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Laboratory Diagnosis of Latent and Active Tuberculosis Infections in Trinidad & Tobago and Determination of Drug Susceptibility Profile of Tuberculosis Isolates in the Caribbean

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1. Introduction

Tuberculosis (TB) is a life-threatening, infectious disease caused by the bacteria *Mycobacterium tuberculosis*. The disease has plagued human beings for many centuries as signs of tubercular damage have been found in Egyptian mummies and bones dating back at least 5,000 years ago **[1].** Today, despite advances in diagnosis and treatment, TB is still a global pandemic, fueled by the spread of the Human Immunodeficiency Virus (HIV), the Acquired Immunodeficiency Syndrome (AIDS), poverty and a lack of proper health services in many developing countries **[2].** As a developing nation, many of the Caribbean countries face serious challenges in the diagnosis, treatment, care and management of patients with TB. Some of these challenges include TB/HIV co-infection, drug resistance, inadequate laboratory services, growth of inequity stemming from rising poverty and the presence of weak health systems in many countries **[3].** A major challenge that affects the Caribbean is the lack of proper facilities for laboratory diagnosis of TB; and there is a dire shortage of laboratory facilities and capability for culture and drug susceptibility testing. Because of this, many cases of TB with low bacillary load may be missed by smear microscopy if culture is not routinely performed. This is even more so in HIV/AIDS patients where smear microscopy may be negative due to the small numbers of bacilli being produced as a result of reduced pulmonary cavity formation **[4].**

Weak laboratory service is one of the major obstacles to reducing the global burden of TB. The clinical mycobacteriology laboratory plays a major role in the prevention strategies and control measures of TB **[5].** A wide spectrum of laboratory techniques has been developed to confirm the diagnosis of active and latent TB infection. No single laboratory test method is perfect, and unfortunately, some of the methods of diagnosis on which clinicians still rely on were developed more than a century ago.

It was based on these challenges that the aims of this study were conceived - to compare the available screening and investigative methods for detection of latent TB in Trinidad and Tobago; and also to evaluate methods for detection of drug resistance to *Mycobacterium*

tuberculosis isolates recovered from clinical and cultured specimens from several Caribbean countries.

2. Materials and methods

Study setting and Site: The overall study design and methods have been described previously [6, 7]. Briefly, this prospective observational cross sectional population and laboratory based study was carried out at the Mycobacteriology laboratory at the Caribbean Epidemiology Centre (CAREC) in Trinidad and Tobago. The materials used for the study included individuals and clinical specimens from patients managed for TB infection in Trinidad and Tobago collected over a twelve month period as well as convenient clinical specimens and *M. tuberculosis* isolates from several countries in the Caribbean that were referred to CAREC for culture, identification and drug susceptibility testing over a twenty four month period.

Specimen collection: The specimens used included sputum and other clinical specimens from several Caribbean countries including Antigua, Belize, Dominica, Jamaica, Montserrat, St. Kitts, Nevis, St. Lucia, St. Vincent and the Grenadines, Trinidad and Tobago and Turks and Caicos Islands. While sputum and clinical specimens were referred from countries that did not perform culture or was not performing culture for TB during the study, cultures on Lowestein Jensen (LJ) slants were referred from countries that had the capability to perform culture for mycobacteria but were unable to perform identification and drug susceptibility testing. These countries included The Bahamas, Barbados, Trinidad and Tobago, Suriname and Guyana. For specimens coming from Trinidad and Tobago, both clinical specimens and cultures on LJ were included in the study. Diagnosis of latent TB infection was performed using the Quantiferon Gold Assay. For this test blood samples were collected in heparinized tubes. Individuals for this assay included contacts of confirmed tuberculosis patients, health care workers from the Caura Chest Hospital and the Chest Clinic at the Eric Williams Medical Sciences Complex, inmates of the maximum security prison (where a case of TB was identified) and HIV positive patients attending routine care and treatment clinic.

All specimens referred to CAREC over a two year period (September 2006 – August 2008) from the CAREC Member Countries except Trinidad and Tobago were included in the study. Only specimens from Trinidad and Tobago referred to CAREC over a one year period (September 2006 to October 2007) were included in the study. Clinical specimens were collected from hospitalized patients, chest clinics and sometimes from patients attending the offices of their private physicians and sent to the hospital laboratory or public health laboratory in each country for acid fast bacilli. For culture and drug susceptibility testing (DST), a portion of the specimen was referred, while for laboratories that are able to culture for mycobacteria, clinical specimens were processed using the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method, inoculated and incubated until visible growth was seen on the LJ slants. Slants showing positive growth were then referred for identification and DST. Patients from Trinidad and Tobago in addition to giving blood specimens placed in heparin and transported at room temperature to the laboratory for detection of latent TB also had tuberculin skin tests (Mantoux test) administered on their forearm. Results of the reaction were was read after 72 hours.

Inclusion and exclusion criteria: (a) Only specimens showing positive growth on LJ or BACTEC 460 TB system were further analysed. (b) Repeat specimens or culture were not included. (c) Specimens without basic demographic data were excluded. (d) Cultures that showed growth of contaminating organisms were also not included in the study.

Data collection: For specimens originating from Trinidad & Tobago, a standardized questionnaire was used to obtain additional information of the test subjects. The questionnaire was divided into several sections including demographics, clinical information, medical history, laboratory investigation, radiographic findings, risk factors and treatment. Several methods were used to collate data, including going through the patient's file at the hospital and speaking to the County and the Public Health Nurses. Information (usually age, gender, type of specimen, HIV status and nationality) of specimens from other countries was taken from the patient's form that accompanied the specimens when they were received at the laboratory for culture, identification and DST. Information obtained was then entered into Excel spreadsheet for analysis.

Digestion and Decontamination of the specimens: All clinical specimens were processed in a Biological Safety Cabinet (BSC) using the NALC-NaOH method as previously described in literature **[8].** Specimens on LJ slants were also processed in a BSC observing all safety precautions that are applicable when working with live tuberculosis cultures as has been described **[9].** From the LJ slants colonies of growth were removed with a sterile disposable loop and placed in tubes containing glass beads and 0.5ml sterile distilled water. The tubes were vortexed for approximately 10 seconds to break up the large clumps of mycobacteria and then left undisturbed for 5-10 minutes. Following this, 0.1 ml portions of the supernatant were used to inoculate into BACTEC 12B vials only.

Processing of blood sample for latent TB detection: Blood specimens in heparin tubes were incubated overnight with antigens according to the manufacturer's (Cellestis Inc., USA) instructions. To do this the blood was mixed at least 20 times by gently inverting the tube, then in a 24 well culture plate 1.0ml of blood was placed in each of 4 wells. To each well 3 drops of the respective reagent was added; saline (NIL), early secretory antigen target-6 (ESAT-6), culture filtrate protein-10 (CFP-10) and phytohemaglutinin (Mitogen control), mixed thoroughly into the blood using a plate shaker for 1-2 minutes and then incubated overnight at 37^{o}C. After overnight incubation, the plasma was removed from each well, placed in labeled tubes and stored at 4^{o}C after which the enzyme linked immunosorbent assay (ELISA) test for the detection of IFN-γ was performed.

Culture of clinical specimens: Clinical specimens were cultured using two types of media, the LJ media and 12B media using the BACTEC 460 TB system (Becton Dickenson). For this 0.1 ml of sediments were added aseptically into each of these two media using a tuberculin syringe and needle. Supernatant from LJ cultures were only inoculated on 12B media as above. Both LJ slants and 12B vials were incubated at 37^{o}C. 12B vials were read twice weekly for the first 2 weeks and then once a week for the next 6 weeks for the presence of growth while LJ slants were read weekly for up to 8 weeks. 12B cultures showing positive growth (between 50 to 100 growth units) or LJ slants showing colonies of mycobacteria were removed and identified using the NAP (p-nitro-α-acetylamino-β-hydroxyl-propiophenone) test. Cultures that showed growth of contaminating organisms were discarded. The supernatant was reprocessed and reinoculated. If the cultures were still contaminated, they were discarded.

