state that women use health services more frequently than men and men have higher rates of reactivation of LTBI when older.
In this study, risk factors for progression of LTBI to TB diseases were not investigated. Factors that could be investigated in a future study include HIV status, presence of comorbidities, immunosuppressive factors (stress level) and some biomarkers, such as Vitamin D whose deficiency has been associated with reactivation of LTBI (127).

Other specific characteristics of the study population such as education, occupation in Mexico, type of housing in Mexico, smoking, alcohol drinking, TB knowledge, Mexican state of origin and number of seasons working in Canada with SAWP were evaluated for association with LTBI.

Education was explored because higher education could reflect (and it generally does) higher socio-economic status (SES) and it is well known that TB is intimately related to poverty (128). With almost half of the study population having completed secondary or technical school, one could assume those participants might have been less exposed to TB disease, having, therefore, a lower probability of LTBI. The findings of the study, however, do not prove that education had any protective effects against LTBI but as the OR shows (OR 0.91 95% CI=0.1-1.6, p=0.8), a trend can be observed that perhaps would have been significant should the sample had been larger. By the same token, even with the high level of education of the study sample, a high prevalence of LTBI was observed. One can only wonder what the rates of LTBI would be in a sample of lower SES.

Smoking was explored because it is well-known that cigarette smoking due to its anti-tumour necrosis factor-α effect is a risk factor for progression to TB disease (129). The purpose of requesting this information was to inform the educational component of
the study and design sessions emphasizing a healthy life style. In general, 29.2% research participants reported daily smoking (1-5 cigarettes per day) and 62.2% alcohol consumption once a week. It was not asked if those habits were the same in Mexico or developed while in Canada in the process of adjusting to their new lifestyle. Neither was asked for how long they had those habits. Notwithstanding, cigarette smoking came up as a slightly protective factor (OR 0.13 95% CI=0.01-1.1, \(p=0.06\)). As explained earlier, asking about smoking was more a qualitative enquiry than a quantitative assessment, therefore, the reliability of data is questionable. In any case, any protective effect of cigarette smoking for LTBI may be confounded by another risk factor that cannot be identified with the present data (more means to buy cigarettes, urban residence, etc).

The lack of associations between LTBI and other possible characteristics of the study population is likely due to the small sample size: As it was mentioned, due to the many challenges found when implementing this study, it was decided that the best approach would be using a convenience sample. Moreover, being the first biomedical study, this study was not only a preliminary study but also a feasibility study which demonstrated that despite the challenges, biomedical studies can be done with this particular population. In summary, despite of the study limitations, important scientific knowledge was produced and valuable lessons were learned so future similar studies can achieve an even greater level of success.
5.5 Study Limitations

Some of the potential limitations of this study include:

*Recruitment of Research Participants and Sample Size*

The major challenge for this study was to recruit participants among Mexican workers in the Niagara Region. As it was discussed, Mexican workers are considered a vulnerable population with many restrictions while in Canada. The access to this population depended on the farms’ participation, which granted permission to enter their properties to meet the workers in their houses, after work hours. For this study, there was no other method to access Mexican workers as research participants. Therefore, this preliminary study used a convenience sample with voluntary Mexican workers among farms which agreed to collaborate. Notwithstanding, although the response rate among farm’s owners was low (22%), the response rate among Mexican workers was high (74%).

*Biases in Data Collection*

Since this study used face to face meetings to administered the questionnaire, it is natural to expect some biases particularly as it relates to the lack of anonymity when collection information. During this type of procedure in data collection, it is more frequent to obtain socially accepted responses. This could have influenced answers regarding TB history, TB contact and lifestyle questions. In addition, some information provided by participants may have not been 100% accurate, for instance, having received BCG vaccination or previous TST results, etc. Another type of bias that may have happened in the study was selection bias. Workers who volunteered for this study may have had a special interest that encouraged participation. Furthermore, given the
communal setting in which the participant was enrolled and interviewed, some may have felt peer-pressure to participate when the majority of their co-workers accepted to do so; and the opposite could be also true: workers may have declined to participate because their fellow workers also declined or did not agree at the beginning (which was the case for one participant who dropped-out).

In addition to the above mentioned limitations, the present study had also to overcome some challenges that are worth mentioning so future researchers can draw from this experience:

*Using Tuberculin Skin Test in a Population of Difficult Access*

To comply with Canadian TB standards, diagnosis of LTBI required TST administration. Trained nurses from the Public Health Department of Niagara Region were contracted to administer and read the TST, following directives from the Medical Officer of Health. The TST procedure (one-step) requires two visits (administration and reading) which complicated the methodology design. Several extra meetings had to be held in order to complete the TST administration and reading.

*Time Constrains*

By the time REB clearance was obtained, the research laboratory certified and researchers met extra requirements for obtaining phlebotomy certification and received specialized training for QFT™, it was the middle of the agricultural season (July 24th) and Mexican workers were mostly scheduled to depart in September or October, depending on the harvest. Due to this time limitation, researchers could enrol fewer workers from fewer farms than initially planned.
5.6 Study Strengths

- This was a co-operative study with a research group from Puebla, Mexico. Our group in Canada provided expertise in infectious disease and laboratory technology whereas the group in Puebla was stronger in immigration health and social issues. This way both groups complemented each other at all stages of their respective studies.

- The Hispanic heritage of the researchers and interviewers was crucial in the design, implementation and successful completion of the study. Spanish language proficiency was important but more important was the cultural background that allowed not only the collection of reliable data but also the smooth exchange of information and ideas, sharing of experiences, and in depth discussions between participants and researchers.

- This study used state-of-the-art technology for LTBI determination, a novel interferon-γ release assay (QuantiFERON-TB Gold In-Tube) which is regarded as more accurate indicator of LTBI than traditional TST.

- Complying with the current Canadian TB standards, this study made use of the TST administered by trained TB nurses under the medical directive of the Medical Officer of Health. This assured standardization in the administration and reading of the procedure and therefore validity as per the Public Health Department’s guidelines.
CHAPTER SIX - CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. This was a successful first attempt in conducting biomedical research in Mexican agricultural workers in the Niagara Region. In many ways this was a groundbreaking study; there were many barriers and challenges but the rewards and lessons learned are numerous. Because of the nature of the study and the target population, the preparations of instruments as well as the planning and execution of data collection were extremely important.

2. The methodology and implementation procedures used in this study may be used as a general working platform and are transferable to other studies in similar populations and settings.

3. The health education component as a crosscutting theme throughout the study was essential and should be part of any similar study. In our experience, this component played a key role in dispelling the fears of being LTBI positive and the perceived implication for further permanence in the SAWP. As a result, response rate among Mexican migrant workers was high.

4. To increase farm owners response rate and provided sufficient time, several strategies could be implemented that include building up from the existing network created by this study and naturally, more follow-up calls and visits.

5. Our study demonstrated a high prevalence of LTBI in healthy Mexican workers: 34% using TST and 18% using QuantiFERON-TB Gold In-Tube.

6. Tuberculin skin test using a 10 mm cut-off compared to QTF™ was confirmed as an acceptable screening test with high sensitivity and low
specificity. If 5 mm was used as a cut-off, specificity will be very low with same sensitivity and if 15 mm were used, sensitivity will decrease considerably. Using ROC, the best cut-off for this sample was confirmed as 10 mm of induration.

7. Our results suggest that QuantiFERON-TB Gold In-Tube may be used as confirmatory test for TST positive persons. In countries with BCG vaccination scheme, QFT™ could be advantageous because of its specificity. In countries with low transmission of TB, the scheme of TST followed by QFT™ as a confirmatory test will target true LTBI positives for treatment, therefore will reduce the possibility of emergence of drug resistance strains.

