EVALUATION OF TB-ST TUBERCULOSIS RAPID TEST IN ADULT PULMONARY TUBERCULOSIS IN RWANDA

By MURAMIRA M. Nobert

Supervisor: Dr OLAF MÜLLER
Co-Supervisor: Dr MUSEMAKWERI André

Huye, October 2007
DEDICATION

From my heart of hearts I dedicate this peace of work to the Most HOLY TRINITY;

To the Most Virgin Mary;
To all the angels and Saints;

My dear Family; Parents, Sisters, Brothers, Cousins, In-laws;

Uncle’s Family;

To “les Petits Frères de Jesus” and associates;

To all Tuberculosis patients.
ACKNOWLEDGEMENT

In the first place, I thank the almighty good Lord for what I 'am and for this great achievement.

I thank the management of Lionex Diagnostics and Therapeutics GmbH and Diavita through Dr Olaf Müller for the TB-ST test kits used in this evaluation.

The Ministry of health; PNIILT in particular for funding this evaluation.

Faculty of Medicine and The National University of Rwanda in general for all the training to make me what I’ am.

I thank the Supervisors of this thesis; Dr Olaf Müller and Dr Musemakweri André for the fabulous work done, a vote of thanks also to Dr. Tugirimana Pierrot for the help rendered.

To my family, my Uncle Justus’s Family, for having made me what I’ am, for having stood by me in thin and thick throughout my life and for the unbeatable multi-pronged support rendered during my education career.

My sincere thanks go to Mbaraga, Edwin, Havyarimana and Semwaga for the incomparable contribution to make this evaluation a success, I really appreciate and there is no way to thank you except to ask God to reward abundantly.

“Les Petits Frères des Jesus”, you know how much you have done to make this work a success; I really thank you more than words can say.

The families of Mzee Kalimba, Mr Balyinyonza Hannington, Mr Maringi and Gen. Levi for the great contribution in my career, I have no words to use to thank you.

To all my dear classmates.

The staff of the departments of internal Medicine CHUB and CHK-CHUK and all those who have contributed to the success of this peace of work.
THE HIPPOCRATIC OATH

I promise that my medical knowledge will be used to benefit people’s health.
Patients are my first concern. I will listen to them, and provide the best care I can.
I will be honest, respectful, and compassionate towards patients.
I will do my best to help anyone in medical need, in emergencies. I will make every effort to ensure that the rights of all patients are respected, including vulnerable groups who lack the means of making their needs known.
I will exercise my professional judgment as independently as possible, uninfluenced by political pressure or by social standing of my patient. I will not put personal profit or advancement above my duty to my patient.
I recognize social value of human life, but I also know the prolongation of human life is not the only aim of health care. If I agree to perform abortion, I agree that it should take place only within an ethical and legal framework.
I will not provide treatments which are pointless or harmful, or which an informed or competent patient refuses. I will help patients find the information and support they want to make decisions on their care. I will answer truthfully as I can, and respect patients’ decisions, unless that puts others at risk of substantial harm. If I can not agree with their requests, I will explain why.
If my patients have limited mental awareness, I will still encourage them to participate in decisions as much as they feel able. I will do my best to maintain confidentiality about all patients.
If there are overriding reasons, which prevent my keeping a patient’s confidentiality, I will explain them. I will recognize the limits of my knowledge and seek advice from colleagues when needed. I will acknowledge my mistakes.
I will do my best to keep myself and colleagues informed of new developments and ensure that poor standards or bad practices are exposed to those who can improve them.
I will show respect for all those with whom I work and be ready to share my knowledge by teaching others what I know. I will use my training and professional standing to improve the community in which I work.
I will treat patients equitably and support a fair and human distribution of health resources. I will try to influence positively authorities whose policies harm public health.
I will oppose policies, which breach internationally accepted standards of human rights. I will strive to change laws, which are contrary to patients’ interests or to my professional ethics.
While I continue to keep this oath inviolate, may it be granted to me to enjoy life and practice of the Art, respected by all, in all times.
LIST OF ABBREVIATIONS

TB-ST: Tuberkulose Schenlltest = Tuberculosis Rapid Test.
TB: Tuberculosis
BCG: Bacilli Calmette Guerrin
PPD: Purified protein derivative
CHUB: Centre hospitalier universitaire- Butare
CHK-CHUK: Centre hospitalier Universitaire –Kigali.
IGRA: A whole-blood interferon-γ release assay.
ELISPOT: An enzyme-linked immunospot assay
PPD-S: Standardized purified protein derivative.
BCG: Bacilli Calmette-Guerin.
WHO: World health Organization.
IUATLD: The International Union against Tuberculosis and Lung Disease
HIV: Human Immuno-surpresion Virus.
PNILT: Programme national intégré de lutte contre la lèpre et la Tuberculose.
PCR: Polymerase Chain Reaction
M. Tuberculosis: Mycobacterium tuberculosis
Ig: Immunoglobulin
% Percentage
USA: United States of America.
ICT: ImmunoChromaTography
AIDS: Acquired Immunodeficiency Syndrome
AFB: Acid-Fast Bacilli
Background:
Rapid and accurate diagnosis of Tuberculosis (TB) is crucial to facilitate early treatment of infectious cases and thus to reduce its spread. Tests for the diagnosis of pulmonary tuberculosis vary in sensitivity, specificity, speed, and cost. Smear-negative pulmonary tuberculosis is a diagnostic challenge in resource-limited settings. To improve the diagnosis of TB, more rapid diagnostic techniques are being developed such as the TB-ST tuberculosis rapid test. The study was designed to evaluate the sensitivity and specificity of TB-ST tuberculosis rapid test.

Design and Setting: It was a prospective study conducted in the Departments of Internal Medicine, University Teaching Hospital, Butare (CHUB) and Kigali Central Hospital (CHK-CHUK) between November 2006 and August 2007.

Methods and Patients:
Sera from 150 patients with active pulmonary tuberculosis (108 smear-positive and 42 smear-negative cases) and 100 controls who did not present with any signs symptoms nor a past history of TB were tested with TB-ST Tuberculosis rapid test.