Identification of mycobacteria using the NAP test: When mycobacterial growth was detected from either the 12B growth media or the LJ slants, each isolate was further identified using the NAP test. This test was performed by adding 1.0 ml of positive culture media to a vial containing p-nitro-α-acetylamino β-hydroxyl-propiophenone. This vial together with the original culture vial was incubated at 37^{0}C and read daily for 4 consecutive days. The culture was identified as *M. tuberculosis* Complex (MTBC) if the tube containing NAP did not allow growth of the mycobacteria while the original tube continued to grow. If growth was detected in both tubes, then the culture was identified as non-tuberculous mycobacteria (NTM); and these were further identified to species level using the Common Mycobacteria (CM) genotyping line probe assay from Hain Lifesciences (Germany). For quality control, a Clinical and Laboratory Standards Institute (CLSI) strain of *M. tuberculosis,* H37Rv was tested along with test specimens each week and for each new lot number of reagents that was used.

Drug Susceptibility Testing using BACTEC 460 TB System: Drug susceptibility tests (DSTs) using the BACTEC 460 TB system was performed on all isolates that belonged to the MTBC group. Only DST to 4 first line drugs and their concentrations - Streptomycin (2.0mg/L), Isoniazid (0.1mg/L), Rifampicin (2.0mg/L) and Ethambutol (2.5mg/L) were used for the study. For DSTs, 0.1 ml of broth from each positive specimen was inoculated to 12B vials containing fixed concentrations of the antibiotics listed above. A control vial without antibiotics was also inoculated with a 1:100 dilution of the respective growth media. All vials were incubated at 37^{o}C and read daily in the BACTEC 460 machine until the control tube read 30 growth units.

The result of each test was determined as resistant if they were above or susceptible if below of the control GI reading. For quality control, once a month and when new antibiotics were prepared, cultures with known resistance patterns were tested along with the test specimens.

Identification of Mycobacteria using Hain Genotyping Assay: This procedure consisted of the following summarized steps: (a) DNA extraction from mycobacterial culture; (b) Preparation of Master Mix for PCR procedure; (c) Amplification Procedure; (d) Hybridization and Detection and; (e) Interpretation of results

DNA extraction: DNA extraction was performed using mycobacteria from liquid cultures (i.e. from positive 12B BACTEC culture vials). DNA extraction was performed when there was heavy growth of mycobacteria (when the liquid culture read at least 900 growth units). In a BSC 1.0ml of the culture was removed from the vial with a tuberculin needle and syringe and placed in a 2.5ml micro-centrifuge tube. The tube was then closed and centrifuged in a micro-centrifuge for 15 minutes at 13,000 r.p.ms. After centrifugation the supernatant was removed and the sediment re-suspended in 300µl molecular grade water. This suspension was then boiled at 96^{o}C in a water bath for 15 minutes to lyse and inactivate the bacilli. After boiling the suspension was then placed in a sonicating water bath for a further 15 minutes. 5µl of this supernatant was used for the amplification reaction.

Preparation of Master Mix: In a sterile room specifically used for preparing master mixes, the master mix was prepared by combining the following reagents in a micro tube: 35µl of PNM (containing a mixture of triphosphate deoxynucleoside and primers marked with

biotin). PNM is included in kit; 5µl of 10X buffer for polymerase incubation; 2.0µl of 2.5mM $MgCl_2$; 0.2µl of HotStart Taq polymerase (Qiagen, Germany); 2.0µl of distilled water. The final volume was 45µl and this is the amount used for 1 sample. This amount was multiplied by the number of samples and controls. After preparation, 45µl amounts of the master mix were aliquoted in 0.5ml centrifuge tubes and labeled with the specimen number before the addition of DNA.

Amplification Procedure: In another room and under a BSC, 5µl of each DNA solution was added to the respective labeled tube containing the master mix prepared above to reach a final volume of 50µl. After addition of the DNA, the tubes were mixed properly by vortexing for 10 seconds. Amplification was performed in a thermal cycler (Perkin Elmer 9600 thermal cycler; Applied Biosystem, CA, USA) using the following amplification protocol: one denaturation cycle of 15 min at 95^{o}C, followed by 10 denaturation cycles of 30s at 95^{o}C and ten elongation cycles of 2 min at 58^{o}C, followed by 20 additional denaturations of 25s at 95^{o}C and annealing of 40s at 53^{o}C, continuing with an elongation step of 40s at 70^{o}C and finishing with an extension cycle of 8 minutes at 70^{o}C. After amplification the amplified DNA was kept at 4^{o}C until hybridization was done.

Hybridization and Detection: Hybridization and detection were performed as described by the manufacturers using a semi-automated method in a TwinCubator (Hain Lifesciences, Nehren, Germany).

Reverse Hybridization Process: In the Reverse Hybridization process, each strip has a total of 17 reaction zones. The first band contains the conjugate control designed to indicate that the conjugate has been effectively united with the substrate, thereby facilitating correct visualization. The second band includes a universal control designed to detect all known mycobacteria and members of the group of gram-positive bacteria with a high G+C content. This band is used for checking the presence of the amplified product after hybridization. The third band contains a sequence that amplifies a fragment of the 23S rRNA region, which is common to all known members of the tuberculosis complex. Amplification bands 4-17 include probes specific for each of the mycobacteria species. A combination of these bands enables identification of the different species of mycobacteria, including *M. tuberculosis* complex.

Drug susceptibility using MTBDRplus (Line probe assay): This procedure was performed exactly as that for identification of mycobacteria except for the difference in the primers used and the type of specimen. While cultured material was used for the Common Mycobacterium CM assay, clinical specimens were used for the MTBDR*plus* assay. The primers used were specific to detect presence of wild types and mutations. Each strip contained bands that detected *M. tuberculosis* Complex, locus controls (*rpo*B, *kat*G and *inh*A) as well as Wild Types (WT) and mutations for *rpo*B, *kat*G and *inh*A. There were eight WT for *rpo*B gene (WT 1-8) and four mutations (MUT 1, 2A, 2B and 3). For *kat*G gene there was one WT and 2 mutations (MUT 1and 2) and for *inh*A gene 2 WT (1 and 2) and 4 mutations (MUT 1, 2, 3A and 3B). The isolate was identified as *M. tuberculosis* complex when the TUB band was present. Resistance was determined when a wild type was missing and or a mutation present for each of the gene on the strip.

ELISA test for detection of gamma interferon (IFN-γ): For each ELISA test run, two strips or 16 wells were required for standards and 4 wells were required for each patient sample.

All reagents except the conjugate were brought to room temperature before the test. For each run the required number of strips were removed from the kit and placed on a strip holder. The standard dilutions were prepared (8.0IU/ml – 0.125IU/ml) as well as the conjugate dilution (5µl conjugate concentrate to 1.0ml of diluent). To each well 50µl conjugate dilution was added followed by 50µl standard dilution and 50µl respective patient samples. The contents of the wells were mixed for 1-2 minutes using a plate shaker and then incubated for 2 hours at room temperature to enable the antigen-conjugate complex to adhere to the surface of the microwells. After incubation, the wells were washed with wash buffer 5 times using an automated plate washer to remove excess conjugate complex. This was followed by the addition of 100µl enzyme substrate (kit component). The wells were mixed as before and incubated for a further 30 minutes at room temperature. The reaction was stopped after this time with 50µl enzyme stop solution and the optical densities of each well measured using wavelengths of 450 and 620. Results were calculated by plotting the results on a graph using Microsoft Office Excel. Results greater than 0.35 in the ESAT-6 and CFP-10 wells were recorded as positive while results less than 0.35 were recorded as negative.

Data Analysis: Statistical analysis of results of culture, identification, DST as well as questionnaire information were performed using Epi Info 3.5.1 version, Centers for Disease Control & Prevention software **[10]** and Open Epi version 2.3 **[11].** Associations between variables were assessed using Chi-square analysis and Fisher's exact test. *P*-values of ≤0.05 were considered statistically significant.