8. These findings at the moment cannot predict the probability of progression to active TB in those LTBI positive individuals; only a prospective study of such populations can ascertain this outcome. However, based on recent publications, IGRA positive individuals may have up to 14% probability of reactivation within the next two years, hence the importance of LTBI monitoring by the right test.

9. The study findings suggest that there were no other risk factor for LTBI in this population other than age and being Mexicans. Lack of statistical significance of known risks factors or specific characteristic of this sample was likely due to the small sample size.

10. None of the LTBI positive workers were aware of their LTBI status. After the educational sessions, participants became aware of the several risk factors for TB reactivation, particularly HIV/AIDS, alcohol abuse and smoking. All
participants who completed the study expressed interest in keeping their annual check-up including chest X-ray to confirm being free of active TB, which is crucial for their continued enrolment in SAWP.

11. Despite the small sample size and other limitations, the findings and conclusions of this pioneer study are a valuable starting point in filling the current gap in the body of knowledge of LTBI epidemiology.

Recommendations

In Mexico

1. It is important for migrant workers to know whether or not they have LTBI so they can take actions to minimize the risk for reactivation and if this happened, to recognize the symptoms of TB disease and seek prompt medical care to protect both their health and their employment. Efforts should be made to implement health promotion activities for TB and other infectious diseases.

2. Preventive measures should be available for those LTBI positives. Chest X-ray should be taken yearly, preventing a reactivation case to go undetected.

3. Screening of LTBI should be done for populations at high risk for reactivation. As per the 2007 Canadian TB standards recommendation, TST screening followed by IGRA confirmation could be a cost effective approach (26).

In Canada

1. More health promotion activities should be in place for this population in particular related to infectious diseases. As well, informative written material
that is in Spanish and culturally appropriate should be distributed among workers.

2. More research aimed to address and solve the particular health issues of this population is warranted.
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Appendix A – Letter of Invitation for Farm Owners

July 2007

Dear Sir or Madame (Farm Owner)

Every year the Niagara Region receives thousands of seasonal agricultural workers including hundreds from Mexico through the Seasonal Agricultural Workers Program (SAWP). The health of these workers is the most valued treasure for themselves, their families and their employers in Canada. Infectious diseases are a significant health concern for these workers, and two in particular warrant considerable attention: Tuberculosis (TB). Researchers from Brock University, in collaboration with the Public Health Department (PHD) would like to conduct research study regarding latent TB infection in a sample of Mexican workers. Following is a brief rational the study and our respectful request for your permission to recruit participants in your premises.

One health issue that could impede these workers from coming or returning to Canada is tuberculosis (TB). In fact, as you are aware, these workers are screened for TB in their home country so they can qualify for the program. A worker that comes to Canada is certainly free of Active TB.

However, TB is a deceiving infection. Many people from areas where TB is frequent may harbor it in a dormant (latent) and non-communicable form for years, without having symptoms and without being a source of infection to others. But in a small percentage of these persons, the infection becomes active again (reactivation of latent TB) with the ensuing consequences to their health and the risk of transmission to others around them.

Therefore, it would be highly beneficial for all (workers, their families and the community where they live in and work) to make sure that the workers do not have the latent form of TB and if they did, facilitate their treatment or follow up. If the person does not take treatment, latent TB could be monitored easily by a yearly chest X-ray test. This would protect their health, their jobs and eliminate the risk of reactivation in a near future.

The Governments of Canada and Mexico have established a cooperation agreement for TB research and, at this time, researchers from Brock University and the Benemérita University of Puebla have secured funding to conduct studies on latent TB with these workers: Brock University would like to conduct a study with workers enrolled in the SAWP program and Benemérita University of Puebla with candidates to the program in Mexico. For this study, we count with the support from the Niagara Region's PHD, which will be overseeing our activities and procedures.

We are aware that biomedical research involving these workers is not common and may prove complicated and difficult to accomplish due to the special circumstances they encompass as temporary workers. For that reason we are seeking the collaboration of wineries, farms and nursery owners that employ Mexican workers across the Niagara Region to carry out these projects.

If you kindly decide to collaborate we would request your permission to access the workers’ living quarters within your property. If granted permission, we would visit the workers after work hours as to not cause any disruption in their job obligations. The workers who voluntarily agree to participate would fill out a questionnaire about their health and knowledge about TB and undergo
laboratory testing to determine if they have a reaction to latent TB infection. The results of these tests will be informed to the participants under the supervision of the PHD. The tests themselves or their results will not affect the participants' health nor will they interfere with their work obligations whatsoever.

Naturally, the research study will follow strict scientific and ethical guidelines (it have the clearance from Brock University's research Ethics Board (Tuberculosis study file# 06-268 Duarte et al) and we will not be allowed to disclose any information to the employers. Additionally, the information collected from workers will only be for the research study purposes and will not be shared with the public except when a scientific publication is made with aggregated and anonymized data. At the end of the study we will provide you with a general report of activities and aggregated general findings (without any workers' personal identifier). As well, the name of your establishment will remain anonymous in all reports we produce at the end of the study.

We have thought of your establishment as a potential venue for this study due to your tradition of community involvement and service in the Region and to your genuine interest for the wellbeing of your seasonal agricultural workers. We believe your participation would send a positive message to others about the scientific rigor and the humanitarian benefits of the study and foster a new era of collaboration between Brock University, PHD, and the pillars of the Niagara Region Community.

We would be grateful if you consider this request and give us the opportunity to explain with further detail the benefits of the study as well as the protocols to follow, or other information you consider necessary. We will get in touch with you by phone in a few days to follow up on this letter; or you could contact us directly at the contact information below.

Sincerely,

Angela Duarte, BSc
Graduate student (MSc)
Dept. of Community Heath Sciences
Tel: (905) 646 5395 (H)

Ana L Sanchez, PhD
Associate Professor
Department of Community Heath Sciences
Thesis Supervisor

cc: Niagara Region Public Health Department
Appendix B- Brock University Research Ethics Clearance

DATE: March 15, 2007
FROM: Linda Rose-Krasnor, Chair
       Research Ethics Board (REB)
TO: Ana Sanchez, Community Health Sciences
    Angela Duarte,
FILE: 06-268 DUARTE et al
TITLE: Preliminary epidemiological study of latent tuberculosis infection in Mexican agricultural workers in the Niagara Region, Canada

The Brock University Research Ethics Board has reviewed the above research proposal.

DECISION: Accepted as is.

This project has received ethics clearance for the period of March 15, 2007 to November 30, 2007 subject to full REB ratification at the Research Ethics Board's next scheduled meeting. The clearance period may be extended upon request. The study may now proceed.

Please note that the Research Ethics Board (REB) requires that you adhere to the protocol as last reviewed and cleared by the REB. During the course of research no deviations from, or changes to, the protocol, recruitment, or consent form may be initiated without prior written clearance from the REB. The Board must provide clearance for any modifications before they can be implemented. If you wish to modify your research project, please refer to http://www.brocku.ca/researchservices/forms to complete the appropriate form Revision or Modification to an Ongoing Application.

Adverse or unexpected events must be reported to the REB as soon as possible with an indication of how these events affect, in the view of the Principal Investigator, the safety of the participants and the continuation of the protocol.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of any research protocols.

The Tri-Council Policy Statement requires that ongoing research be monitored. A Final Report is required for all projects upon completion of the project. Researchers with projects lasting more than one year are required to submit a Continuing Review Report annually. The Office of Research Services will contact you when this form Continuing Review/Final Report is required.

Please quote your REB file number on all future correspondence.

