COMPARATIVE STUDY OF COMMERCIAL IMMUNO-CHROMATOGRAPHIC TEST STRIP AGAINST ZIEHL-NEELSEN STAIN IN THE DIAGNOSIS OF TUBERCULOSIS

BY

UTHMAN, Taofeek Adegoke

M.Tech/SSSE/2008/1958

DEPARTMENT OF MICROBIOLOGY

FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA.

MAY 2012

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THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN MICROBIOLOGY

MAY 2012

ABSTRACT

A comparative study of Immuno-chromatographic test (Serology) with Ziehl-Neelsen (Z-N) staining technique was carried on patients who are suspected of having tuberculosis in the General Hospital, Minna, Niger state, Nigeria. The study was done to compare their performance in the diagnosis of tuberculosis. Three Hundred (300) patients were enlisted for the study but only Two Hundred and Fifty-two (252)patients completed the study. Three (3) Sputum samples were collected from each patient for Z-N staining and 5ml of whole blood was collected from each patient concurrently for serological testing. At the end of the studies the result shows a Raw Agreement of 76% (range of 75-77%), Positive Agreement 1.4% (range of 1.2-1.6%), and a Negative Agreement of 75% (range of 74-76%) between the 2 methods. The Coefficient of Agreement ranged between 0.006 and 0.015(range of κ is 0.006-0.014; range of T is 0.0056-0.0154). The Sensitivity of the serological test was 12.76%, while it Specificity was 92.42%. It has a Positive Predictive value of 16.22%, while the negative predictive value was 80.53%. With these results the level of Agreement (0.009) between the Immuno-chromatographic Test Strip and Sputum Z-N stain in the diagnosis of PTB is very low and hence Immuno-chromatographic Test Strip cannot replace Z-N staining in the diagnosis of PTB.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Epidemiology

Tuberculosis (TB) is a granulomatous disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) that affects all organs of the body with varying frequencies (Kumar *et al.*, 2007). The primary mode of infection is by inhalation of droplets laden with the organism (Konstatinos, 2010), hence the vast majority of tuberculosis disease is pulmonary; that is, in the lungs, this accounted for about 75% of all TB infections (WHO, 2007). Other sites of affectations include the Central Nervous System, the Musculo-skeletal System, the Uro-genital system, the Gastrointestinal System and other Systems in the Human body; these other sites outside of the lungs are collectively referred to as extra-pulmonary tuberculosis (Kumar *et al.*, 2007).

A third of the world's populations are said to be infected with *M. tuberculosis*, (WHO, 2009). And new infections occur at a rate of about one per second (WHO, 2009). The proportion of people who become sick with tuberculosis each year is stable or falling worldwide but, because of population growth, the absolute number of new cases is still increasing (WHO, 2009).

In 2007 there were an estimated 13.7 million chronic active cases, 9.3 million new cases, and 1.8 million deaths, mostly in developing countries (WHO, 2009). In addition, more people in the developed world are contracting tuberculosis because their immune systems are compromised by immunosuppressive drugs, substance abuse, or Acquired Immune Deficiency Syndrome (AIDS). The distribution of tuberculosis is not uniform across the globe; about 80% of the population in many Asian and African countries test positive in tuberculin tests, while only 5-10% of the US population test positive (Kumar *et al*., 2007).

About 90% of those infected with Mycobacterium tuberculosis have asymptomatic, latent TB infection (sometimes called LTBI), with only a 10% lifetime chance that a latent infection will progress to TB disease (Kumar *et al.,* 2007). However, if untreated, the death rate for these active TB cases is more than 50% (Onyebujoh, P. & Graham, A. W., 2004).

Accurate identification of LTBI is the key to prevention of the disease among persons at risk (Payam *et al*., 2006). However, diagnosing this category of Tuberculosis infection i.e. latent Tuberculosis infection, has been a real problem because, hitherto, the modality being used is the Tuberculin Skin Test (TST) using Purified Protein Derivative (PPD) of *M. tuberculosis* antigen, the response of the host is then measured to determine the state of the patient bearing in mind the risk status (CDC, 2000). However, cross reactivity of this antigen with other strains of the mycobacterium species, especially the Non Tuberculous Mycobacterium (NTM) species raised the issues of false positivity; also previous vaccination with Baccille Calmette-Guerin (BCG) (an attenuated extract of *M. bovis*) gives a false positive reaction; whilst chronic illnesses including active tuberculosis disease can give a false negative reaction to tuberculin skin test even amongst active sufferers (Pai, 2005).

To resolve these issues, that is, cross reactivity with the Non Tuberculous Mycobacterium (NTM) specie, there is a need to evolve a diagnostic method that will measure response to antigen specific to *M. tuberculosis* alone, this is what the relatively new Immunologic test for TB infection seeks to resolve. These tests seek to demonstrate the production of Antibody in the infected individual in response to some specific Antigens e.g. Early Secreted Antigen Target 6 kilodalton (ESAT6) and Culture Filtrate Protein 10 kilodalton (CFP10) that are unique to Mycobacteria of the Mycobacterium Tuberculosis Complex (MTC). MTC include *M. tuberculosis, M. bovis, M.*

africanum, M. canetti and M. microti. These antigens are not present in common NTM (e.g. *M. avium*) as well as BCG vaccination strains (Kang*et al.*, 2007).