Results:
The specificity, sensitivity (smear-positive) and sensitivity (smear negative) of TB-ST Tuberculosis rapid test for pulmonary TB were 96%, 88.9%, and 42.9% respectively. The positive and negative predictive values were 95.7% and 89.63% respectively. There was no statistically significant difference between the HIV positive and HIV negative patients’ TB-ST tuberculosis rapid test results (P-values = 0.166, 0.189).

Conclusion
With its high specificity and relatively high sensitivity, TB-ST tuberculosis rapid test is a good complementary diagnostic tool to the current available tools.
RESUME
Le diagnostic rapide et précis de la tuberculose (TB) est très important pour faciliter le traitement à temps et ainsi réduire sa diffusion. Les tests pour le diagnostic de la tuberculose pulmonaire diffèrent selon la sensibilité, la spécificité, la rapidité et le coût. La tuberculose pulmonaire à bacilloscopie négative constitue un défi diagnostique en cas de ressources limitées. Pour améliorer le diagnostic de la TB, des techniques diagnostiques plus rapides sont en train d’être développées tel que la test rapide de la tuberculose (TB-ST). Cette étude a été conçue pour évaluer la sensibilité et la spécificité du test rapide de la tuberculose appelé TB-ST.


Méthodes et patients : Notre échantillon est constitué de trois groupes: 150 patients présentant la tuberculose pulmonaire active dont 108 patients à bacilloscopie positive et 42 patients à bacilloscopie négative et 100 patients qui ne présentaient aucun symptôme ou signe ni l’histoire de la TB ont été testés avec le test rapide de la tuberculose (TB-ST).

Résultats: La spécificité, la sensibilité (bacilloscopie positive) et la sensibilité (bacilloscopie négative) du test rapide de tuberculose (TB-ST) étaient respectivement de 96%, 88.9%, et 42.9%. Les valeurs prédictives positives et négatives étaient de 95.7% et 89.63% respectivement. Il n’y avait aucune différence statistiquement significative entre les résultats de TB-ST test rapides de la tuberculose en comparaison avec ceux du VIH sero-positifs et VIH sero-négatifs (P-values = 0.166, 0.189).

Conclusion. Avec sa spécificité élevée et sa sensibilité relativement élevée, le test rapide de tuberculose (TB-ST) est un bon outil diagnostique complémentaire aux outils disponibles courants.
# TABLE OF CONTENTS

**DEDICATION** ................................................................................................................. i

**ACKNOWLEDGEMENT** ............................................................................................... ii

**THE HIPPOCRATIC OATH** ........................................................................................ iii

**LIST OF ABBREVIATIONS** ........................................................................................ iv

**RESUME** ..................................................................................................................... vi

**TABLE OF CONTENTS** .............................................................................................. vii

**LIST OF FIGURES** ......................................................................................................... x

**LIST OF TABLES** .............................................................................................................. x

**CHAPTER I: INTRODUCTION** ....................................................................................... 1

1.1. Background ............................................................................................................. 1

1.2. Evaluation hypotheses ......................................................................................... 2

1.3. Objectives. ............................................................................................................ 2

1.3.1. General objective. ............................................................................................ 2

1.3.2. Specific objective............................................................................................. 2

**CHAPTER II: GENERAL CONSIDERATION** ................................................................. 3

2.1. TB-ST TUBERCULOSIS RAPID TEST ................................................................... 3

2.1.1. TB-ST Description ......................................................................................... 3

2.1.2. Sample collection and preparation.................................................................. 3

2.1.3. Properties of the Test .................................................................................... 4

2.2. TUBERCULOSIS ..................................................................................................... 5

2.2.1. DEFINITION .................................................................................................... 5

2.2.2. EPIDEMIOLOGY .............................................................................................. 5

2.2.3. PATHOPHYSIOLOGY ..................................................................................... 5

2.2.4. CLINICAL MANIFESTATIONS ....................................................................... 6

2.2.5. DIAGNOSIS .................................................................................................... 7

2.2.5.1. Active disease ........................................................................................... 7

2.2.5.2. Latent infection ......................................................................................... 9

**CHAPTER III: MATERIALS AND METHODS** ............................................................... 10
3.1. Materials ............................................................................................................. 10
  3.1.1: The study site ................................................................................................. 10
  3.1.2. Study population ............................................................................................ 10
  3.1.3. The Apparatus ............................................................................................... 10
  3.2.1. Type of evaluation .......................................................................................... 11
  3.2.2. Evaluation period ........................................................................................... 11
  3.2.3. Selection criteria ............................................................................................ 11
    3.2.3.1. Inclusion criteria ......................................................................................... 11
    3.2.3.2. Exclusion criteria ....................................................................................... 11
  3.2.4. Data collection ............................................................................................... 12
  3.2.5. Evaluation approval ....................................................................................... 12
  3.2.6. Test procedure (Appendix C) ....................................................................... 12
  3.2.7. Data Analysis ............................................................................................... 12
  3.2.8. Interpretation of results Appendix D, ............................................................ 13
  3.2.9. Evaluation limitations .................................................................................... 13

CHAPTER IV: PRESENTATION OF RESULTS .......................................................... 14

4.1. SOCIO-DEMOGRAPHIC FACTORS .................................................................. 14
  4.1.1.1. Distribution according to age of the control group .................................. 14
  4.1.1.2. Distribution according to age of Patients with a Positive Sputum Smear 15
  4.1.1.3. Distribution according to Age of Patients with a negative Sputum Smear .... 16
  4.1.2.1. Distribution according to sex of the control group ...................................... 17
  4.1.2.2. Distribution according to Sex of Patients with a Positive Sputum Smear18
  4.1.2.3. Distribution according to sex of Sputum Smear negative Patients .......... 18