LRK/bb

Brenda Brewster, Research Ethics Assistant
Office of Research Ethics, MC D250A
Brock University
Office of Research Services
500 Glenridge Avenue
St. Catharines, Ontario, Canada L2S 3A1
phone: (905)688-5550, ext. 3035   fax: (905)688-0748
email: reb@brocku.ca
http://www.brocku.ca/researchservices/ethics/humanethics/
Appendix D - Circular of invitation for Mexican workers
English Version

Mexican Friend,

You are invited to protect your health
From infectious diseases

Come and participate in a research studies from Brock University to detect if you have been exposed to:

WEST NILE VIRUS
TUBERCULOUS BACTERIA

We will administered a questionnaire, and we will do some lab test
You will have your results and a

In addition, you will have special information
About some infectious diseases, useful for you and your family

Don’t miss this opportunity

Where:
When:

Your supervisor will give you permission and we will bring delicious sandwiches!!!
Appendix D - Circular of invitation for Mexican workers
Spanish Version

Amigo Mexicano,

Estas invitado a proteger tu salud de las enfermedades infecciosas

Participa en un estudio de investigación realizado por la universidad de brock para saber si te has expuesto a:

VIRUS DEL NILO OCCIDENTAL Y LA BACTERIA DE LA TUBERCULOSIS

Llenaras un cuestionario, te haremos unas pruebas de laboratorio, tendrás tus resultados y una consejería,

ADEMAS RECIBIRAS EDUCACION ESPECIAL SOBRE COMO PROTEGERTE A TI Y A TU FAMILIA

NO TE PIERDAS LA REUNION INFORMATIVA

CUANDO:
DONDE:

TIENES PERMISO DE TU JEFE Y HABRAN BOCADILLOS!!
LETTER OF INVITATION
TO PARTICIPATE IN A RESEARCH STUDY

Preliminary epidemiological study of latent tuberculosis infection in Mexican agricultural workers in the Niagara Region, Canada.

Principal Investigator: Angela Duarte, BSc. Graduate student, Department of Community Health Sciences, Brock University
Faculty Supervisor: Ana L. Sanchez, PhD. Professor associated, Department of Community Health Sciences, Brock University

I, Angela Duarte, BSc, Graduate student, and my supervisor Dr. Ana Sanchez, PhD, from the Department of Community Health Sciences, Brock University invite you to participate in a research project about latent Tuberculosis in Mexican agricultural workers that are in the Niagara Region this year (2007) through the Seasonal Agricultural Workers Program (SAW).

The purpose of this research project is to evaluate latent tuberculosis (TB) in a group of Mexican workers who agree to volunteer. If you are a Mexican agricultural worker, you are screening in Mexico for active TB and other infectious diseases before you come. However, latent TB is not part of the screen tests. Latent TB is an infection with the bacteria that causes TB which is in the body in a dormant form. You may not feel any symptom and you are not contagious if you have this form of TB. However, there is a probability that this form reactivate to pulmonary TB and you become sick and contagious. Because the SAW program is continuous for workers like you through years, this study pretend to establish if there is exposure to TB while you are in Mexico or a possible reactivation while you are in Canada. In addition, the project will identify and assess your current knowledge/attitudes in respect to pulmonary TB to implement an educational program on TB and other infectious diseases for all Mexican workers.

The expected duration of the whole study is one year, but you are asked for 3 encounters with us for the study that will be in private but we would also like to implement additional group sessions in one or two more meetings.

1. During the first meeting, called “information meeting”, we will explain the purpose of the study, what will be involved, an explanation of benefits and risks, manage of information (anonymity and confidentiality), the laboratory tests that will be done and the meaning of the results. At the end of the meeting and following a period of questions and answers, we will ask...
for your voluntary participation. If you agree, you and us will set a date and place for the next meeting (preferable in your place and after work at your better convenient). This meeting may take one hour approximately.

2. During the second meeting, called “the data collection meeting” we will administer in a private and in Spanish a questionnaire to you in which you will be asked questions about your health and living conditions both in Canada and in Mexico. At the end of the meeting and with your permission we will draw a blood sample from you, which will be collected in 2 tests tubes.

3. The third meeting will be set 72 hours after the application of the TST and is called “Reading meeting”. We will come back with to read the skin reaction. This process may take 15-20 minutes.

4. The last private meeting, “Results meeting”, a collaborator for Public Health Department will hand in the results to all participants but it will be in private with you. In this short session, we will explain the results and also we will provide more information or counselling depending on the results. This meeting may take 15 minutes.

And finally, all together will select the best date and place to carry out the first educational gathering.

This research study has several benefits:
TO YOU: because you will get a complete screening for Tuberculosis (together with the Chest X-ray required for the program and done before you come). And the educational component will give you important knowledge about infectious diseases and TB and will promote healthy behaviours for the future.

TO THE SAWP PROGRAM: This research also should benefit the Seasonal Agricultural Workers program. It will confirm the successful of the program considering that migrant workers do not have any threat for both other migrants and Canadian population around them.

FOR THE SCIENTIFIC COMMUNITY: because this study will generate new knowledge of latent TB, which it is important for the developing of new vaccines.

This project is sponsor by CIHR and it is a complementary program with Mexico.

IF YOU HAVE ANY PERTINENT QUESTIONS ABOUT YOUR RIGHTS OR BENEFITS AS A RESEARCH PARTICIPANT, PLEASE CONTACT THE BROCK UNIVERSITY RESEARCH ETHICS OFFICER (905 688-5550 EXT 3035, reb@brocku.ca) OR IN SPANISH WITH MISS JANET MACLAUGHLAN, CELL PHONE.
IF YOU HAVE ANY QUESTIONS ABOUT THE STUDY, PLEASE FEEL FREE TO CONTACT ME.

Thank you!

Angela Duarte, BSc
Graduate student
Department of Community Health Sciences
Health Sciences

Ana L. Sanchez, PhD
Associate Professor
Department of Community
Co-investigators:

Dr. Douglas Sider, M.D., M.S.c, F.R.C.P.
Associate Medical Officer of Health program,
Niagara Region Public Health Department
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Heather Hague,
Manager Infectious Disease
Niagara Region Public Health
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heather.hague@regional.niagara.on.ca

This study has been reviewed and received ethics clearance through Brock University’s Research Ethics Board (file 06-268 DUARTE et al.)
Appendix E – Letter of Invitation for Mexican Workers
Spanish version

CARTA DE INFORMACIÓN
PARA PARTICIPAR EN LOS ESTUDIOS DE INVESTIGACIÓN

“Estudio epidemiológico preliminar de tuberculosis latente en Mexicanos
pertenecientes a SAWP en la Región del Niagara, Canada”

Investigador Principal: Angela Duarte, BSc, Estudiante de Post-grado. Departamento de Ciencias
de la Salud Comunitaria, Universidad de Brock
Supervisor Académica: Ana L. Sanchez, PhD, Profesora Asociada. Departamento de Ciencias de
la Salud Comunitaria, Universidad de Brock

Nosotros, Angela Duarte, BSc estudiante de post-grado de la Maestría en Ciencias, bajo la
supervisión de la Dra. Ana Sanchez, profesora del Departamento de Ciencias de la Salud
Comunitaria, Universidad de Brock, por este medio te INVITAMOS A PARTICIPAR en un
estudio de investigación sobre la tuberculosis latente en trabajadores agrícolas migrantes
Mexicanos que están en la Región del Niagara este año de 2007 a través del programa temporal
de trabajadores agrícolas [Seasonal Agricultural Workers Program (SAWP)].