ESAT6 and CFP 10 are located on Region of Difference 1 (RD1) on the *M. tuberculosis* genome hence they are referred to as RD1 based (Payam *et al*., 2006)**.**

Current evidence on the performance of Interferon gamma (IFN-γ) assays suggests that RD1-based IFN-γ assays have higher specificity, better correlation with surrogate measures of exposure to *M. tuberculosis,* and less cross-reactivity due to BCG vaccination than the Tuberculin Skin Test (TST) (Payam *et al*., 2006). RD1-based IFN-γ assays that use a mixture of ESAT-6 and CFP-10 seem to be at least as sensitive as the PPD-based TST in active TB (Payam *et al*., 2006)**.**

1.2 Statement of the Problem

A third of the World's population is said to be infected with Mycobacterium tuberculosis (MTB). With about eight Million cases of active disease occurring each year, Tuberculosis (TB) is therefore regarded as a global health problem (WHO, 2007).

Furthermore, the continual migration of people from the developing world with a high incidence of Tuberculosis to highly industrialized countries with less incidence of TB has made MTB a global emergency (Dye *et al*., 2005).

Also with improvement in the quality of healthcare worldwide, people with chronic illnesses now survive longer albeit, with low immunity, this will enhance the reversion from latent (asymptomatic) MTB infection to active Tuberculosis.

However, despite this prevalence, diagnosis of TB infection in Human has remained a thorny medical issue; all the currently available diagnostic modalities have their drawbacks;

Diagnosis of MTB based on Clinical evaluation, Chest radiography and Sputum staining and Microscopy are sensitive but not specific (Anderson *et. al*., 2000), Culture of bacteria is

time-consuming and the yield is low (Sarmiento *et al.,* 2003), nucleic acid-based methods such as polymerase chain reaction (PCR) are not consistently accurate enough for the diagnosis of smear-negative pulmonary TB (Roba *et al.*, 2010). Tuberculin Skin Test (TST) using Purified Protein Derivative (PPD) largely used for screening has a major drawback of cross-reactivity with antigens derived from several Mycobacteria species, including attenuated *M. bovis* (Bacille Calmette Guerin (BCG)) used for vaccination, greatly decreasing the specificity of the TST (Roba *et al*., 2010). Moreover, 10-20% of patient with TB have a negative result with TST due to reduction in their immune competence; this percentage increases with advanced disease or with immunodeficiency due to Human Immune Deficiency Virus (HIV) infections (Converse *et al.,* 1997).

However, it was discovered that MTB infections evoke strong cell-mediated immune response, and detection of specific immune cells might be a mean to detect infection (Delgado *et al.*, 2002).

1.3 Justification for the Research

Immunological tests are Convenient and fast, they can therefore be adopted as a point of care test.

Immunological test do not have the subjectivity inherent in measurements of skin indurations produced by the TST.

Furthermore, there is only one patient's visit, unlike Ziehl-Neelsen (Z-N) stain/microscopy where three (3) samples collected at three different times are needed per patient.

Also the ability to perform repeat testing without boosting the response, as may occur with serial tuberculin testing, makes serological testing a more attractive option (Payam *et al*., 2006).

1.4 Aim

- To determine if Serological Test (Commercial Immuno-chromatographic Test Strip) can replace Z-N stain and Microscopy in the diagnosis of Tuberculosis.

1.4.1 Specific Objectives

1 Compare the performance of Serological Test using Commercial Immuno-chromatographic Test Strip against Sputum Ziehl-Neelsen (Z-N) stain and Microscopy in the diagnosis of Pulmonary Tuberculosis (PTB) in the Directly Observed Treatment strategy (DOTS) Centre (General Hospital, Minna, Niger State).

1 To find the level of Agreement between the Sputum Z-N stain/Microscopy and Serological Test using Commercial Immuno-chromatographic Test Strip in diagnosis of PTB.

1.5 Study Area

The study was done between February 2011 and August 2011, on 300 patients suspected of having Tuberculosis; they were drawn from the Out-patients Clinics of the General hospital, Minna and patients referred from other Hospitals in Minna and its environs to the Directly Observed Treatment Strategy (DOTS) TB Clinic of the General Hospital, Minna. Minna is the capital city of Niger state, which is one of the States in the North-central zone of Nigeria.

The Hospital is one of the designated centres for the National Leprosy and Tuberculosis programme, where diagnosis of Tuberculosis are done free of charge for all cases. Confirmed cases are also given free treatment courtesy of the Federal Ministry of Health in collaboration with the World Health Organization.