4.2. THE RELATIONSHIP BETWEEN TB-ST AND OTHER PARAMETERS. .... 19
  4.2.1. HIV Status ..................................................................................................... 19
    4.2.1.1. Distribution according to the Sputum Smear Positive Patient’s HIV Status ................................................................. 19
    4.2.1.2. Distribution according to Sputum Smear Negative Patient’s HIV status 19
  4.2.2. BCG Vaccination .......................................................................................... 20
    4.2.2.1. Distribution according to BCG vaccination ............................................. 20
4.3. THE RELATIONSHIP BETWEEN THE TB-ST RESULT AND OTHER VARIABLES ................................................... 20
  4.3.1. Relationship between TB – ST Test result and HIV status of Patients with a Positive Sputum Smear ........................................................................................................... 20
  4.3.2. Relationship between TB – ST Test and HIV status of Patients with a negative Sputum Smear ........................................................................................................... 21
4.4. CALCULATIONS OF SENSITIVITY, SPECIFICITY AND PREDICTIVE VALUES OF TB-ST .................................................................................................................. 22
  4.4.1. Distribution according to TB-ST Tuberculosis rapid test result of the control group .............................................................................................................................. 22
  4.4.2. Distribution according to TB-ST Test result among the Smear Positive Patients .............................................................................................................................. 22
  4.4.3. Distribution according to TB – ST Tuberculosis rapid test result among the Smear negative Patient’s ......................................................................................... 23
4.5. PREDICTIVE VALUES .................................................................................................................. 23

CHAPTER V: DISCUSSION ........................................................................................................ 24

CHAPTER VI: CONCLUSIONS AND RECOMMENDATIONS .................................................................. 26
  6.1. CONCLUSIONS .................................................................................................................. 26
  6.2: RECOMMENDATIONS ...................................................................................................... 27
REFERENCES ............................................................................................................................... 28
APPENDIX .................................................................................................................................. 32
LIST OF FIGURES

Figure 1: Distribution according to Age. ................................................... 14
Figure 2: Distribution according to age. ................................................... 15
Figure 3: Distribution according to age. ................................................... 16
Figure 4: Distribution according to Sex. ................................................... 17
Figure 5: Distribution according to sex. ................................................... 18
Figure 6: Patient’s sex. .............................................................................. 18
Figure 7: Patient's HIV status. ................................................................. 19

LIST OF TABLES

Table 1: Patient's HIV status. ................................................................... 19
Table 2: Relationship between TB-ST Test result and Patient's HIV status. .... 20
Table 3: Relationship between TB-ST Test and Patient’s HIV status. ............. 21
Table 4: The TB-ST tuberculosis rapid test results. .................................... 22
Table 5: TB-ST Tuberculosis rapid test results. ........................................... 22
Table 6: TB-ST Test results. ....................................................................... 23
Table 7: Table for the calculation of the predictive values. ....................... 23
CHAPTER I: INTRODUCTION

1.1. Background

There is an estimated 8 million new cases annually of Tuberculosis world-wide. In Rwanda, 6,667 registered Tuberculosis cases were reported in 2004 and Global Database and Country Profile, WHO reported 32,949 estimated incidence in 2004. (52) In 2007, PNILT registered 4066 cases of tuberculosis for the 1st six months. The Global case fatality rate is ~23% and is >50% in some African countries with high HIV rates. (10) Tuberculosis kills about 2 million people each year. An estimated 14 million people all over the world were living with dual HIV-TB, 70% of these in Africa. (13)

Tests for the diagnosis of pulmonary tuberculosis vary in sensitivity, specificity, speed, and cost. (6, 7) Rapid and accurate diagnosis of Tuberculosis is crucial to facilitate early treatment of infectious cases and thus to reduce its spread. (3)

The diagnostic tool available to the clinician in the work-up for Tuberculosis includes Chest X-ray, smear, culture of secretions and tissues, biopsy of lymph nodes and other organs, the current diagnostic projects like polymerase chain reaction (PCR) plus other serological tests. Tuberculosis however, is a disease with a variety of manifestations and often presents diagnostic problems. (14)

The chest x-ray findings may be inconclusive. (10) Diagnosis by means of radiographic examination in patients suspected of tuberculosis is unreliable. Abnormalities identified on a chest radiograph may be due to tuberculosis or to a variety of other conditions, and the appearance on the radiograph is not specific for tuberculosis. (14) HIV patients may present normal chest X-ray.

Smear-negative pulmonary tuberculosis is a diagnostic challenge in resource-limited settings. The sensitivity of an AFB smear is estimated to be 50%-60 %. (27, 28) Different criteria, clinical scoring systems and algorithms have been developed to facilitate the diagnosis of pulmonary tuberculosis in people with suspected tuberculosis that have
repeated smear-negative sputum. All of these criteria, systems, and algorithms have methodological problems, which limit their validity and usefulness.

1.2. Evaluation hypotheses

- TB-ST Tuberculosis Rapid Test is a good and efficient completion tool to the current diagnostic methods.
- With its high sensitivity and specificity, can the test help in the continuous difficult in finding the diagnosis of pulmonary tuberculosis.

1.3. Objectives.

1.3.1. General objective.

- To contribute to the better diagnosis of adult Pulmonary Tuberculosis.

1.3.2. Specific objective.

- To determine the sensitivity and specificity of TB-ST Tuberculosis Rapid Test.
CHAPTER II: GENERAL CONSIDERATION

2.1. TB-ST TUBERCULOSIS RAPID TEST

2.1.1. TB-ST Description
The Tuberculosis Rapid Assay (‘Tuberkulose Schnelltst [TB-ST]’) from Diavita is a serological test method aiming at detecting, within 10-15 minutes, tuberculosis-specific antibodies in whole blood or serum of tuberculosis patients. TB-ST is a membrane-based screening test. This rapid screening test is based on lateral flow immunochromatography.