El objetivo del estudio en Tuberculosis es evaluar tuberculosis latente por medio de dos pruebas,
una es la Tuberculina y la otra es una prueba nueva que se realiza en sangre, en un grupo de
trabajadores mexicanos del SAWP, que decidan participar voluntariamente. Si tu perteneces al
programa de trabajadores temporales migratorios, tu has sido examinado para la tuberculosis
activa en México antes de venir a Canadá y si estas aquí, es porque estas libre de ese tipo de
tuberculosis. Sin embargo, la tuberculosis latente no esta dentro de los exámenes que te hacen
para venir. La tuberculosis latente es una infección causada por la bacteria de la tuberculosis,
pero la infección está dormida dentro de tu cuerpo y tu no tienes ninguna sintomatología, ni te
sientes enfermo, ni eres “contagioso”. Pero existe una probabilidad que esa bacteria “se
despierte” o se reactive y se te enfermes de la tuberculosis pulmonar. Este estudio pretende
determinar cual es la proporción de Mexicanos que tienen tuberculosis latente, eso quiere decir,
determinar si han tenido exposición a la tuberculosis en México y determinar si hay alguna causa
que puede hacer que esa bacteria se reactive mientras están en Canadá. Esto por supuesto NO
VA A INTERFERIR CON TU CONTRATO DE TRABAJO, ni con tu participación en el
programa para un futuro. Por lo contrario, te daremos un resultado que te va a servir para que
tengas continuidad en el programa. Además queremos darte mas información de la tuberculosis,
y de otras enfermedades infecciosas en unas charlas informales para que cuides tu salud.
La duración de todo el estudio es de un año, y a cada participante le pediríamos que se reúna con nosotros al menos cuatro veces. Estas reuniones serán en privado pero también nos gustaría hacer reuniones de grupo para las charlas informativas. A continuación te explicamos más acerca de las reuniones:

1. Durante la primera reunión, llamada “reunión informativa”, explicaremos el propósito del estudio y lo que implican, se explicarán las ventajas y los riesgos, cómo se manejará la información (anónimo y en confidencialidad), las pruebas de laboratorio que serán hechas y qué significan sus resultados. También explicaremos cosas sobre la tuberculosis, especialmente tuberculosis latente. También nos gustaría informarte en caso de un resultado positivo, la manera en que se puede manejar. Al final de la reunión y después de un período de preguntas y de respuestas, pediremos tu participación voluntaria. Si estás de acuerdo, juntos fijaremos una fecha y un lugar para la reunión siguiente (preferible en donde vive después de horas de trabajo, a tu conveniencia). Esta reunión puede durar una hora más o menos.

2. Durante la segunda reunión, llamada “reunión de la recolección de datos” usaremos un cuestionario para hacerte preguntas sobre tu salud y condiciones de vida en Canadá y en México; también habrán preguntas relacionadas con tuberculosis. Al final del cuestionario y con tu permiso te tomaremos una muestra de la sangre que será recogida en varios tubos de laboratorio. El proceso entero puede tomar 30-40 minutos.

3. En la tercera reunión, llamada la “reunión de aplicación de la Tuberculina” volveremos con enfermeras del Departamento de Salud Pública de la Región de Niagara para que ellas apliquen la prueba de la tuberculina. Esta reunión será realizada en grupos y posiblemente durará una hora.

4. En la cuarta reunión, llamada la “reunión de lectura”, las enfermeras y una persona del equipo de investigación volveremos para medirte la reacción de la tuberculina en tu brazo. Es una medición con una regla de la parte endurecida que se formó después de la prueba. En ésta reunión se te entregará el resultado de la prueba de la Tuberculina.

5. En la última reunión, llamada “reunión de resumen”, te daremos los resultados del resto de las pruebas que estén listas y también proporcionaremos más información o consejos de cómo mejor manejar tu salud. Esta reunión puede durar 15 minutos.

Además, si tú y tus compañeros de trabajo estuvieran interesados, quisiéramos arreglar reuniones del grupo para desarrollar actividades educativas sobre otras enfermedades infecciosas.

**ESTE ESTUDIO DE INVESTIGACIÓN TIENE VARIAS VENTAJAS:**

**PARA USTED:** En lo que respecta a la tuberculosis, los estudios que te harémos complementarán los que ya tenías y así tendrás un conocimiento completo de tu situación. También sabrás cómo protegerte mejor para que no te den estan infecciones o qué hacer en el caso de que te infectes. También obtendrás bastante información y educación para la salud. El componente educacional te dará conocimientos importantes sobre estas infecciones, y otras, y como adoptar hábitos saludables para tu mejor salud.
Latent Tuberculosis in Mexican Migrant Workers

PARA MEXICO: Con el estudio de Tuberculosis, porque vas a estar atento a los cambios de tu salud y los de tu familia y vas a conocer los síntomas de la tuberculosis y también vas a aprender a tomar medidas para evitar que te contagies.

PARA LA COMUNIDAD CIENTÍFICA: porque gracias a su participación usted ayudará a dar respuestas a preguntas importantes sobre estas dos enfermedades en la región del Niagara.

SI TIENE ALGUNA PREGUNTA O DUDA EN RELACIÓN A SUS DERECHOS O BENEFICIOS DE ESTE ESTUDIO POR FAVOR NO DUDE EN LLAMAR A LA OFICINA DE ÉTICA PARA LA INVESTIGACIÓN EN LA UNIVERSIDAD DE BROCK AL TEL. 905 688-5550 ext 3035, reb@brocku.ca) O EN ESPAÑOL CON LA SENORITA JANET MACLAUGHLAN A SU TELEFONO CELLULAR # 416 876-9453

SI TIENE ALGUNA PREGUNTA SOBRE LOS ESTUDIOS SOBRE EL VIRUS DEL NILO OCCIDENTAL Y TUBERCULOSIS POR FAVOR NO DUDE EN CONTACTARNOS,

GRACIAS POR SU COLABORACION!

Angela Duarte, BSc
Estudiante de Maestría
Departamento de Ciencias de la Salud Comunitaria
Universidad de Brock
905 6885550 ext. 3882-5036
angela.duarte80jq@brocku.ca

Ana L. Sanchez, PhD
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Co-investigators:
Dr. Douglas Sider, M.D., M.Sc, F.R.C.P.
Associate Medical Officer of Health Program
Niagara Region Public Health Department
Department
905-688-8248
douglas.sider@regional.niagara.on.ca

Heather Hague, RN, MA
Manager Infectious Disease
Niagara Region Public Health
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heather.hague@regional.niagara.on.ca
Appendix F – Consent Form

English version

INFORMED CONSENT

TO PARTICIPATE AS A VOLUNTEER IN THE RESEARCH STUDY

“Preliminary epidemiological study of latent tuberculosis infection in Mexican agricultural workers in the Niagara Region, Canada”

Principal Investigator: Angela Duarte, BSc, Graduate student, Department of Community Health Sciences, Brock University
Faculty Supervisor: Ana L. Sanchez, PhD, Professor associated, Department of Community Health Sciences, Brock University

This study has been reviewed and received ethics clearance through Brock University’s Research Ethics Board file # 06-268 DUARTE et al.

Name of the participant: ___________________________ Code ______________

I have been explained in detail the specific aspects of the study by the researchers.

I understand that my participation is voluntary; that I can refuse to participate with no penalty and that I may stop my participation at any time.

I understand that I will participate in a study for screening latent tuberculosis infection.

I understand that I will be asked some questions (personal information and Tuberculosis related) of a questionnaire and that it will be administered by a Spanish-speaker researcher.

I understand that Tuberculin skin test will be applied and that it consists of an intradermic injection with 0.1 ml of tuberculin in my forearm. The reaction (induration) has to be measured 72 hours after the application.