Ethical approval: The Ethics committee of the Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago, approved the study while written permission for use of the specimens was received from the Chief Medical Officers in the Ministry of Health from each of the countries represented in the study.

3. Results

Specimens and patients: A total of 1,262 specimens comprising 43% culture materials and 57% clinical samples obtained from 15 Caribbean countries were used for this study. The highest number of specimens 28% were obtained from Trinidad & Tobago and the least 0.2% was from Dominica as depicted on Table 1. For latent TB detection, 560 subjects were recruited from Trinidad and Tobago.

Culture and Identification: The BACTEC 460-TB culture method used for culturing the specimens yielded 773 positive cultures and from this, 79.04% (611/773) were identified to belong to the *Mycobacterium tuberculosis* complex group, while 20.96% (162/773) were NTMs using NAP test. The Hain Common Mycobacteria (CM) genotyping assay was used to further identify the NTMs isolates that consisted of *Mycobacterium fortuitium* (34.6%), followed by *Mycobacterium intracellulare* (12.3%), *Mycobacterium gordonae* (6.8%), and *Mycobacterium kansassi* (6.2%). The genotyping assay was unable to identify 16.7% of the NTMs, while 7.4% showed characteristics of mixed infection with *M. tuberculosis* and *M. fortuitium* (referred to Hain Lifesciences for confirmation). The distribution of the NTMs isolates from the various Caribbean countries is highlighted in Table 2.

Country	Specimens		Total (%)
	Clinical samples	Culture materials	
Antigua	3	-	3 (0.24)
Bahamas	-	28	28 (2.22)
Barbados	-	26	26 (2.06)
Belize	54	-	54 (4.28)
Dominica	2	-	2 (0.16)
Guyana	-	214	214 (16.96)
Jamaica	208	-	208 (16.48)
Montserrat	25	-	25 (1.98)
St. Kitts	3	-	3 (0.24)
Nevis	5	-	5 (0.40)
St. Lucia	77	-	77 (6.10)
St. V & G	7	-	7 (0.55)
Suriname	-	240	240 (19.02)
T&T	328	34	362 (28.68)
TCI	8	-	8 (0.63)
TOTAL	720 (57.05)	542 (42.95)	1,262 (100)

St. V&G = St. Vincent and the Grenadines, T&T = Trinidad & Tobago, TCI = Turks & Caicos Islands

Table 1. Distribution of specimens (clinical samples and cultures) received from fifteen (15) Caribbean countries used for this study (%).

Species	**N (%)**	**Countries**
M. fortuitium	56 (34.6)	BDS, BLZ, GUY, SUR, T&T
M. intracellulare	20 (12.3)	ANT, GUY, JAM, STL, SUR, T&T
M. gordonae	11 (6.8)	BRB, GUY, SUR
M. kansassi	10 (6.2)	BRB, JAM, T&T
M. abscessus	8 (4.9)	BRB, JAM, SUR
M. avium	6 (3.7)	JAM, SUR, TCI, NVS
M. intermedium	4 (2.5)	SUR
M. scrofoleceum	3 (1.9)	BLZ, SUR, T&T
M. interjectum	3 (1.9)	SUR, TCI
M. simiae	1 (0.6)	JAM
M. chelonae	1 (0.6)	SUR
Mycobacterium species	27 (16.7)	GUY, JAM, SUR, MNT
Mixed infection (MTB and *M. fortuitium*)	12 (7.4)	JAM, SUR
TOTAL	162(100)	

N = total number, MTB = Mycobacterium tuberculosis, T&T = Trinidad & Tobago, TCI = Turks and Caicos Islands, JAM = Jamaica, SUR = Suriname, GUY = Guyana, MNT = Montserrat, BLZ = Belize, NVS = Nevis, BRB = Barbados, ANT = Antigua

Table 2. Distribution of non tuberculosis mycobacteria (NTM) species identified in specimens from Caribbean countries using the Common Mycobacteria (CM) genotyping assay

3.1 Tuberculin Skin Test and QuantiFERON-TB Gold for the detection of Latent TB infection

A total of 560 subjects were recruited from Trinidad & Tobago for this component of the study. They all had blood samples drawn from them and equally had TST administered on their forearm. Of these 560, only 530 of the subjects met the study criteria and were therefore included in the final analysis of the results. Summary of the result of the detection of latent TB infection using the tuberculin skin test (TST) and the QuantiFERON-TB Gold assay is given on Table 3.

The majority of the subjects were males (73.5%) and between the ages of 40 and 49 years (32.8%), The TST results surprisingly revealed that only 1.8% (3/165) of the TB patient (control group) readings were <5 mm and only 3% (5/165) were 5-9 mm. As expected, 95.2% of the TB patients had a wheal reaction ≥10 mm. None of the HIV subjects had a reaction of ≥ 10 mm but most of them (90.6%) had a reaction < 5 mm; the rest (9.4%) had a reaction measuring 5-9 mm. Among the health care workers, there were no TST readings ≥ 15mm, but most (73.2%) were <5 mm. In Trinidad and Tobago, a TST reading ≥ 10 mm among uncompromised individuals is considered a positive result. For individuals with HIV or any other underlying condition, such as malignancy, the positive cutoff threshold drops to 5-9 mm. Therefore, the 9.4% of HIV-positive subjects in the current study with readings at that level were considered to have positive TST results. Cutoff thresholds for interpretation of actual TST results (which are not considered biologically meaningful) range from 5 mm to 15 mm, depending on the type of high-risk group being surveyed and the level of TB prevalence in the study setting. The positive cutoff values used in the current study are relatively high but did not affect the final study results due to the clustered distribution of the induration values described above.

The comparative results of the two test methods used for the diagnosis or screening for latent TB infection among high risk groups in Trinidad & Tobago (Table 3) revealed that the QFT-G assay detected a significantly higher proportion of latent TB infected individuals than the TST in all high-risk groups except TB patients (the study controls), among whom the TST appeared to be more effective. The differences were statistically significant among all target groups studied for each testing method.

There was no significant age difference between the TST positive subjects and those with positive results for the QFT-G assay, who ranged from 20 to 60 years and 21 to 59 years respectively (with a mean age of 33.1 versus 34.5 years; $p > 0.05$). Most of the HIV and TB positive subjects (68.8% and 69.7% respectively) were in the 30-59 year age group.

The average number of hours required to complete the TST was 70.1 hours versus 23.4 hours for the QFT-G assay ($p < 0.0001$). The average cost to perform each TST was US $3.70 (for a total cost of US $2,065.00), whereas US $18.60 was required to carry out the QFT-G assay (total cost of US $10,440.00). These differences were significant (US$3.70 versus US$18.60; $p = 0.0008$) and favored use of the TST method for latent TB infection (LTBI) detection.

When the results of both tests were combined, the rate of LTBI detection increased to 88.7%. In the prison inmate group, concomitant results for both tests were available for 62 subjects. Of these, 24.2% (15/62) tested positive based on the TST and 56.5% (35/62) tested positive based on the QFT-G assay. The rate of concordance between the two tests for this target

group was 49.7% (32/62) for negative results, and 15.6% (10/62) for positive results, and overall agreement of 76%. For all discordant results, subjects were more likely to be TST-positive and QFT-G – negative (92.1%) versus TST-negative and QFT-G – positive (7.0%). Overall, 39.6% of all subjects had a positive TST result and 51.3% had a positive QFT-G assay result. The significant differences obtained for TAT favored the QFT-G assay, whereas the cost of material required to perform the tests favored the TST.

High risk group	N	No. cases detected (%)		P-value
		TST	QFT-G	
TB patients' contacts[a]	200	35 (17.5)	78 (39.0)	<0.001
Health Care Workers	40	3 (7.5)	15 (37.5)	<0.003
Prison inmates	62	15 (24.2)	35 (56.5)	<0.006
HIV+ patients	60	12 (20.0)	26 (43.3)	<0.0006
TB Patients (controls)	168	157 (93.5)	118 (70.2)	<0.008
Total	530	210 (39.6)	272 (51.3)	0.08
Cost per test[b]		$3.70	$18.60	<0.0008
Turnaround time[c]		70.0	23.0	<0.0001

N = total number of subjects tested. [a] Individuals who came into contact with active TB patients - friends and family members. [b] In 2010 US dollars ($1 = 6.35 TTD). [c] Average number of hours from time of intradermal injection of tuberculin on subjects' forearms to the time wheal reaction at puncture was read within 72 hours.