The TB-ST is an in vitro test for the detection of antibodies (IgG, IgA and IgM) against *Mycobacterium tuberculosis*. The test uses recombinant antigens (38kDa and 16kDa) of only *Mycobacterium tuberculosis*. The advantage is that other mycobacteria do not have any influence on the test result because the “antigen cocktail” was designed especially for the detection of antibodies to *M. tuberculosis*. Samples taken from patients who have been treated recently or are taking antibiotics can lead to faulty results. Antibodies in blood diminish rapidly after treatments with antibiotics, sometimes antibody levels in the blood can be so low in patients that antibodies cannot even be detected at all in blood/serum even when infection is present.

The TB-ST can recognize active tuberculosis, i.e. the results are neither falsified by a BCG inoculation (Tuberculosis protective inoculation), nor by latent Tuberculosis.

2.1.2. Sample collection and preparation
The blood used up to now is venous blood since a study on the capillary blood is currently in progress. This blood is collected in a tube containing an anticoagulant, example EDTA, Citrate and Heparin; this is to allow easy pipetting of blood. The test should be used with the freshest possible samples. In case the test cannot be performed immediately after sample collection; samples may be stored at 2-8° Celsius for up to 3 days.
2.1.3. Properties of the Test

1. A special antibody binding protein affixed to a gold particle (conjugate).
2. A membrane with a unique combination of immobilized TB-specific antigens.

After the serum or whole blood sample and diluent are put into the well on the card using a pipette or dropper, the diluent sample passes through the gold-marked antibody binding protein (conjugate). The conjugate attaches to the immunoglobulins contained in the sample. This antibody-conjugate complex then flows through the membrane to the point where the TB-specific antigens are. If the sample has antibodies for \textit{M. tuberculosis}, then the antibody–conjugate complex attaches itself to the TB antigens immobilized on the membrane. A pink-purple band then appears in the “T” zone of the test card. The rest of the antibody-conjugate complex then passes through the card until it reaches the control zone “C”. Again, a pink-purple band appears, indicating that the test has been performed properly.

Stability of the test kit is for 24 months from the date of manufacture.
2.2. TUBERCULOSIS

2.2.1. DEFINITION
Tuberculosis is a chronic infection caused by the bacteria Mycobacterium tuberculosis (and occasionally other variants of Mycobacterium). It usually involves the lungs, but other organs of the body can also be involved.

2.2.2. EPIDEMIOLOGY
There were an estimated 8-9 million new cases of tuberculosis in 2000, fewer than what was reported; 3-4 million cases were sputum-smear positive, the most infectious form of the disease. (2) Most cases (5–6 million) are in people aged 15-49 years. Sub-Saharan Africa has the highest incidence rate (290 per 100,000 populations), but the most populous countries of Asia have the largest numbers of cases: India, China, Indonesia, Bangladesh, and Pakistan together account for more than half the global burden. 80% of new cases occur in 22 high-burden countries (Appendix A).

There has been striking increases in countries of the former Soviet Union and in sub-Saharan Africa (Appendix B1). (3)

The increase in tuberculosis incidence in Africa is strongly associated with the prevalence of HIV infection. (6) Rates of HIV infection among tuberculosis patients are correspondingly high, exceeding 60% in Botswana, South Africa, Zambia, and Zimbabwe. (Appendix B2) About two million people died of tuberculosis in 2000; about 13% of these people were also infected with HIV. (2)

2.2.3. PATHOPHYSIOLOGY
Tuberculosis is spread by airborne droplet nuclei, which are particles of 1–5 µm in diameter that contain Mycobacterium tuberculosis. Because of their small size, the particles can remain airborne for minutes to hours after expectoration by people with pulmonary or laryngeal tuberculosis during coughing, sneezing, singing, or talking. (7, 9) The infectious droplet nuclei are inhaled and lodge in the alveoli in the distal airways. M. tuberculosis is then taken up by alveolar macrophages, initiating a cascade of events
that result in either successful containment of the infection or progression to active
disease (primary progressive tuberculosis). Although the risk of development of active
disease varies according to time since infection, age, and host immunity, the estimated
lifetime risk of disease for a newly infected young child is 10%, with roughly half of that
risk occurring in the first 2 years after infection. (10, 11)

After being ingested by alveolar macrophages, *M. tuberculosis* replicates slowly but
continuously and spreads via the lymphatic system to the hilar lymph nodes. In most
infected individuals, cell-mediated immunity develops 2–8 weeks after infection.
Activated T lymphocytes and macrophages form granulomas that limit further replication
and spread of the organism. (12) *M. Tuberculosis* is in the centre of the characteristically
necrotic (caseating or cheese-like) granulomas, but it is usually not viable. Unless there is
a subsequent defect in cell-mediated immunity, the infection generally remains contained
and active disease may never occur.

The development of cell-mediated immunity against *M tuberculosis* is associated with the
development of a positive result in the tuberculin skin test. At the cellular level, an
effective host immune response occurs as follows. Alveolar macrophages infected with
*M. tuberculosis* interact with T lymphocytes via several important cytokines.

Several studies have suggested that some patients have a genetic predisposition to
tuberculosis. This idea has arisen from studies among monozygotic and dizygotic twins
(16) and in an assessment of tuberculosis risk according to ancestral history. (17)

### 2.2.4. CLINICAL MANIFESTATIONS
The most common clinical manifestation of tuberculosis is pulmonary disease; extra
pulmonary tuberculosis accounts for about 20% of disease in HIV sero-negative people
but is more common in HIV sero-positive individuals. (18) Among people not infected
with HIV, extra-pulmonary disease, particularly lymphatic tuberculosis, is particularly
common in women and young children. (19, 20)
Pleural tuberculosis occurs as a result of either primary progressive *M. tuberculosis* infection or reactivation of latent infection. A chest radiograph generally reveals a unilateral pleural effusion. Unlike other clinical manifestations of tuberculosis, pleural disease probably represents an increased, rather than diminished, immune response. In fact, primary serofibrinous pleural effusion resolves without treatment in up to 90% of cases; however, if untreated, nearly two-thirds of patients will subsequently have relapses with tuberculosis at other organ sites. (21)