I understand that I will provide a sample of my blood, that will be taken by the researcher who is a certified phlebotomist in a blood collection tube and it will be analyzed for latent tuberculosis. I understand that minimum bleeding and hematoma may occur.

I understand that my general demographic information and a portion of my blood sample will be shared with the West Nile study conducted by Ron Mergl.

I understand that all tests will be done at Brock University, but if necessary my sample may be sent to another laboratory to complement results. This other laboratories are located either in Karolinska Institute (Sweden) or in Honduras.

I understand that all skin reactions DO NOT mean POSITIVE and that Tuberculin test has specific interpretation for each person.
I understand that all information will be kept confidential in a secure location and that my personal information will be protected from any person other than the researchers.  

____ have read and understand this information  
____ agree freely to participate  

______________________________________________  
Signature  

Date: ________  ________, 2007  
Month  Day  

IF YOU HAVE ANY PERTINENT QUESTIONS ABOUT YOUR RIGHTS OR BENEFITS AS A RESEARCH PARTICIPANT, PLEASE CONTACT THE BROCK UNIVERSITY RESEARCH ETHICS OFFICER (905 688-5550 EXT 3035, reb@brocku.ca) OR IN SPANISH WITH MISS JANET MACLAUGHLAN, CELL PHONE # 416 8769453 

IF YOU HAVE ANY QUESTIONS ABOUT THE STUDY OR ABOUT TUBERCULOSIS, PLEASE FEEL FREE TO CONTACT ME.  

Thank you!  

Angela Duarte, BSc  
Graduate student  
Department of Community Health Sciences  
Health Sciences  
Brock University  
905 6885550 ext. 3882-5036  
Angela.duarte@brocku.ca  

Ana L. Sanchez, PhD  
Associate Professor  
Department of Community Health Sciences  
Brock University  
905 685550 ext. 4388  
apa.sanchez@brocku.ca  

Co-investigators:  

Dr. Douglas Sider, M.D., M.S.c, F.R.C.P.  
Associate Medical Officer of Health  
program,  
Niagara Region Public Health Department  
Department  
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Heather Hague,  
Manager Infectious Disease  
Niagara Region Public Health  
Department  
905-688-8248 ext. 7329  
heather.hague@regional.niagara.on.ca
Appendix F – Consent Form

Spanish version

CONSENTIMIENTO INFORMADO
PARA PARTICIPAR COMO VOLUNTARIO EN EL ESTUDIO DE INVESTIGACIÓN

“Estudio epidemiológico preliminar de tuberculosis latente en Mexicanos pertenecientes a SAWP en la Región del Niagara, Canada”

Investigador Principal: Angela Duarte, BSc Estudiante de Post-grado. Departamento de Ciencias de la Salud Comunitaria, Universidad de Brock
Supervisora Académica: Ana L. Sanchez, PhD, Profesora Asociada. Departamento de Ciencias de la Salud Comunitaria, Universidad de Brock

Estos estudios han recibido aprobación por el Comité de Ética de la Universidad de Brock, expediente # 06-268 DUARTE et al

Nombre del participante: _____________________________ Código ______________

Los investigadores me han explicado detalladamente todos los aspectos específicos de los estudios.

Entiendo que mi participación es voluntaria; que puedo rechazar participar sin penalidad alguna y que me puedo retirar de los estudios en cualquier momento.

Entiendo que participaré en un estudio para ver si tengo tuberculosis latente.

Entiendo que se me harán preguntas de carácter personal y relacionadas a TUBERCULOSIS mediante un cuestionario el cual será aplicado en Español.

Entiendo que me sacarán una muestra de sangre en VARIOS tubos. Esta muestra será tomada por los investigadores que están certificados y serán analizadas en el laboratorio para los estudios de tuberculosis. Yo entiendo que puedo tener un poco de sangrado en el sitio de la inyección que se pasara en unos minutos o un morado, que pasará en un par de días.

Entiendo que las muestras serán analizadas en la Universidad de Brock, pero si es necesario, un análisis adicional para estas muestras podría ser realizado en otro laboratorio fuera o dentro de Canadá, pero sin ninguna identificación personal (por ejemplo, mi nombre), solo el código asignado.

Entiendo que me aplicarán la prueba de la Tuberculina, que consiste en una inyección de una pequeña cantidad (0.1 ml) de tuberculina en mi brazo y que la reacción que tenga, será medida a las 48-72 horas después de la aplicación.
Latent Tuberculosis in Mexican Migrant Workers

Entiendo que solo la enfermera podrá interpretar la reacción de la Tuberculina como positiva o negativa, de acuerdo a un método estandarizado. El enrojecimiento en el área de aplicación no significa que la reacción sea positiva.

Entiendo que toda mi información será mantenida confidencial una localización segura y que mi información personal será protegida de personas ajenas al estudio.

Entiendo que los estudios también me proveerán información extensa sobre estas y otras enfermedades infecciosas para protegerme mientras estoy en Canadá.

Consentimiento para participar en un estudio de investigación sobre el virus del Nilo Occidental y sobre la Tuberculosis latente de la Universidad de Brock

---

HE LEÍDO Y ENTIENDO ESTA INFORMACIÓN

ACEPTO PARTICIPAR VOLUNTARIAMENTE

Firma

Fecha: _______ _______ 2007
Mes Dia

---

SI TIENE ALGUNA PREGUNTA O DUDA EN RELACIÓN A SUS DERECHOS O BENEFICIOS DE ESTE ESTUDIO POR FAVOR NO DUDE EN LLAMAR A LA OFICINA DE ÉTICA PARA LA INVESTIGACIÓN EN LA UNIVERSIDAD DE BROCK AL TELEFONO 905 688-5550 extension 3035, reb@brocku.ca O EN ESPAÑOL CON LA SEÑORITA JANET MACLAUGHLAN A SU TELEFONO CELULAR 416 876-9453

SI TIENE ALGUNA PREGUNTA SOBRE ESTOS ESTUDIO DE INVESTIGACION, POR FAVOR NO DUDE EN CONTACTARNOS,

GRACIAS POR SU COLABORACION!

Angela Duarte, BSc
Estudiante de Maestría
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program,
Niagara Region Public Health Department
Department
905-688-8248
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Heather Hague, RN, MA
Manager Infectious Disease
Niagara Region Public Health
905 688-8248 ext. 7329
heather.hague@regional.niagara.on.ca
# Appendix G Personal Result Card

## Research Study on Latent Tuberculosis

<table>
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**TUBERCLEIN SKIN TEST**

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<th>1/2 TUBERCULIN SKIN TEST</th>
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**INTERFERON RELEASE ASSAY**

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**Date of Administration**

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<th>Date not performed, 2007</th>
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**Interpretation**

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**Date of Investigation**

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**Latent Tuberculosis Positive (LTP)**

<table>
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**Authorised Signatory**

<table>
<thead>
<tr>
<th>Authorised Signatory</th>
<th>Brock Niaagara Region Public Health</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>September 24, 2007</td>
</tr>
</tbody>
</table>

**Department of Community Health Services, Brock University, 3580 Telford Drive, Oshawa, ON L1H 7K4; Phone: 905-436-3390, Fax: 905-436-2424; Email: info@brockuniversity.ca**
Appendix H- Questionnaire.

LATENT TUBERCULOSIS SCREENING QUESTIONNAIRE
CUESTIONARIO DE TAMIZAJE TUBERCULOSIS LATENTE

Date: __________________ Code of interviewer: __________________
Fecha Nombre del entrevistador

INCLUSION-EXCLUSION CRITERIA/CRITERIOS DE INCLUSIÓN-EXCLUSIÓN

INCLUSION - INCLUSIÓN
If NO to any, cannot be enrolled/Si dice que NO a alguna, no puede participar

Are you Mexican? Es usted mexicano?