Table 3. Comparison of QuantiFERON® TB-Gold (QFT-G) assay and tuberculin skin test (TST) in diagnosis/screening for latent tuberculosis (TB) infection among high-risk groups from Trinidad & Tobago

The average number of hours required to complete the TST was 70.0 hours versus 23.0 hours for the QFT-G assay ($p < 0.0001$). The average cost to perform each TST was US $3.70 (for a total cost of US $2,065.00), whereas US $18.60 was required to carry out the QFT-G assay (total cost of US $10,440.00). These differences were significant (US$3.70 versus US$18.60; $p = 0.0008$) and favored use of the TST method for LTBI detection.

Drug Susceptibility Testing (DST): The BACTEC 460 TB System was used to successfully determine the drug susceptibility tests (DST) of 91.3% (558/611) cultures identified as *M. tuberculosis* Complex from 12 of the Caribbean countries as revealed on Table 4. Overall, a total of 42 (7.5%) isolates from 8 countries showed resistance to at least one or more anti-TB drugs.

Analysis of the susceptibility pattern to the anti TB agents revealed that 73.8% (31/42) of the isolates were resistant to isoniazid (INH), 66.7% (28/42) were resistant to rifampicin (RIF), 38.9 (16/42) were resistant to both RIF and INH while 28.6% (12/42) were either resistant to streptomycin or ethambutol. The highest number of isolates subjected to DST analysis were obtained from Trinidad & Tobago and then followed by Guyana. Although no multidrug resistance was seen in isolates from several of the Caribbean countries, the highest frequency of resistance and multidrug resistance were noted among isolates from Guyana.

The Hain Genotyping line probe assay (MTBDR*plus*) was further used to genotypically analyze a total of 33 isolates that had initially been identified to be resistant to INH (26 isolates) and/or RIF (24 isolates) by the phenotypic method. This result is shown on Table 5. Overall, of the 24 isolates showing resistance to RIF using the phenotypic method, 23 (95.8%) showed resistance with the genotypic method. Of the 26 isolates showing resistance to INH with the phenotypic method, 9 (34.6%) showed resistance with the genotypic method. Additionally, 2 isolates sensitive to INH with the phenotypic method showed resistance with the genotypic method and 1 isolate resistant to RIF with the phenotypic method was sensitive with the genotypic method.

Resistance to RIF was identified genotypically by the presence or absence of mutations in the *rpo*B gene, while resistance to INH was identified by the presence or absence of mutations in the *kat*G and *inh*A genes. The codons most frequently involved in RIF mutations were S-531L (57.1%) and codon S-516L (20%). Twenty (20) isolates carried the most common mutation, Ser531 → Leu. As for INH resistance, of the 9 isolates that the Genotype MTBDR*plus* detected, 78% of them carried mutation at S315T1 codon of the *kat*G gene, showing AGC → ACC mutation, and 22% showed AGC → ACA mutation. Equally, 64.7% of these mutation occurred at these bands at the *inh*A gene in the MDR isolates from the region.

Country	N	S	R	MDR
Bahamas	21	15	6 (14.3)	0
Barbados	14	12	2 (4.8)	1 (6.25)
Belize	5	3	2 (4.8)	1 (6.25)
Dominica	1	1	0	0
Guyana	141	117	24 (57.1)	13 (81.25)
Jamaica	95	92	3 (7.14)	0
St. Lucia	10	10	0	0
St. Kitts	2	2	0	0
St. V&G	6	6	0	0
Suriname	115	112	3 (7.1)	1 (6.25)
T&T	145	144	1 (2.4)	0
TCI	3	2	1 (2.4)	0
Total	558	516	42 (100)	16 (100)

N = number of isolates tested, S = isolates fully susceptibile to all first line drugs, R = isolates resistant to any of the first line drugs, MDR = isolates resistant to both isoniazid and rifampcin, T&T – Trinidad and Tobago, TCI – Turks and Caicos Islands, St. V&G – St. Vincent and the Grenadines.

Table 4. BACTEC 460 TB System susceptibility results of 558 TB isolates (patients) from the Caribbean (%)

BACTEC 460 MTBDRplus	rpoB		katG		InhA			
	MWT	Mut	MWT	Mut	MWT	Mut	RIF	INH
RIF	WT7	2A	-	-	-	-	R	-
R	WT8	3	-	-	-	-	R	-
R	N	2A, 3	-	-	-	-	R	-
R	WT8	3	-	-	-	-	R	-
R	WT8	3	-	-	-	-	R	-
RE	WT8	3	-	-	-	-	R	-
I	WT8	3	N	N	N	N	R	S
I	-	-	N	N	N	N	-	S
I	-	-	N	N	N	N	-	S
I	-	-	WT1	1	N	N	-	R
I	-	-	N	N	N	N	-	S
I	W3,4	-	N	N	N	N	R	S
I	-	-	N	N	WT1	1	-	R
IE	_	-	N	N	N	N	-	S
IR	WT8	3	N	N	N	N	R	S
IR	WT8	3	N	N	W	1	R	R
IR	WT4,5,7,8	N	N	N	N	N	R	S
IR	WT7	2B	N	N	N	N	R	S
IR	N	N	N	N	WT1	1	S	R
IR	WT8	3	N	N	N	N	R	S
IR	WT2.3	N	WT1	N	N	N	R	R
IRE	WT8	3	N	N	N	N	R	S
IRE	WT3.4	1	N	N	WT1	1	R	R
IRE	WT8	3	N	N	N	N	R	S
IRE	WT8	N	WT1	1	N	N	R	R
SIR	WT3, 4		N	N	N	N	R	S
SIRE	WT3, 4		1	N	N	N	R	S
SIRE	WT8	3	N	N	N	N	R	S
SIRE	WT8	N	WT1	1	N	N	R	R
IRE	WT8	N	WT1	1	N	N	R	S
SIR	WT3, 4		N	N	N	N	R	S
SIRE	WT3, 4		1	N	N	N	R	S
SIRE	WT8	3	N	N	N	N	R	S
SIRE	WT8	N	WT1	1	N	N	R	R

MWT = Missing wild type, MUT = mutation, N = none, R = resistant, S = susceptible, R-IRE = resistant to isoniazid, rifampicin and Ethambutol, R-SIRE = resistant to Streptomycin, isoniazid, rifampicin and ethambutol

Table 5. Results of MTBDR*plus* for isolates that showed resistance to INH and RIF using BACTEC 460 assay

4. Discussions

One of the major objectives of this study was to evaluate the tuberculin skin test (TST), the method currently being used in Trinidad & Tobago, with that of the Quantiferon TB-Gold Test (a new IFN-γ based test that has now been introduced in the market for LTBI detection) to determine the cost and efficiency of these methods in detecting latent TB infection (LTBI). Unlike the TST and IFN-γ analysis, most diagnostic assays for detecting *M. tuberculosis* infection are based on either isolation or identification of the bacteria, which makes them inapplicable for diagnosis of latent infection. The development of IFN-γ tests to detect T-cells specific for *M. tuberculosis* antigens addressed this important issue. The current study was carried out among individuals from various groups with a high risk of developing TB due to either exposure to or contact with TB patients, lack of isolation facilities, or weak infection control. In the current study, the two selected testing methods (QFT-G and TST) detected LTBI among the various target groups at different rates.