### 2.2.5. DIAGNOSIS

#### 2.2.5.1. Active disease

Criteria for the diagnosis of active tuberculosis vary according to the setting. Patients with persistent cough (e.g. lasting longer than 2 weeks) should be assessed for tuberculosis. (22, 23) Other common symptoms include fever, night sweats, weight loss, shortness of breath, haemoptysis, and chest pain. (24)

The sputum smear is an inexpensive test that can be carried out rapidly; fluorochrome, Ziehl-Neelsen, and Kinyoun staining methods can be used. The International Union against Tuberculosis and Lung Disease (IUATLD) and WHO recommend the Ziehl-Neelsen method under most circumstances. (23, 26) Although the smear is positive in only 50–80% of individuals with culture-confirmed pulmonary tuberculosis, cases with organisms on the smear are more infectious than smear negative cases and have higher case-fatality rates. (27, 28) Nonetheless, smear-negative disease accounts for 15–20% of *M tuberculosis* transmission (28, 29). In countries with a high prevalence of tuberculosis, a positive direct smear is due to *M. tuberculosis* in more than 95% of patients suspected of having tuberculosis; (30) routine cultures are generally neither practicable nor necessary for disease control. Non-tuberculous mycobacteria, particularly in HIV-infected patients, tend to be present in much lower concentrations and are therefore rarely seen on a direct sputum smear. Concentrated smears (i.e., those made from samples that have been decontaminated, liquefied, and centrifuged) may be more sensitive and are routinely used in laboratories that also routinely culture all specimens, because decontaminated and concentrated specimens are needed for culturing. (26, 31)
less-developed countries, a diagnostic algorithm for sputum-smear-negative patients is commonly used, based on response to antibiotics and results of chest radiography.

Although the organism can take 6 weeks or longer to grow on solid culture media (e.g. the egg-based Lowenstein-Jensen medium or the agar-based Middlebrook 7H10 or 7H11), growth generally occurs within 7–21 days with liquid culture media. (32) Ideally, when cultures are done, both solid and liquid culture media should be used, because the former allow examination of colony morphology and the identification of mixed cultures, and the latter enable more rapid diagnosis.

Radiographic findings suggesting tuberculosis include upper-lobe infiltrates, cavitary infiltrates, and hilar or Para tracheal adenopathy. In many patients with primary progressive disease and those with HIV infection, radiographic findings are more subtle and can include lower-lobe infiltrates or a miliary pattern. HIV-infected patients, particularly those late in the course of HIV infection, generally experience greater weight loss and fever but are less likely to have cavitary disease and positive smears for acid-fast bacilli (33) than those not infected with HIV, and in one study, 8% of HIV-infected patients with pulmonary tuberculosis had normal chest radiographs. (34)

About 15–20% of adults with tuberculosis (on the basis of clinical, radiographic, and histopathological findings, as well as response to antituberculosis treatment) (24) have negative sputum cultures.

Nucleic-acid amplification assays can be used directly on clinical specimens; they are most reliable in smear positive respiratory samples from patients with previously untreated tuberculosis. In such samples, the sensitivity and specificity can be as high as 95% and 98%, respectively. The sensitivity is 48–53% in smear-negative respiratory samples, but the specificity is roughly 95%. (24, 35) In areas of high tuberculosis prevalence, there is no need to confirm a heavily positive sputum smear, which will in most cases reflect \textit{M. tuberculosis}. 

\textit{M. tuberculosis}. 

\textit{M. tuberculosis}. 

Amplification tests do not replace the sputum smear (which provides a gauge of infectiousness) or culture (which is necessary for drug-susceptibility testing). The assays can still give positive results after effective treatment (because of detection of residual genetic material), so they may not be as useful in people with previous disease or in monitoring response to therapy.

In addition to advances in clinical laboratory tests, research methods of DNA fingerprinting can be useful to identify laboratory cross-contamination and elucidate the epidemiology of tuberculosis. (36)

2.2.5.2. Latent infection

The intradermal administration of tuberculin has been used as a diagnostic test for tuberculosis infection since the early 1900s (37); the more consistent form of tuberculin, standardized purified protein derivative (PPD-S), has been used to assess latent *M. tuberculosis* infection since 1939. (38,39) Although the tuberculin skin test is the best available way to diagnose latent *M. tuberculosis* infection, it has limitations, including low sensitivity in immunocompromised patients, cross-reactivity with bacilli Calmette-Guerin (BCG) vaccine and environmental mycobacteria (resulting in decreased specificity), and a requirement that patients must return 48–72 h after the test is done to have the result read. (40) The criteria for a positive test vary according to the population group being tested; they are influenced by the likelihood of being infected with *M. tuberculosis* and the risk of developing active disease if infected. (15)

A whole-blood interferon-γ release assay (IGRA), like the tuberculin skin test, assesses cell-mediated immunity to tuberculin. (41) IGRA responses are diminished in HIV-infected individuals, resulting in low sensitivity in this important population (43), but they may aid in detecting latent infection among certain populations who are at increased risk (e.g. recent migrants from countries with high incidence of tuberculosis). (41) Although the IGRA is less sensitive and specific than the tuberculin skin test, (42) responses are less affected by previous BCG vaccination. (44) An enzyme-linked immunospot (ELISPOT) assay has recently been developed that is relatively sensitive and specific in detecting latent *M. tuberculosis* infection. (45)
CHAPTER III: MATERIALS AND METHODS

3.1. Materials

3.1.1: The study site
The Evaluation was carried out in the departments of Internal Medicine at Kigali Central Hospital (CHK-CHUK) and the University Teaching Hospital-Butare (CHUB).

3.1.2. Study population
Our evaluation population was composed of two classes of people; they were all Adults that were equal or older than 15 years as in Rwanda these are classed as Adults;

- **Group 1(Control):**
  - 100 Subjects who were not suffering from pulmonary Tuberculosis by the time of the evaluation. These individuals were selected randomly. All members of the control group had no previous history of Tuberculosis, no signs or symptoms suggestive of pulmonary Tuberculosis.

- **Group 2:**
  - 108 Sputum smear positive subjects who were diagnosed with pulmonary tuberculosis before they started on the anti-Tuberculosis treatment.