Are you enrolled with the SAWP* program?
Perteneces al programa de trabajadores agrícolas temporales?

Are you 18 years old or older?

Are you freely and voluntarily participating in this study?

EXCLUSION - EXCLUSIÓN
If YES OR DON'T KNOW to any, cannot be enrolled/ SI dice que SI O NO SE a alguna, no puede participar

(Only women) Are you currently pregnant?
(Solo mujeres) Esta embarazada?

Have you had a recent viral vaccine for example to Measles, Mumps, or Rubella in the last 2 months?

Have you had a tuberculosis skin test done in the last 2 months?

Have you been told that you had a positive TB skin test in the last year?

*Seasonal Agricultural Workers Program/Programa de trabajadores agrícolas temporales

INCLUSION - INCLUSIÓN
If NO to any, cannot be enrolled/Si dice que NO a alguna, no puede participar

Are you Mexican? Es usted mexicano?

Are you enrolled with the SAWP* program?
Perteneces al programa de trabajadores agrícolas temporales?

Are you 18 years old or older?

Are you freely and voluntarily participating in this study?

EXCLUSION - EXCLUSIÓN
If YES OR DON'T KNOW to any, cannot be enrolled/ SI dice que SI O NO SE a alguna, no puede participar

(Only women) Are you currently pregnant?
(Solo mujeres) Esta embarazada?

Have you had a recent viral vaccine for example to Measles, Mumps, or Rubella in the last 2 months?

Have you had a tuberculosis skin test done in the last 2 months?

Have you been told that you had a positive TB skin test in the last year?

*Seasonal Agricultural Workers Program/Programa de trabajadores agrícolas temporales

Name of the participant: ________________________________
Nombre del participante

Age: ______ Date of Birth: ______________________________ Sex: ___
Edad Fecha de nacimiento Sexo

Address: ______________________________________________ Telephone: ______________
Dirección de la finca Teléfono

Please answer the following questions.
Por favor responda las siguientes preguntas

Seasonal Agricultural Workers Program

1. Is this your first with the Seasonal Agricultural Workers Program (SAWP)?
   Es ésta la primera vez que viene a Canadá con el programa de trabajadores agrícolas?
   Y / N ________

   *IF YES JUMP TO QUESTION # 9 ↓

2. What was the first year you came to Canada with the Seasonal Agricultural Workers Program?
   En qué año vino por primera vez a Canadá con el programa de TAT? ________

3. How many times have you worked in Canada?
   Cuántas temporadas ha trabajado en Canadá? ________ (#)

4. Have you ever worked in provinces other than Ontario?
   Ha trabajado en otras provincias fuera de Ontario? Y / N ________

   *IF YES,

   5. If yes, which one?
      Si contestó que sí, cual (es):
      Quebec ( ) British Columbia ( ) Alberta ( ) Manitoba ( )
      Others: ________________________________

6. Have you worked in cities other than the Niagara Region?
   Ha trabajado en que otras ciudades de esta región? Y / N ________
   Other: ________________________________

7. Have you worked in other farms in the Niagara Region beside the current one?
   Ha trabajado en otras fincas aquí en Niagara? Y / N ________

   *IF YES

   8. If yes, how many/Cuántas ______

   →9. When did you arrive in Canada this year?
      En qué fecha llegó a Canadá este año? __________________________(exact date: mm/dd)

LIVING CONDITIONS WHILE IN CANADA/ CONDICIONES DE VIDA EN CANADÁ

10. While in Canada, where do you live?
    Mientras está en Canadá, dónde vive?
      • Farm house ( )
        Casa en la finca, hacienda
      • Trailer ( )
        Carro-casa
      • Other (please specify): ________________________________
        Otro (Por favor especifique)
11. Including yourself, how many persons live in the same house or facility? 
   Incluyéndolo a usted, cuantas personas viven en esta casa? __________ # people

12. How many people sleep in the same room/quarters with you? ________ # people
   Incluyéndolo a usted, cuantas personas duermen en la misma habitación o área?

13. Are there any windows in the room you sleep in? Y/N ______________ # windows
   Hay ventana en su dormitorio?

**GEOGRAPHICAL RESIDENCE/MOBILITY / RESIDENCIA Y MOBILIDAD**

14. Where were you born (city and state in Mexico)?
   Cómo se llama el lugar donde nací? (guiarse por el mapa)
   ______________ State:

15. What Mexican city and state are you coming from?
   De qué ciudad y Estado de México viene?
   __________________________ State:

16. For how long have you lived in your current address in Mexico?
   Por cuánto tiempo ha vivido en su actual dirección en México? __________ (años)

**SOCIO-DEMOGRAPHIC DATA IN MEXICO / DATOS SOCIODEMOGRÁFICOS EN MÉXICO**

17. Including yourself, how many people live permanently or for more than 6 months in the same household with you in Mexico?
   Incluyéndolo a usted, cuántas personas viven con Ud en su casa en México?
   
   Children (0-12) _____ Teenagers (13-19) ______ Adults 1+ ________
   Niños Adolescentes Adultos

18. In your home in Mexico, do you have the following
   En su casa de México tiene lo siguiente?
   Indoor Potable water (agua potable adentro) Y / N
   Indoor flushing toilet (Servicio sanitario) Y / N
   Indoor electricity (electricidad en toda la casa) Y / N
   Floor made of cement or tiles in whole house (piso de cemento o cerámica/loza en toda la casa) Y / N

19. What type of job do you have in Mexico?
   Cúal es su ocupación o oficio mientras está en México?
   _______________
20. What is the highest school grade you successfully completed? (check as many as apply)
Qué nivel de educación tiene COMPLETA?
- Elementary/ Primaria _________
- High School/ Secundaria _________
- Technical/Técnica (oficio) _________
- Other/otro_____________________

21. How do you consider your health in general?
Cómo considera su salud en general en este momento? (solo una )
- Excellent/Exelente__________
- Good/Buena ___________
- Fair/Mas o menos ___________
- Poor/Mala ________________

22. If other than excellent, what health problems do you think you have?
Si respondió menos que excelente, qué problemas de salud tiene?

________________________________________________________________________

**TUBERCULOSIS- KNOWLEDGE & AWARENESS**

**SALUD Y CONOCIMIENTO / CONCIENTIZACION SOBRE TUBERCULOSIS**

23. Before this study, have you ever heard of Tuberculosis?
Antes de participar en este estudio, habia oído hablar de la Tuberculosis? Y / N

*IF YES,*

24. What other names you know Tuberculosis for? None: _____
Con qué otros nombres conoce a la Tuberculosis? Ningun otro nombre: _____

25. What symptoms of Tuberculosis do you know?
Cuales son los sintomas de la Tuberculosis que usted sabe?

26. How do you think Tuberculosis is transmitted?
Como cree que se transmite la Tuberculosis?

Don’t know ( ) Water ( ) Food ( ) Air ( ) Mosquitos ( ) Other: _____
No se _____ Agua Comida Aire Mosquitos Otros

27. Do you know about Latent TB?
Antes de este estudio ha oído hablar de la Tuberculosis latente?
Y / N Don’t know ( )

*IF YES,*
28. What do you think is TRUE about Latent Tuberculosis? Si respondio si, que cree que es cierto acerca de Tuberculosis latente?