Among the TB patient [control] group, the rate of TB detection by the QFT-G assay (70.2%) was significantly different from that of the TST. This rate of detection was similar to that observed by Lee *et al*., who reported a sensitivity of 70% among 87 patients diagnosed with TB **[12],** and higher than both the 64.4% rate of detection observed by Kobashi *et al*. in Japan **[13]** and the rate observed by Dewan *et al*., who reported a sensitivity of 60% in culture-confirmed cases **[14].** However, the 70.2% rate was lower than that reported by both Kang *et al*. (81% sensitivity in 54 patients) and Mori *et al*. (89% sensitivity among 118 patients) **[15, 16].** More recently, Kobashi *et al*. demonstrated significant differences in the quantitative responses of IFN-γ to *M. tuberculosis* between patients with active TB disease and those with LTBI **[13].**

Combining the results for the QFT-G assay and the TST in the current study increased the overall sensitivity for detection of LBTI among the culture-confirmed TB-infected control group. This confirms and reinforces recommendations that negative results should not be used alone to exclude active TB but should be interpreted in conjunction with other clinical and diagnostic findings **[17].** It also underscores the fact that the QFT-G assay has a limited role in the evaluation of patients with culture-confirmed TB. The authors of the current study agree with Kobashi *et al*.'s conclusion that it would be difficult to use the QFT-G assay to completely discriminate active TB disease from LTBI **[13].**

In the current study, 43.3% of all HIV patients included in the analysis had a positive result for the QFT-G assay. This was in huge contrast to the earlier study by Kobashi *et al***. [13],** in which all HIV patients produced QFT-G–positive results. The indeterminate or nonreactive results observed in some of the HIV-positive subjects in the current study also contrast with those found by Ferrara *et al*. **[17].** In the current study, the QFT-G assays were run several times to minimize the effect of laboratory and procedural errors. However, the indeterminate and nonreactive results persisted, with test results continuing to produce low mitogen levels. Although all indeterminate or nonreactive results were excluded from the final analysis, the QFT-G assay results should be interpreted with caution, bearing in mind the high prevalence of HIV in Trinidad & Tobago and the Caribbean region. Several possible explanations for a high rate of indeterminate and nonreactive results have been adduced

and these include the presence of lymphocytopenia and/or inflammatory and immunosuppressive conditions, as well as hypoalbuminemia, which suggests poor nutritional status **[13],** and there is a high probability that some of these conditions could have existed among the subjects of the current study. Lymphocytopenia (especially the CD4 strain) has been shown to depend on the elaboration of inflammatory cytokines by T-cells previously sensitized to *M. tuberculosis*–specific antigens in QFT-G assays. In the blood, mononuclear cells from peripheral blood are stimulated in vitro, and the production of IFN-γ from sensitized T-lymphocytes by *M. tuberculosis*–specific antigen is measured by ELISA in the QFT-G **[18,19].**

In the current study, however, only 58% of TST-positive subjects had a positive QFT-G result. More than half of this group consisted of prison inmates with a documented TST > 10 mm. In Trinidad & Tobago, TST is likely to be a very good indicator of latent infection in recently exposed individuals because of the following reasons (a) most individuals under the age of 20 years did not receive the BCG vaccination, which was discontinued during the early 1990s, and (b) BCG vaccination has been observed to significantly increase the likelihood of a positive TST in subjects without LTBI.

Multiple outbreaks of TB, including those involving the multi-drug-resistant strain (MDR-TB), have been reported in prisons and jails, especially among HIV-infected inmates, a population regarded as having moderate risk of acquiring TB **[20].** The results of the current study from this moderate-risk population show that prevalence of LTBI was 24.2% and 56.5% based on the TST and the QFT-G assay respectively. These values were quite high compared to those observed in correctional facilities in the United States, where prevalence was less than 10%. However, the QFT-G values obtained in the current study were in line with the current rate of TB in Trinidad & Tobago, which is estimated to be about 17 per 100 000 population **[21].** It has been suggested that annual TB screening of prison inmates using the TST may account for the increase in the number of TST-positive results, due to the "boosting" effect caused by repeat use of the test. However, this type of screening is not carried out among prison inmates in Trinidad & Tobago. Therefore, the high rate of TST-positive results in the current study could be attributed mainly to exposure to the disease.

The prevalence of LTBI among health care workers using the TST in the current study was a mere 7.5%. This value was very low compared to those reported by studies in Portugal (33%) and Germany (10%)**[22, 23].** The low value found in the current study may have been due to a smaller sample size and the use of a higher positive cutoff. Like the studies in Portugal and Germany, the current study showed that the QFT-G assay was more useful than the TST in identifying LTBI among health care workers. As this target group may be exposed to TB more frequently than the local population, screening of staff exposed to the disease is frequently recommended to identify infected individuals and treat them adequately and promptly. Because the QFT-G assay was more sensitive than the TST in detecting LTBI, the authors of the current study strongly support its use in screening health care workers in Trinidad & Tobago.

The TST may also be less desirable due to complications in interpreting its results caused by the above-mentioned boosting effect (from repeat testing) as well as conversions and reversions (changes in results from negative at baseline to positive and vice versa,

respectively). In a study on health care workers in India, Pai *et al*. suggested that individuals with recent exposure to TB usually presented with large increases (≥ 10 mm) in TST indurations that were always accompanied by substantial increases in IFN-γ **[24].** This finding was in line with the results of the current study.

The QFT-G assay also fared better than the TST in terms of TAT. In terms of cost, however, the TST appears best suited for the resource-strapped environment of Trinidad & Tobago (if the calculation of this variable is based mainly on the cost of the materials required to perform the test versus the cost of labor and other inputs). This argument is partly supported by Pooran *et al*., who concluded in a recent report that screening for LTBI using TST alone was the most cost-effective testing strategy but ultimately incurred the highest cost due to test inaccuracies **[25].** Another factor that may make the TST less cost-effective over time is high replacement costs, since the Mantoux test solution is often not accessible in developing countries and would have to be replaced with the relatively labor-intensive IFN-γ release assay. Minimizing cost for TB testing has become increasingly important because prevalence of the disease has fallen dramatically in developed countries and more than 90% of all cases worldwide occur in resource-strapped developing countries **[26].** However, as pointed out by both Diel *et al*. and Marra *et al*., use of the IFN-γ release assay alone or in combination with the TST for screening close TB contacts prior to LTBI treatment is highly cost-effective in reducing the TB disease burden **[27, 28, 29].**

4.1 Profiles of drug susceptibility patterns of *Mycobacterium tuberculosis* isolates encountered in the Caribbean

Drug resistance using the Hain Lifescience MTBDR*plus* line probe assay revealed that this method performed very well for the detection of RIF resistance isolates in the region. This is in agreement with the high sensitivity reported elsewhere [**30, 31, 32**]. However, the results for detection of resistance to INH were much lower using this assay. This observation is not unique since the molecular mechanisms for INH resistance are not fully understood and about 25-30% of phenotypic INH-resistance associated mutations are still unaccounted for **[32]**.

This study revealed that the codon most frequently involved in the mutation was the S-531L of the *rpo*B gene among the RIF resistant isolates. Similar result has been reported by Cavusoglu *et al*., 2007 **[34]**, but Barnard *et al*., 2008 **[35]**, reported that most mutations in the isolates tested in their study occurred at several other codons. Also, a high proportion of the mutational changes were detected in the S-315T1 codon of the *kat*G gene for the INH and RIF monoresistant isolates in this study in contrast to regions reported elsewhere **[35]**. This was a trend reported in a high burden setting that seem to be a different trend among the *M. tuberculosis* isolates seen here in the Caribbean region.

The MTBDR*plus* genotype assay allowed for the rapid and specific detection of most mutations conferring resistance to RIF and to a lesser extent INH. Collective observations have indicated that mutations to the *rpo*B gene may account for the greater than 96% of the resistance to RIF **[30,35]**. This present study indicated that this is also true for Caribbean TB isolates that showed an overall resistance detection of 95.8% with the MTBDR*plus* assay.