- **Group 3:**
  - 42 Sputum smear negative subjects who were diagnosed with pulmonary tuberculosis before starting on anti-Tuberculosis treatment. (Smear negative because the sputum smear was negative, no response to broad-spectrum antibiotics and Clinicians’ decision to start on anti-Tuberculosis treatment).

3.1.3. The Apparatus.
1. Tuberculosis Patients
2. Health individuals for the control
3. TB-ST Tuberculosis rapid test testing kit.
   - TB-ST Tests
   - Diluent in dropper vials
   - 1 set of instructions
5. Needles.
The TB-ST rapid test kit costs about 4 euros in Germany and this kit contains 10 tests which means that a single test can cost about 500Frws.

3.2. Methods

3.2.1. Type of evaluation
It was a prospective evaluation.

3.2.2. Evaluation period
The evaluation was conducted between November 2006 and August 2007.

3.2.3. Selection criteria

3.2.3.1. Inclusion criteria
Group 1 (Control):
- Subjects who were not presenting with signs and symptoms of Tuberculosis.

Group 2:
- Positive sputum-smear pulmonary Tuberculosis Subjects:
  A patient with at least 2 sputum specimens positive for AFB; OR a patient with only one sputum specimen positive for AFB by microscopy and a chest radiography consistent with pulmonary TB.

Group 3:
- Negative sputum-smear pulmonary Tuberculosis Subjects:
  A patient who presents with at least 3 sputum specimens negative for AFB, absence of response to treatment with broad spectrum antibiotics, and chest radiography with abnormalities suggestive of active pulmonary TB and a decision by a physician to give the patient a full course of TB treatment.

3.2.3.2. Exclusion criteria
Subjects who did not fully fulfill the above-mentioned criteria according to the above outlined groups.
3.2.4. Data collection
A data form was used to collect age, sex, TB-ST results, date of TB-ST reading for each study subject, BCG vaccination, and HIV status. (Appendix E). Each study subject was assigned an abbreviation for her/his names; names were collected for medical follow-up purposes only where it was necessary. Names were not entered into the computerized database.

3.2.5. Evaluation approval
The National University of Rwanda Medical Faculty approved this study. The approval of this study was also obtained from the Research committee of Kigali central Hospital (CHU-CHK) prior to beginning patient enrollment.

3.2.6. Test procedure (Appendix C).
The TB-ST rapid test detects tuberculosis-specific antibodies in whole blood or serum of tuberculosis patients. TB-ST is a membrane- based screening test and is based on lateral flow immunochromatography. The TB-ST is an in vitro test for the detection of antibodies (IgG, IgA and IgM) against Mycobacterium tuberculosis. The blood used up to now is venous blood. This blood is collected in a tube containing an anticoagulant, example EDTA, Citrate and Heparin; this is to allow easy pippeting of blood.

Two drops of whole blood or serum were put in a well on the test card and two drops of the diluent added to the well too. The results were read after 15 minutes, but maximum after 25minutes.

3.2.7. Data Analysis.
The determination of the Specificity and Sensitivity was done using the following formulae;

1- SPECIFICITY (%)  

\[ = \frac{TN}{TN + FP} \times 100 \]

TN = true negative samples  
FP = false positive samples
2- SENSITIVITY (%)

\[
= \frac{TP}{TP + FN} \times 100
\]

FN = false negative samples
TP = true positive samples

3- PREDICTIVE VALUES

A- Positive Predictive Value

\[
= \frac{TP}{TP + FP} \times 100
\]

TP = true positive.
FP = false positive

B- Negative Predictive Value

\[
= \frac{TN}{TN + FN} \times 100
\]

TN = true negative
FN = false negative

3.2.8. Interpretation of results Appendix D.

3.2.9. Evaluation limitations

- There is little Data to compare with on the evaluation of this particular test therefore this partly limits the scientific arguments concerning the test.
- Limited time and means to carry out a more detailed evaluation for example we did not get the required number of Patients previously planned in some groups.
- Lack of the means to rule-out active TB infection in the control group.
- There was no enough time to make the culture as the gold standard.
CHAPTER IV: PRESENTATION OF RESULTS

4.1. SOCIO-DEMOGRAPHIC FACTORS

4.1.1. Age

4.1.1.1. Distribution according to age of the control group

Figure 1: Distribution according to Age.

The age range of our patients was between 16 and 48 years with the mean age at 28.19 and standard deviation of 7.46.
4.1.1.2. Distribution according to age of Patients with a Positive Sputum Smear

Figure 2: Distribution according to age.

The age of our patients ranges between 17 years and 52 years with a mean age of 31.87 (SD 10.22)

Most patients lie in the class interval of 25 – 34 with 38 (35.2%) and least patients lie in the class interval of 45 – 55 with 16 (13%). However 87.1% of our patients are between 17 and 44 years.
4.1.1.3. Distribution according to Age of Patients with a negative Sputum Smear

Figure 3: Distribution according to age.

The age range of our patients was 18 years to 56 years with the mean age 32.55. Most patients lie in the class interval of 15–24 with 18 (38.1%) and least patients lie in the class interval of 35–44 and 55–64 with 2 (4.8%). However 87.1% of our patients are between 18 and 34 years.
4.1.2. SEX

4.1.2.1. Distribution according to sex of the control group

Figure 4: Distribution according to Sex.

Most of our subjects were Females 56 (56%)

The Male/Female ratio was 1: 1.21.
4.1.2.2. Distribution according to Sex of Patients with a Positive Sputum Smear

Figure 5: Distribution according to sex.

Most of our patients were Females 68 (63%). The Male /Female ratio was 1:1.7.

4.1.2.3. Distribution according to sex of Sputum Smear negative Patients

Figure 6: Patient’s sex.

Most of our patients were Males 23 (52%). Our patients had a sex ratio of 1.1:1.
4.2. THE RELATIONSHIP BETWEEN TB-ST AND OTHER PARAMETERS.