Very contagious ( ) Could reactivate ( ) You are very sick ( ) Dormant bacteria ( ) Don’t know ( )
Muy contagiosa Puede ractivarse Uno se siente muy enfermo Es un bacteria dormida No se

29. Do you think TB there are drugs to treat and cure TB? Sabe Ud si la TB se cura con tratamiento? Y / N Don’t know ( )

30. Have you ever been diagnosed as having Tuberculosis? Ha sido diagnosticado con Tuberculosis? Y / N Don’t know ( )

*IF NO/DON’T KNOW, JUMP TO QUESTION # 40*

*IF YES,

31. In what year or how old were you? En que año o que edad tenia? _______________ Don’t know ( )

32. Were you treated with medicines for TB? Ha tomado medicamentos para la tuberculosis? Y / N Don’t Know ( )

33. For how long the treatment lasted (in months) Cuánto tiempo duró el tratamiento (en meses) _______________
Don’t know ( )

34. Did you complete the recommended treatment? Terminó el tratamiento? Y / N Don’t know ( )

35. Before you were accepted to SAWP, have you ever been rejected because you had TB? Antes de que viniera a Canada con el programa, lo rechazaron porque tenia TB? Y / N

36. Have you ever lived in the same household or had close contact with someone with active TB? Alguna vez ha vivido en la misma casa o ha tenido contacto con alguna persona enferma con Tuberculosis? Y / N Don’t Know ( )

*IF YES,

37. If yes, in total, for how many years did you have contact?
- Hace cuanto fue ese contacto (o en que año)? _______________
- Por cuanto tiempo estuvo usted en contacto con esa persona (meses o años)? _______ Don’t know ( )

38. In the last 12 months, how many months have you had contact with one or more individuals that were sick of Tuberculosis?
Ha estado en contacto con alguna persona enferma con Tuberculosis en los últimos 12 meses? Cuántos meses? ___________________________ months

39. Have you been vaccinated with the TB vaccine also called BGC?
Tiene la marca en el hombro izquierdo de la vacuna de la tuberculosis?
Y / N Don’t know ( )

40. Have you been tested with the Skin Tuberculin test?
Le han practicado la prueba de la Tuberculina, la prueba en el brazo? Y / N Don’t know ( )

*IF YES,

41. If yes, when? (year or how old were you)
En qué año o que edad tenía? ___________________________ don’t know ( )

42. If yes, how many millimeters was reaction to the PPD test (If known)?
Cuántos milímetros midió su PPD (si sabe)? ________ don’t know ( )

43. When was your last chest X-ray exam?
Cuando le practicaron la última radiografía de pulmón?
Year ______ month _______ where ______________________

44. Do you know how the result of the chest X-ray was?
Sabe cual fue el resultado de la radiografía?

  Normal ( ) Abnormal ( ) Don’t know ( )
  Normal   Anormal   No se

45. Have you had special medical care for chest symptoms in the last 12 months?
Ha tenido atención medica por síntomas en el pecho/pulmón en los últimos 12 meses?
Y / N

*IF YES,

46. Which type of care?
Qué tipo de atención requirio?

_________________________________________________

47. Have you experienced any of the following symptoms in last 2 weeks and in the last 12 months or since 2002?
Ha tenido alguno de los siguientes síntomas en las últimas 2 semanas/últimos 12 meses/desde 2002?

<table>
<thead>
<tr>
<th>Last 2 weeks</th>
<th>Last 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Últimas 2 semanas</td>
<td>Últimos 12 meses</td>
</tr>
<tr>
<td>CAN</td>
<td>CAN</td>
</tr>
</tbody>
</table>

• Persistent productive cough lasting?
Latent Tuberculosis in Mexican Migrant Workers

- Tos productiva (con gargajo) persistente?
- Coughing with blood?
- Tos con sangre?
- Chest pain?
- Dolor en el pecho?
- Unexplained fever?
- Fiebre sin causa aparente?
- Unexplained loss weight? How much?
- Perdida de peso sin causa? Cuanto?
- Fatigue?
- Cansancio todo el tiempo?
- Night sweats?
- Sudoracion nocturna sin explicacion?

48. Do you currently smoke?
Fuma usted? Y / N

*IF YES,

49. If yes, on an average day, how many cigarettes do you smoke daily?
Como promedio, cuantos cigarrillos fuma al dia? _________ cigarettes per day

50. Do you drink alcoholic beverages regularly?
Usted bebe bebidas alcohólicas frecuentemente? Y / N

*IF YES,

51. With what frequency?
Con que frecuencia bebe?
Occasionally ( ) every two weeks ( ) Once a week ( ) Twice a week ( )
every day ( )

52. What type of beverages mostly?
Que tipo de bebida prefiere? Beer ( ) Wine ( ) Tequila ( ) Other:

53. When you drink, how much do you usually drink?
Cuanto generalmente usted toma? One serving ( ) 1-3 ( ) >4 ( )

→ 54. Are you taken any medication?
Esta tomando algun medicamento? Y / N

*IF YES,

55. What type of medicine do you take?
Que tipo de medicina o para que ?

THANK YOU!!!

CODE: ___________________

FOR RESEARCH USE ONLY
Venipuncture performed by (initials) 

Blood with heparin for TB study □

Blood without anticoagulant □

Record of any Special Observations or complications:


RESEARCHER WHO COMPLETED THIS INTERVIEW:


RESEARCHER WHO CHECKED FOR INFORMATION COMPLETENESS
## Appendix I – Interpretation of Results for QuantiFERON® TB Gold In-Tube

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<tbody>
<tr>
<td>≤ 8.0</td>
<td>&lt; 0.35</td>
<td>≥ 0.5</td>
<td>Negative</td>
<td><em>M. tuberculosis</em> infection NOT likely</td>
</tr>
<tr>
<td></td>
<td>≥ 0.35 and &lt; 25% of Nil value</td>
<td>≥ 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 0.35 and ≥ 25% of Nil value</td>
<td>Any</td>
<td>Positive²</td>
<td><em>M. tuberculosis</em> infection likely</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.35</td>
<td>&lt; 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 8.0¹</td>
<td>Any</td>
<td>Any</td>
<td>Indeterminate¹</td>
<td>Results are indeterminate for TB Antigen responsiveness</td>
</tr>
</tbody>
</table>

¹ If Nil < 8.0 IU/mL, the result is indeterminate.
## Appendix J - Informed Consent for Administration of Tuberculin Skin Test (Niagara Region Version)

**Niagara Region**

**PUBLIC HEALTH**

**CONSENTIMIENTO INFORMADO PARA LA PRUEBA CUTANEA DE LA TUBERCULINA (INFORMED CONSENT FOR TB SKIN TEST)**

<table>
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<tr>
<th>APPELLATION (FIRST NAME)</th>
<th>PRIMER NOMBRE (FIRST NAME)</th>
<th>SEXO</th>
<th>NÚMERO DE IDENTIFICACIÓN (IDENTIFICATION NUMBER)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hombre (Male)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mujer (Female)</td>
<td></td>
</tr>
<tr>
<td>D. DE NACIMIENTO (DATE OF BIRTH)</td>
<td>AÑOS/MES/DÍA (YYYY/MM/DD)</td>
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<td></td>
</tr>
<tr>
<td>CIUDAD</td>
<td>CIUDAD</td>
<td>NUMERO DE TELEFONO (TELEPHONE NUMBER)</td>
<td></td>
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</tbody>
</table>

**RAZON PARA HACER LA PRUEBA** (Reason for testing):

- [ ] Empleo/Emloyment
- [ ] Contacto de una caso de TB (Contact of a case)
- [ ] Escuela (School)
- [ ] Otro (Other): __________

**Por favor responda a las siguientes preguntas:** (Please answer the following questions): **SI/YES** **NO**

1. [ ] Ha tenido alguna vez una reacción alérgica a una vacuna? (Have you ever had a reaction to a vaccine?)
2. [ ] Ha tenido tuberculosis? (Have you ever had the disease Tuberculosis?)
3. [ ] Ha tenido la prueba cutánea de la tuberculosis? (Have you ever had a TB skin test?)
4. [ ] Ha tenido la prueba vacuna contra la Tuberculosis (TB) o BCG? (Have you ever had a BCG vaccine?)
5. [ ] Tiene alguna condición médica severa (como cáncer, VIH, transplantes de órgano) o está tomando medicamentos que afectan su sistema inmunológico (como prednisone, medicamentos para trasplantes, quimioterapia) (Do you have a serious medical condition (ie. cancer, HIV, organ transplant) or take medications that affect your immune system (ie. prednisone, transplant medications or chemotherapy)?
6. [ ] Ha tenido alguna vez alguna condición médica que afecte su sistema inmunológico (como prednisone, medicamentos para trasplantes, quimioterapia) (Do you have a serious medical condition (ie. cancer, HIV, organ transplant) or take medications that affect your immune system (ie. prednisone, transplant medications or chemotherapy)?