Detection of INH resistance using the MTBDR*plus* assay for the *kat*G and *inh*A gene was disappointing. In this study, only 34.6% resistance to INH was detected using the MTBDR*plus* assay which is less than that reported by Johnson *et al*., 2008 **[36]**. Other studies showed detection of 60-90% within the *kat*G gene and 15-43% within the *inh*A gene **[32, 31]**. Nonetheless, it must be kept in mind that the isolates used in this study were screened using the single drug concentration of 0.1µg/ml of INH in the BACTEC 460 TB system (which detects low levels of resistance to INH at this concentration, mainly useful for therapeutic purposes). The study did not discriminate between strains with low levels of INH resistance with those harboring a high level of resistance, a fact that may indirectly explain the poor agreement between the number of INH-resistant isolates detected using the gold-standard BACTEC 460 TB as compared to the MTBDR*plus* (26 instead of 9). In fact, it has been shown that the MTBDR*plus* assay was unable to detect low levels of INH resistance that was commonly detected using the BACTEC 460 TB system **[32]**.

Previous reports have confirmed that high INH concentration levels of more than 0.4µg/ml can be detected in the *kat*G genes among *M. tuberculosis* isolates that are resistant to the drug **[32].** This detection of low INH resistance among the *M. tuberculosis* isolates seen in this study could perhaps be because of the low concentration of the INH drug used. In addition, the MTBDR*plus* assay only detects those resistances of *M. tuberculosis* that have their origins in the *rpo*B, *kat*G and *inh*A regions (MTBDR*plus* kit insert). Since resistance originating from mutations of other genes or gene regions as well as other RIF and INH mechanisms are not detected by the MTBDR*plus,* it could be that other mechanisms of resistance possessed by the isolates from the Caribbean were not detected. This will definitely require further studies since such was outside the scope of this study.

With the MTBDR*plus* assay, clinical specimens that are AFB positive with moderate to many AFBs, can be reliably tested for drug resistance. Furthermore, the genotypic DST method was able to detect drug resistance in samples that were contaminated as well as in those that had lost viability, circumventing the need to request follow-up sputum thus decreasing the time between specimen collection, results and treatment of the patient. This study also showed that less time was spent using the MTBDR*plus* in detecting INH and RIF resistance in TB isolates in the Caribbean. Using the BACTEC 460 TB system, the mean time for reporting results showing any drug-resistance was 32 days for cultures and 40 days for clinical specimens. This time represented repeating all drug-sensitivities for specimens showing any resistance. For specimens that were sensitive to anti-TB drugs, the mean time was 21 days for clinical specimens and 14 days for isolates.

Determination of drug resistance is difficult due to technical reasons and in several cases; these results are not always accurate **[37].** In addition, it can take up to 6 weeks to get a phenotypic DST result and during this time many transmission events may take place. Therefore, alternative methods need to be evaluated to improve the speed of diagnosis especially drug resistant TB and this is what was achieved using the MTBDR*plus* assay in this study. With the BACTEC 460 system, culture material was not able to give results for non viable or contaminated materials; however in this study the MTBDR*plus* system gave identifiable results. This is in agreement with what has been reported as these tests are able

to perform on specimens that contain non viable bacilli or from specimen that were contaminated by other bacteria and fungi **[32, 35, 36]**.

Although MTBDR*plus* assay has limitations as with any DNA-based screening nucleic acid sequence, it is possible that mutations that do not cause an amino acid exchange (silent mutations) will still produce the absence of one of the wild type probes. In addition, this assay only detects those resistance of the *M. tuberculosis* that have origins in the *rpo*B, *kat*G and *inh*A regions, yet the high sensitivity of RIF resistance detection is a plus point since this test can be used for detecting RIF resistance, a surrogate marker for multiple drug resistance in *M. tuberculosis* isolates. This assay is an excellent test to use on selected clinical samples because the amount of time required in generating a result was within 24 hours after receipt of specimen, culturing of the specimen is not required and contaminated as well as non-viable cultures can be used. Finally, the test method was also cheaper to use in resource-poor countries like in the Caribbean region.

Despite the global expansion in coverage of drug-resistance surveillance, data on drug resistance are still unavailable for more than 100 countries throughout the world **[38].** Even in the Caribbean there is a paucity of information or data on the anti TB susceptibility pattern. This study was very important as it provided data on drug resistance that was lacking for most of the countries in the Caribbean. The level of drug resistance observed in most of the countries in this study was quite low with the exception of Guyana. Although drug resistance has been reported from several countries in the Caribbean, data reported to WHO on drug resistance is lacking as only Trinidad and Tobago reported 1 case of MDR-TB to the WHO in 2006 **[39].**

In this study, resistance to anti-TB medications was seen in seven countries, five of which had >5 cases of TB/100,000 population and with one country (Guyana) accounting for 85% of the MDR-TB strains seen. This data confirms the continued existence of drug resistance in Guyana, as an earlier report by Menner *et al*., 2005 **[40]** also reported a high frequency of drug resistance in this country with 22.2% of the isolates tested showing resistance to at least one anti-tuberculosis drug and 11.1% showing resistance to INH and RIF **[40]**. The reason for the continued persistence of MDR-TB in Guyana according to Menner *et al* is the lack of human resources to adequately follow up and monitor patient treatment as well as poor management of the tuberculosis control programme **[40]**. In this present study similar results were seen as 20.5% of isolates tested showed resistance to at least one anti-TB drug and 14.5% showed resistance to INH and RIF (MDR-TB).

Unlike previous studies from Africa, Haiti and Guyana that showed high levels of drug-resistance with high TB/HIV co-infection rates **[40, 41, 42, 43, 44]**, the moderate TB incidence seen in the rest of the Caribbean was not accompanied by any substantial level of drug-resistance. For example, there was no case of drug resistance among TB isolates from Trinidad and Tobago. This was very surprising especially with the high levels of TB/HIV co-infection (30.6% of the TB positive cases) and the high defaulter rate (22.7% of the TB positive cases). As reported in the literature, drug resistance was commonly seen in other countries where there was inadequate chemotherapy and also where HIV co-infections was present **[45, 46];** but this is in contrast to Trinidad & Tobago where despite the high prevalence of HIV co-infection with TB, drug resistant cases were almost non- existent.

The absence of drug resistance in Trinidad and Tobago may be attributed to the excellent care and treatment TB programme such as direct observed treatment (DOT), adequate provision and supply of TB drugs that exists in the country. Additionally, all patients with tuberculosis are admitted and managed at the Caura Chest hospital until they become non-infectious, after which they are monitored on a regular basis by public health officials from the TB programme.

In Suriname drug resistance was rarely seen and when it occurred, only mono-resistance was seen. Mono-resistance was also recorded for isolates from Jamaica and The Bahamas, two countries where HIV infection is also relatively high and where low levels of resistance are seen. As in Trinidad and Tobago, the TB programmes in these countries are well managed and there is a very good collaboration between the TB and the HIV programmes.

A review of the literature for drug resistance in other parts of the Caribbean showed that similar low levels of drug resistance have been seen in the French Caribbean Islands of Guadeloupe and Martinique where there is significant migrant population from Haiti, an area of high drug resistance. The incidence of monoresistance in the French Caribbean Islands was 12.9%, however the incidence of MDR-TB was much lower with a rate of 0.9% **[47]**.

Limitations of the study: An inherent limitation designed to compare cost and turnround time against an imperfect conventional test such as TST is that no gold standard has been established for resolution of discordant results. The MTBDR*plus* assay has the limitation that as a DNA assay based procedure that screens for nucleic acid sequence and not amino acid sequence, it is possible that mutations that do not cause amino acid exchange (silent mutations) will result in the absence of one of the wild types probes. Besides the assay detects only resistance that originate in the *rpo*B, *kat*G and *inh*A regions of the *Mycobacterium tuberculosis*. Hence other regions where resistance occurs will completely be missed.

The lack of adequate facilities for manipulation of solid and liquid cultures of *M. tuberculosis* is a major challenge in the Caribbean. Because of this drug susceptibility testing information for proper management of patients infected with TB as well as identification of species is limited.