4.2.1. HIV Status.

4.2.1.1. Distribution according to the Sputum Smear Positive Patient’s HIV Status

Figure 7: Patient’s HIV status.

64 (59.3%) of our patients were HIV positive, 24 (22.2%) HIV negative, where as 20 (18.5%) their HIV status was unknown.

4.2.1.2. Distribution according to Sputum Smear Negative Patient’s HIV status

Table 1: Patient’s HIV status

<table>
<thead>
<tr>
<th>HIV STATUS</th>
<th>FREQUENCY. N=42</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>26</td>
<td>61.9</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>12</td>
<td>28.6</td>
</tr>
<tr>
<td>UNKNOWN</td>
<td>4</td>
<td>9.5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>42</td>
<td>100</td>
</tr>
</tbody>
</table>

26 (61.9%) of our patients were HIV positive, 12 (28.6%) HIV negative, where as 4 (9.5%) their HIV status was unknown.
4.2.2. BCG Vaccination.

4.2.2.1. Distribution according to BCG vaccination
All the subjects tested were found to have undergone vaccination with BCG vaccine.

4.3. THE RELATIONSHIP BETWEEN THE TB-ST RESULT AND OTHER VARIABLES

4.3.1. Relationship between TB – ST Test result and HIV status of Patients with a Positive Sputum Smear

Table 2: Relationship between TB-ST Test result and Patient's HIV status

<table>
<thead>
<tr>
<th>PATIENT'S HIV STATUS</th>
<th>TB – ST Test.</th>
<th></th>
<th></th>
<th>Total</th>
<th>P-value=0.307</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>56</td>
<td>8</td>
<td></td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>87.5%</td>
<td>12.5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>24</td>
<td>0</td>
<td></td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>100.0%</td>
<td>.0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>8</td>
<td></td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>90.9%</td>
<td>9.09%</td>
<td>100.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Among the HIV positive patient’s tested, 56 (87.5%) had a positive TB – ST Test and 8 (12.5%) a negative TB – ST Test. There is no statistically significant difference between a positive HIV status and TB – ST Test result. (P-value = 0.307)
4.3.2. Relationship between TB – ST Test and HIV status of Patients with a negative Sputum Smear

Table 3: Relationship between TB-ST Test and Patient's HIV status

<table>
<thead>
<tr>
<th>PATIENT’S HIV STATUS</th>
<th>TB – ST RESULT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>33.3%</td>
<td>66.7%</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>33.3%</td>
<td>66.7%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>33.3%</td>
<td>66.7%</td>
<td></td>
</tr>
</tbody>
</table>

P-value=0.189

Among the HIV positive patient’s tested, 8 (33.3%) had a positive TB – ST Test and 16 (66.7%) a negative TB – ST Test. There is no statistically significant difference between a positive HIV status and TB – ST Test result. (P-value = 0.189)
4.4. CALCULATIONS OF SENSITIVITY, SPECIFICITY AND PREDICTIVE VALUES OF TB-ST

4.4.1. Distribution according to TB-ST Tuberculosis rapid test result of the control group

Table 4: The TB-ST tuberculosis rapid test results

<table>
<thead>
<tr>
<th>TB-ST Test</th>
<th>Frequency (n=100)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Negative</td>
<td>96.0</td>
<td>96.0</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100</td>
</tr>
</tbody>
</table>

Out of 100 subjects tested 96 (96%) were negative therefore giving a specificity of 96% with a 4% of probable false positives.

4.4.2. Distribution according to TB-ST Test result among the Smear Positive Patients

Table 5: TB-ST Tuberculosis rapid test results

<table>
<thead>
<tr>
<th>TB – ST TEST.</th>
<th>FREQUENCY (n=108)</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>96</td>
<td>88.90</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>12</td>
<td>11.10</td>
</tr>
<tr>
<td>TOTAL</td>
<td>108</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Out of 108 patients tested 96 (88.9%) were positive giving a sensitivity of 88.9%, with 11.1% of false negatives.
4.4.3. Distribution according to TB – ST Tuberculosis rapid test result among the Smear negative Patient's

Table 6: TB-ST Test results

<table>
<thead>
<tr>
<th>TB – ST</th>
<th>FREQUENCY. N=42</th>
<th>PERCENTAGE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>18</td>
<td>42.90</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>24</td>
<td>57.10</td>
</tr>
<tr>
<td>TOTAL</td>
<td>42</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Out of 42 patients tested 18 (42.9%) were positive giving a sensitivity of 42.9%.

4.5. PREDICTIVE VALUES

Table 7: Table for the calculation of the predictive values

<table>
<thead>
<tr>
<th>TB DISEASE.</th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB – ST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>96</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>96</td>
<td>108</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>100</td>
<td>208</td>
</tr>
</tbody>
</table>

1- Positive predictive value

= 95.7%

2- Negative predictive value

= 89.63%
CHAPTER V: DISCUSSION

The need for improved TB diagnostics has long been recognized. Local diagnostic needs vary markedly, and it is unlikely that a single diagnostic test can be ideal for all situations. However, an accurate, simple, rapid, point-of-care assay would be widely useful and could be expected to improve case holding, empower health-care workers at a peripheral level, and decrease diagnostic confusion and delay.

This study was designed to evaluate the diagnostic potential of TB-ST Tuberculosis rapid test for the detection of Ig G, Ig M and Ig A to \textit{M. tuberculosis} antigens (38kDa and 16 kDa) in adult pulmonary tuberculosis.