**He leído la información sobre la prueba cutánea de la tuberculina. He tenido oportunidad de hacer preguntas que fueron respondidas a mi satisfacción. Entiendo completamente la razón por la cual se me está haciendo la prueba de la tuberculina. (I have read the information about the TB skin test. I have had a chance to ask questions which were answered to my satisfaction. I fully understand the reason why I am having a TB skin test.)**

Firma de la persona a quien se le hizo la prueba (Signature of person to receive test) __________

Fecha (Año/mes/día) (Date (YY/MM/DD)) __________

La información personal se recolecta de acuerdo a la ley de Protección y Promoción de la Salud, es solo para los fines del programa de control de enfermedades infecciosas y se mantendrá confidencial. Usted tiene derecho a preguntar u obtener esta información contactando al encargado de los registros del Servicio de Archivos al teléfono # (905) 685-1571, ext. 3741. (The personal information that is being collected is done so in accordance with the Health Protection and Promotion Act. (The information collected will be used solely for the purpose of infectious disease control programs and is held in strict confidence. You have the right to access and to make an enquiry relating to this information by contacting the Region's Manager of Corporate Records and Archives Services at (905) 685-1571, ext. 3741.)

---

**Latent Tuberculosis in Mexican Migrant Workers**

145
La prueba cutánea de la tuberculina

¿Qué pasa si mi reacción es mayor de 10 mm (1 cm)?

La reacción de la tuberculina puede ser positiva por las siguientes razones:

- Vacuna contra la Tuberculosis
- Otras bacterias

**PASOS A SEGUIR:**

1. No se preocupe 😊
2. Esperar los resultados de la prueba de sangre
3. Si la prueba de sangre y la del brazo están positivas, usted puede hacer lo siguiente:

<table>
<thead>
<tr>
<th>POSITIVA PRUEBA DEL BRAZO</th>
<th>POSITIVA PRUEBA DE SANGRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO HACER NADA</td>
<td>IR AL MEDICO EN CANADA</td>
</tr>
<tr>
<td></td>
<td>IR AL MEDICO EN MEXICO</td>
</tr>
</tbody>
</table>

No hacer nada de inmediato...

PERO cuidar su salud:
- Como dejar de fumar,
- Beber alcohol en exceso...
- y estar alerta de los síntomas de la TUBERCULOSIS por si algún día se le despierta:
  - Tos con más de 2 o 3 semanas de duración
  - Fiebre
  - Pérdida de peso
  - Sudoración nocturna

Mostrarle los resultados al médico...

El le va a mandar unas pruebas para confirmar que usted NO tiene TUBERCULOSIS activa:
- Radiografía de pulmón
- Prueba de esputo (gallo)
- Examen clínico

Cuando confirmen que usted NO tiene TUBERCULOSIS ACTIVA, entonces el médico le puede recomendar:

1. Medicina por 6 a 9 meses (es gratis en Ontario y México)

2. Hacer una radiografía de pulmón cada año para ver que usted sigue bien.

3. Y SIEMPRE CUIDAR SU SALUD !!!!!

Preparado por: Dr. Ana Sanchez, PhD and Angela Duarte, Universidad de Brock, 2007©
ESTUDIO DE TUBERCULOSIS LATENTE EN TRABAJADORES AGRICOLAS MEXICANOS

INFORMACION SOBRE LA PRUEBA CUTANEA DE LA TUBERCULINA PARA EL DIAGNOSTICO DE LA TUBERCULOSIS

¿Qué es la tuberculosis?
La tuberculosis (TB) es una infección causada por una bacteria específica. Cuando tú compartes el aire que respiras con alguien que padece de la enfermedad de la TB, la bacteria puede entrar en tu cuerpo y causar infección en los pulmones o vectes otro lado del cuerpo. Al principio, cuando las bacterias de la tuberculosis entran en tu cuerpo, suelen causar una infección inactiva. O sea que se puede tener la infección pero no padecer de la enfermedad, a esta situación se le llama TB LATENTE. Cuando esto sucede, no eres infeccioso para nadie, y te sientes normal y sano. No hay motivo de preocupación a menos que por otras razones tu cuerpo y tus defensas se debiliten. A veces algunas personas con TB latente se los activa la bacteria de la TB y ésta se empieza a multiplicar y causar daño a los pulmones u otros órganos. Una vez que la infección sea activa, puedes contagiar a otras personas pues la TB se propaga de persona a persona por el aire.

¿Qué es la prueba cutánea de la tuberculina?
La prueba cutánea de la tuberculina, también conocida como el método “Mantoux,” es una manera sencilla y segura de averiguar si has contraído una infección inactiva de tuberculosis.

¿Cómo se aplica la prueba?
Consiste en inyectar entre las capas más superficiales de la piel del antebrazo, una pequeña cantidad de PPD o tuberculina. La reacción que causa es un endurecimiento en la zona donde se inyectó, entre 48-72 horas después.

¿Cómo debo cuidar la parte de mi brazo donde se me hizo la prueba cutánea de la tuberculina?
*No lo tapes ni lo cubras con una venda o curita.
*Sí te pica, colócate un paño o una compresa fría suavidad.
*No lo frotes ni rasgueñas.
*Puedes lavarte el brazo y secarlo con

¿Qué Pasa si la Prueba es Negativa?
La mayoría de veces, una prueba negativa significa que la persona no está infectada. EN algunos casos, es necesario comprobar este resultado, ya sea repitiendo la tuberculina o con otra prueba mas confiable.

¿Qué Pasa si La Prueba es Positiva?
El resultado es positivo cuando el endurecimiento es mayor de 10 milímetros. Un resultado positivo no significa que la persona esté ENFERMA o que sea CONTAGIOSA, solo significa que la persona ha tenido contacto previo con la bacteria y que la tiene en su cuerpo en forma latente.

¿Qué Hacer con un Resultado Positivo?
Como mencionamos anteriormente, la tuberculosis es muy común en nuestros países. Es muy posible que tengamos muchos resultados positivos y es importante saber que medidas se pueden tomar para evitar la reactivación de esta bacteria:
- Los servicios de salud recomiendan una radiografía de torax para confirmar que la bacteria no se ha reactivado.
- Se puede dar tratamiento para prevenir la reactivación.
- Si no se opta por el tratamiento, se debe tratar de llevar una vida saludable y hacerse la radiografía de pulmon cada año
- Evitar el contacto con personas enfermas con tuberculosis.
- Las personas que presentan tos por más de dos semanas de duración, especialmente con sangre en el esputo, fiebre, debilidad y falta de apetito debe consultar con su médico inmediatamente.