5. Conclusions

Despite the several constraints and limitations, this study demonstrated that the QFT-G test was more effective and a quicker turnaround time was achieved over the TST in detecting LTBI among several target groups in the population studied. However, because the QFT-G appears more costly as well as showing indeterminate and non reactive response for immuno-compromised subjects such as HIV positive patients, care must be taken when screening or making a diagnosis of LTBI based on QFT-G results in a poor resource and high HIV prevalence setting like Trinidad & Tobago or any other Caribbean country.

The turnaround time for results for line probe assays is also a major asset. Additionally, the identification of organisms and DST can be performed on contaminated as well as non-

viable specimens. Finally, the cost of this assay makes it ideal for use as it is less than 2 times that of traditional culture methods.

Although the Quantiferon Gold TB test results were comparable in many aspects with other published international studies the use of this test for the Caribbean may still be limited due to the cost involved when compared to the Tuberculin Skin Test. This is so because of the cost of the kit, transportation issues and the laboratory component of the test.

6. Recommendations

The authors therefore support the recommendation that Quantiferon Gold TB test be used in conjunction with the well established TST for the screening of patients suspected of infection. Confirmation of positive TST can then be performed using the QFT-G test if warranted. In cases where the patient is co-infected with HIV, the interpretation of the test should be made in collaboration with the CD4+ count of the patient as this test is dependent on the reaction of T-cells and if the CD4 count is low then the result can be falsely negative.

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8. References

[1] New Jersey Medical School (NJMS), National Tuberculosis Centre [Online]. Brief History of Tuberculosis, July 23, 1996 [*Assessed online March 27, 2009* from: http://www.umdnj.edu-ntbcweb/history.html].

[2] World Health Organization. TB/HIV Research priorities in resource-limited setting: Report of an expert consultation, 2005. WHO/HTM/TB/2005.355.

[3] Health Agenda for the Americas 2008-2017. Text document distributed at the launching ceremony in Panama City, 3 June, 2007.

[4] Getahun, H., Harrington, M., O'Brien, R., Nunn, P. (2007). Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. Available at www.thelancet.com. Published online on February 28, 2007. DOI:10.1016/S0140-6736(07)60284-0.

[5] Hale YM, Pfyffer GE and Salfinger M, 2001. Laboratory diagnosis of mycobacterial infections: new tools and lessons learned. Clin. Infect. Dis. 33:834-846.

[6] Akpaka PE; Baboolal S, Clarke D, Francis L, Rastogi N. (2008) Evaluation of methods for rapid detection of resistance to isoniazid and rifampicin in Mycobacterium tuberculosis isolates collected in the Caribbean. *Journal of Clinical Microbiology; 46 (10):3426-3428*

[7] Baboolal S, Ramoutar D, Akpaka PE. (2010). Comparison of QuantiFERON®-TB Gold assay and Tuberculin skin test to detect latent tuberculosis infection among among target groups in Trinidad & Tobago. *Pan American Journal of Public Health/ Rev Panam Salud Publica; 28(1):36-42*

[8] Mathew, P., Kuo, Y.H., Vazirani, B., Eng, R.H.K and Weinstein, M.P. (2000). Are Three Sputum Acid Fast Bacillus Smears Necessary for Discontinuing Tuberculosis Isolation? J Clin Microbiol 40(9) 3482-3484.

[9] Herman, P., Fauville-Dufaux, M., Breyer, D., van Vaerebergh, B., Pauwels, K., Dai Do Thi, Chuong., Sneyers, M., Wanlin, M., Snacken, R and Moens, W. (2006). Biosafety recommendations for the contained use of *Mycobacterium tuberculosis* complex isolates in industrialized countries. Royal Library of Belgium Deposit Number: D/2006/2505/22.175. Supply, P., Lesjean, S., Savine, E., Kremer, K., van Soolingen, D., Locht, C. (2001). Automated high-throughput genotyping genotyping for study of global epidemiology of *Mycobacterium tuberculosis* based on mycobacterial interspersed repetitive units. J. Clin. Microbiol. 39. 3563-3571.

[10] Centers for Diseases Control and Prevention. Epi Info 3.5.1 version software. Atlanta Georgia, USA

[11] Dean, A.G., Sullivan, K.M., Soe, M.M. OpenEpi. Open Source Epidemiologic Statistics for Public Health version 2.3. www.OpenEpi.com. Updated 2009/20/05

[12] Lee, J. Y., Choi, H. J., Park, I-N., Hong, S-B., Oh, Y-M., Lim, C-M., Lee, S. D., Koh, Y., Kim, W. S., Kim, D. S., Kim, W. D., Shim, T. S. (2006). Comparison of two commercial interferon-γ assays for diagnosing *Mycobacterium tuberculosis* infection. Eur Respir J; 28:24-30.

[13] Kobashi, Y., Mouri, K., Obase, Y., Fukuda, M., Miyashita, N., Oka, M. (2007). Clinical evaluation of QuantiFERON TB-2G test for immunocompromised patients. Eur Respir J; 30:945-950.

[14] Dewan, P. K., Grinsdale, J., Kawamura, L. M. (2007). Low Sensitivity of a Whole-Blood Interferon-γ Release Assay for Detection of Active Tuberculosis. Clin Infect Dis; 44:69-73.

[15] Kang, Y. A., Lee., H. W., Yoon, H. I., Cho, B. L., Han, S. K., Shim, Y-S., Yim, J-J. (2005). Discrepancy Between the Tuberculin Skin Test and the Whole-Blood Interferon γ Assay for the Diagnosis of Latent Tuberculosis Infection in an Intermediate Tuberculosis-Burden Country. JAMA; 293:2756-2760.

[16] Mori, T., Sakatani, M., Yamagishi, F. et al. (2004). Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. Am J Resp Crit Care Med; 170:59-64.

[17] Ferrara, G., Losi, M., Meacci, M., Meccugni, B., Piro, R., Roversi, P., Bergamini, B. M., D'Amico, R., Marchegiano, P., Rumpianesi, F., Fabbri, L. M., Richeldi, L. (2005). Routine Hospital Use of a New Commercial Whole Blood Interferon-γ Assay for the Diagnosis of Tuberculosis Infection. Am J Respir Crit Care Med. 172:631-635.

[18] Stuck, A. E., Minder, C. E., Frey, F. J. (1989). Risk of infection complication in patients taking glucocorticosteroids. Rev Infect Dis; 11: 954–963.

[19] Andersen, P., Munk, M. E., Pollock, J. M., Doherty, T. M. (2000). Specific immune based diagnosis of tuberculosis. Lancet; 256:1099–1104.

[20] Porsa, E., Cheng, L., Seale, M. M., Delclos, G. L,. Ma, X., Reich, R., Musser, J. M., Graviss, E. A. (2006). Comparison of a New ESAT-6/CFP-10 Peptide-Based Gamma Interferon Assay and a Tuberculin Skin Test for Tuberculosis Screening in a Moderate-Risk Population. Clin Vaccine Immunol; 13:53-58.

[21] Francis, M. and Rattan, A. (2006). Tuberculosis in CAREC Member Countries. CAREC Surveillance Report, Vol 26; No. 3

[22] Torres Costa J, Sá R, Cardoso MJ, Silva R, Ferreira J, Ribeiro C, et al. Tuberculosis screening in Portuguese healthcare workers using the tuberculin skin test and the interferon-gamma release assay. Eur Respir J. 2009;34(6):1423–8.

[23] Schablon A, Harling M, Diel R, Nienhaus A. Risk of latent TB infection in individuals employed in the healthcare sector in Germany: a multicentre prevalence study. BMC Infect Dis. 2010;10(1):107.

[24] Pai, M., Kaustubh, G., Joshi, R., Dogra, S., Kalantri, S., Mendiratta, D.K., Narang, P., Daley, C.L., Granich, R.M., Mazurek, G.H., Reingold, A.L., Colford, J.M. (2005). *Mycobacterium tuberculosis* Infection in Health Care Workers in Rural India. Comparison of a Whole-Blood Interferon γ Assay with Tuberculin Skin Testing. JAMA; 293 (22):2746-2754.