POPULATION CHARACTERISTICS

The age ranges of our patients were 16 to 48 years with the mean age at 28.19 (SD 7.46), 17 to 52 years with a mean age of 31.87 (SD 10.22), 18 to 56 years with the mean age 32.55 for the controls, smear-positive cases and smear-negative cases respectively. The above groups studied lie in the same age group and can easily be compared. This may be explained by the fact that it is usually the youth that is most affected by the tuberculosis. We must also notice that from the demographic point of view, the Rwandan population is essentially comprised of the youth. (47, 48)

In our study we also found out that most of the subjects were female; 56 (56%) and 68 (63%) in the control group and among the smear positive patients. This can be explained by the fact that most the Rwandan population is female. (47, 48)

Amongst the patients studied, 64 (59.3%) of the positive sputum smear patients and 26 (61.9%) of the negative sputum smear patients were HIV positive and this can be explained by the fact that there is an increase of TB incidence in Africa due to HIV (6). Furthermore, we found out that 87.5% and 33.3% of the HIV positive patients among smear positive and smear negative cases had a positive TB-ST result respectively and the CD4+ cell count of some of the patients were not available (differences not statistically
significant) p=0.307 and p=0.189 respectively. This is contrary to the expectations of many since the sensitivity of a serological test should be low due to the immuno-suppression which affects the antibody production. This may be explained by the high sensitivity of TB-ST; 88.9% but the fact that the CD4 cell count and/or the stage of AIDS of these patients were not put into consideration stop us from jumping to the conclusion.

**TB-ST RESULTS**

In our study we got a specificity of 96% and sensitivity of 88.9% and 42.9% in sputum smear-positive and sputum smear-negative patients respectively with positive and negative predictive values of 95.7% and 89.63% respectively.

According to the clinical trial carried out in USA, Germany and India (2005), a total number of 322 samples were tested and 153 samples from healthy donors or patients suffering from other diseases. According to Loddenkemper R et al (2005), 169 samples from TB infected individuals were measured and a specificity of 99.3% was obtained, sensitivity from TB confirmed cases and smear-negative patients of 78.1% and 69.2% respectively were also obtained. (46)

A study done in Germany in 2005, according to the unpublished personal information by Prof. Dr. Mahavir Singh of Lionex Diagnostics and therapeutics GmbH the study populations were increased to 1696 for the controls and 107 for TB patients. They found out a specificity of 97.68% in health individuals, specificity of 95.77% in patients suffering from other infections, sensitivity of 70.59% and 42.47% in sputum smear positive and sputum smear negative patients respectively. These studies show some slight differences in the obtained specificities and sensitivities and this can be due to the differences in the sizes and origins of the study populations since the subjects came from different countries but also keeping in mind the fact that there is a high tuberculosis prevalence rate in sub-Saharan Africa. (3).
CHAPTER VI: CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

In this evaluation of Tuberculosis rapid test in adult pulmonary tuberculosis we deduced the following conclusions;

- **TB-ST** Tuberculosis rapid test has relatively high specificity, sensitivity and predictive values.
- **TB-ST** Tuberculosis rapid test is a useful complementary diagnostic tool in the diagnosis of pulmonary tuberculosis and can serve as a good screening test.
- With the TB-ST Tuberculosis rapid test 42.9% of the Smear negative TB can be confirmed.
- In consideration of the difficulties and costs of diagnosing TB especially AFB-negative TB, TB-ST serological test based on multiple antigens with a relatively high specificity and sensitivity, with an easy test procedure, simple equipment, a much lower cost and which do not require invasive procedures such as fiber optic bronchoscopy may play a crucial role in our setting.
6.2: RECOMMENDATIONS

At the end of our study we formulate the following recommendations;

❖ To the Ministry of Health.
  ❖ To consider the use of TB-ST Tuberculosis rapid test in the diagnosis of Adult Pulmonary Tuberculosis.
  ❖ Larger studies with more representative populations and more variables like CD4+ cell count in HIV positive patients need to be carried-out to really be convinced about its utility.

❖ To the Medical Doctors.
  ❖ The following to be put into consideration in the subsequent evaluations;
    o Purified protein derivative test.
    o Onset of clinical manifestations.
    o Sputum culture incase of microscopy negative TB.
    o To consider the stage and/or the CD4 count in HIV positive patients.
REFERENCES


15- American Thoracic Society, Centers for Disease Control and Prevention. Targeted
tuberculin testing and treatment of latent tuberculosis infection. Am J Respir Crit Care Med 2000; 161: S221–47.


41- Mazurek GH, Villarini ME. Guidelines for using the QuantiFeron-TB test for


51- Prof. Dr. Mahavir Singh: CEO LIONEX Diagnostics & Therapeutics GmbH.
APPENDIX

APPENDIX A: ESTIMATED NUMBER OF NEW TUBERCULOSIS CASES BY COUNTRY

Figure 1: Estimated number of new tuberculosis cases by country, 2001
Figure 2: Trends and projections in numbers of tuberculosis cases to 2010 for countries of eastern and southern Africa with high HIV prevalence, and in the former Soviet Union.
APPENDIX.B2: Estimated HIV prevalence in TB cases, 2003
Appendix C: The TB-ST Test procedure

Application

Remove test cards from the pouch and label these with patient’s name or ID.

Fill either 20 µl whole blood or serum into the sample well of the card, marked ‘S’ (1).

Add two drops of diluents from the vial dropper bottle to sample well ‘S’ (2).

Read the results after 15 minutes (maximum 25) as follows:

Negative results: Appearance of a purple coloured band at the control region ‘C’ (3).

Positive results: Two purple coloured bands at the control region ‘C’ and on the test region ‘T’ (4).

Inspection: The purple coloured bands in the test region ‘C’ indicate a successful execution of the test.
Appendix D  Interpretation of results
APPENDIX E DATA COLLECTION FORMS

GROUP A: (CONTROL) PATIENT’S DATA COLLECTION FORM.
1-Name (Abbreviated)…………..
2-Sex /Gender …… 1=Male, 2=Female
3-Age (years) ………..
4-TB-ST test result……… 1=Positive, 2=Negative.
5-Date of the test (dd/mm/yyyy: (…/…/…)
6-BCG Vaccination

GROUP B: PATIENT’S DATA COLLECTION FORM
1-Name (Abbreviated)…………..
2-Sex /Gender ……1=Male, 2=Female
3-Age (years) ………..
4-TB-ST test result …… 1=Positive, 2=Negative.
5-Date of the test (dd/mm/yyyy: (…../…../…..)
6-HIV Status
7-BCG Vaccination