[25] Pooran A, Booth H, Miller RF, Scott G, Badri M, Huggett JF, et al. Different screening strategies (single or dual) for the diagnosis of suspected latent tuberculosis: a cost effectiveness analysis. BMC Pulmo Med. 2010;10(1):7.

[26] Mathema, B., Kurepina, N.E., Bifani, J. and Kreiswirth, B.N. (2006). Molecular Epidemiology of Tuberculosis: Current Insights. Clin Microbiol Rev; 19(4):658-685.

[27] Diel, R., Wrighton-Smith, P. and Zellweger, J-P. (2007). Cost-effectiveness of interferon-c release assay testing for the treatment of latent tuberculosis. Eur Respir J; 30: 321-332.

[28] Marra F, Marra CA, Sadatsafavi M, Morán-Mendoza O, Cook V, Elwood RK, et al. Cost-effectiveness of a new interferon-based blood assay, QuantiFERON-TB Gold, in screening tuberculosis contacts. Int J Tuberc Lung Dis. 2008;12(12):1414–24.

[29] Pai, M., Riley, L.W., Colford, J.M. Jr. (2004). Interferon-gamma assays in the immune diagnosis of tuberculosis: a systematic review. Lancet Infect Dis; 4: 761–776.

[30] Hilleman, D., Rush-Gerdes, S., Richter, E. (2007). Evaluation of the GenoType MTBDRplus Assay for Rifampin and Isoniazid Susceptibility Testing for *Mycobacterium tuberculosis* strains and Clinical Specimens. J Clin Microbiol 45(8): 2635-2640.

[31] Somoskovi, A., Dormandy, J., Mitsani, D., Rivenburg, J., Salfinger, M. (2006). Use of smear-positive samples to assess the PCR-based Genotype MTBDR assay for rapid, direct detection of the *Mycobacterium tuberculosis* Complex as well as its resistance to Isoniazid and Rifampicin. J Clin Microbiol 44(12):4459-4463.

[32] Bang, D., Anderson, A.B., Thomsen, V.Q. (2006). Rapid Genotypic detection of Rifampin- and Isoniazid- resistant Mycobacterium tuberculosis directly in clinical specimens. J Clin Microbiol 44(7):2605-2608.

[33] Wade., M.M., Zhang, Y., (2004). Mechanisms of drug resistance in Mycobacterium tuberculosis. Front Biosci 9975-994.

[34] Cavusoglu, C., Turhan, K., Akinci, P., Soyler, I. (2007). Evaluation of the Genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in Mycobacterium tuberculosis isolates. J Clin Microbiol 44; 2338-2342.

[35] Barnard, M., Albert, H., Coetzee, G., O Brien, R., Bosman, M.E. (2008). Rapid Molecular screening for multidrug-resistant tuberculosis in a high-volume health laboratory in South Africa. Am J Respir Crit Care Med 177:787-792.

[36] Johnson, R., Jordaan, A.M., Warren, R., Bosman, M., Young, D., Nagy, J.N., Wain, J.R., van Helden, P.D., Victor, T.C. (2008). Drug susceptibility testing using molecular techniques can enhance tuberculosis diagnosis. J Inf dev Countries 240-45.

[37] Parsons, L.M., Salfinger, M., Clobridge, A., Dormandy, J., Mirabello, L., Polletta, V.L., Sanic, A., Sinyavskiy, O., Larsen, S.C., Driscole, J., Zickas, G., Taber, H.W. (2005). Phenotypic and molecular characterization of Mycobacterium tuberculosis isolates resistant to both Isoniazid and Ethambutol. JCM 49(6) 2218-2225.

[38] Zignol, M., Hosseini, M.S., Wright, A., Weezenbeek, C.L., Nunn, P., Watt, C.J., Williams, B.G. and Dye, C. (2006). Global Incidence of Multidrug-resistant tuberculosis. J. Infect Dis. 194:479-85.

[39] World Health Organization. Global Tuberculosis Control, Surveillance, Planning and Financing. WHO Report, 2008. WHO/HTM/TB/2008.393

[40] Menner, N., Gunther, I., Orawa, H., Roth, A., Rambajan, I., Wagner, J., Hahn, H., Persaud, S., Ignatius, R. (2005). High frequency of multidrug-resistance Mycobactium tuberculosis isolates in Georgetown, Guyana. Tropical Medicine and International Health 10 (12) 1215-1218.

[41] Diguimbaye, C., Hilty, M., Ngandolo, R., Mahamat, H.H., Pfyffer, G.E., Baggi, F., Tanner, m., Schelling, E, Zinsstag. J. (2006). Molecular characterization and drug resistance testing of *Mycobacterium tuberculosis* isolates from Chad. J Clin Microbiol 44:1575-1577.186. Ferdinand, S., Sola, C., Verdol, B., Legrand, E.Goh, K.S., Berchel, M., Aubery, A., Timothee, M., Joseph, P., Pape, J.W., Rastogi, N. (2003). Molecular characterization and drug resistance patterns of strains of *Mycobacterium tuberculosis* isolated from patients in an AIDS counselling center in Port-au-Prince, Haiti: a 1-year study. J Clin Microbiol 41(2):694-702.

[42] UNAIDS/WHO/ AIDS Epidemic Update (2009). www.unaids.org/en/knowledgeCentre/HIVData/EpiUpdate/EpiUpdArchive/2009. [downloaded 18/02/11]

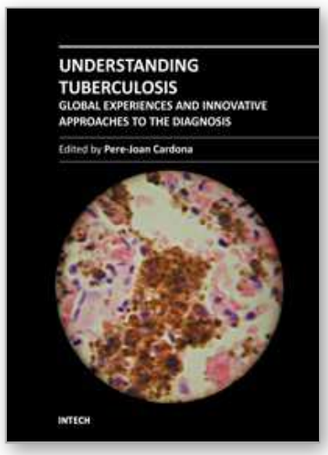
[43] Pratt, R., Robinson, V., Navin, T. (2009). Trends in Tuberculosis – United States, 2008. JAMA 301 (18): 1869-1871.

[44] Streicher, E.M., Warren, R.M., Kewlwy, C., Simpson, J., Rastogi, N., Sola, C., van der Spuy, G.D., van Helden, P.D., Victor, T.C., 2004. Genotypic and phenotypic characterization of drug resistant *Mycobacterium tuberculosis* isolates from rural districts of Western Cape Province of South Africa. J Clin Microbiol. 42, 891-894.

[45] Zagar, E. M., Mc Nerney, R., 2008. Multidrug-resistant tuberculosis. BMC Infectious Diseases 8:10 doi:10.1186/47 1-2334-8-10.

[46] Khue, P.M., Phue, T.Q., Hung, N.V., Jarlier, V., Robert, J. (2008). Drug resistance and HIV co-infection among pulmonary tuberculosis patients in Haiphong City, Vietnam. Int J Tuberc Lung Dis 12(7): 763-768.

[47] Brudey, K., Driscoll, J.R., Rigouts, L., Prodinger, W.M., Gori, A., Al-Hajoj, S.A., Allix, C., Aristimuno, L., Arora, J., Baumanis, V.,et al (2006). *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. BMC Microbiol. 6:6-23.



Understanding Tuberculosis - Global Experiences and Innovative Approaches to the Diagnosis

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Mycobacterium tuberculosis is a disease that is transmitted through aerosol. This is the reason why it is estimated that a third of humankind is already infected by Mycobacterium tuberculosis. The vast majority of the infected do not know about their status. Mycobacterium tuberculosis is a silent pathogen, causing no symptomatology at all during the infection. In addition, infected people cannot cause further infections. Unfortunately, an estimated 10 per cent of the infected population has the probability to develop the disease, making it very difficult to eradicate. Once in this stage, the bacilli can be transmitted to other persons and the development of clinical symptoms is very progressive. Therefore the diagnosis, especially the discrimination between infection and disease, is a real challenge. In this book, we present the experience of worldwide specialists on the diagnosis, along with its lights and shadows.

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