Prior approval of the Ethics and Scientific Committee of the Hospital was sought and permission was granted for the studies.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aetiology

Tuberculosis is caused by the bacteria *Mycobacterium tuberculosis* of the Mycobacterium genus. This genus is composed of slow growing organisms, which are "acid fast". Currently about 55 species of Mycobacterium are recognized. They are non-motile, slightly curved or straight rods (0.2 – 0.6 X 1-10 mm) and may occasionally demonstrate branching. The organisms are aerobic and have a gram positive cell wall, although they do not gram stain well (Southwick, 2007).

The Mycobacterium contains a lipid rich cell surface which includes true waxes and glycolipids. 60-90 carbon, long chain Mycolic acids, unique to the Mycobacterium cell wall are responsible for their:

- Acid fastness;
- Failure to react with Gram stains;
- Resistance to the action of antibodies and complement.

The major cause of Tuberculosis in Human, *Mycobacterium tuberculosis* (MTB) is a small aerobic non-motile bacillus. The high lipid content of this pathogen accounts for many of its unique clinical characteristics (Southwick, 2007). It divides every 16 to 20 hours, an extremely slow rate compared with other bacteria, which usually divide in less than an hour (Cox, 2004). Other species of Mycobacterium implicated in primary Human tuberculosis infection includes: *M. bovis, M. africanum, M. canetti and M. microti*, they are collectively referred to as the Mycobacterium Tuberculosis Complex (MTC) (van Soolingen *et al.*, 1997).

Other Mycobacterium includes; *Mycobacterium leprae* the causative agent of Leprosy, *Mycobacterium avium* and *Mycobacterium kansasii*. The last two are part of the Non-Tuberculous Mycobacteria (NTM) group. Non-Tuberculous Mycobacterium cause neither tuberculosis nor leprosy, but they may cause pulmonary diseases resembling tuberculosis (American Thoracic Society, 1997).

In an immune competent person, tuberculosis is often limited to a site in the body e.g. the lungs, it can however be disseminated widely to involve multiple organs in an immune compromised individuals whence it is usually referred to as miliary tuberculosis, especially in infants (C.D.C., 2000).

2.2 Pathogenesis of Tuberculosis

By aerogenic spread of Mycobacteria, people with active pulmonary tuberculosis are the main source of tuberculosis infection (Feja *et al*., 2005).

After deposition of the infectious, 2–5 μm-sized, aerosol particles "droplet nuclei" in the alveoli the immune system of the host organism begins to confront the Mycobacteria in the alveolar macrophages and in the lymphatic tissue of the lungs. Processing of antigens by M-cells in the respiratory epithelium is also possible (O'Brien, R. J. & Spigelman, M., 2005). After presentation of *M. tuberculosis* antigens, the specific cellular host immunity is stimulated; lymphocyte subpopulations are activated and proliferate (Jacobsen *et al.*, 2007). In the course of this activation, various cytokines are released, in particular interferon-γ and TNF-α, which play a key role in T cell activation and granuloma-formation.

At the site of infection the mycobacteria are phagocytosed by macrophages, in which they can persist for long periods without replicating. As an immuno-pathological substrate very small

epitheloid cell granulomas can develop at an early stage, which does not show up on radiograph (Algood *et al*., 2005). This so-called latent tuberculosis infection (LTBI) is usually clinically unapparent and can remain so for a lifetime.

In adolescents and adults, reactivation of dormant bacilli can occur later in life in about 10% of cases with latent infection, known as post-primary tuberculosis. Immune compromised patients, e.g. HIV-infected individuals, have a higher risk of 5–15% per year to develop active disease after infection (Aaron *et al*., 2004). The infection can spread haematogenously i.e. through the blood stream, or lymphatically (through the lymphatic channels) throughout the body, e.g. in the meninges, bones, joints, kidneys, the urinary tract or peripheral lymph nodes (Sharma *et al*., 2005).

2.3 Pathogenicity of *Mycobacterium tuberculosis*

The pathogenicity of *M. tuberculosis* is thought to be due to the presence of some proteins, particularly Early Secreted Antigen Target 6 (ESAT-6) and Cultural Filtrate Protein 10 (CFP-10). The mechanisms by which ESAT-6 and CFP-10 contribute to virulence are not fully defined, but they are believed to include cytolysis of alveolar epithelial cells and macrophages (Gao *et al*., 2004), favoring intercellular spread of *M. tuberculosis* (Gao *et al*., 2004). ESAT-6 dissociates from CFP-10 under acidic conditions in the phagosome and can destabilize lysosomes, perhaps allowing *M. tuberculosis* and its products to eventually escape the phagosome (de Jonge *et al*., 2007).

2.4 Diagnosis of Tuberculosis

The approach to diagnosis is dependent on the stage of infection i.e. whether active infection or latent infection and the site of infection. In active (symptomatic) infections the preferred methods are based on the demonstration of the pathogen in appropriate specimen while immunologic tests are employed in suspected (latent, asymptomatic) infections (Konstatinos, 2010).

The definitive diagnosis of active Tuberculosis like most infective diseases is based on identification of the bacterium from an appropriate specimen. This is relatively easy in pulmonary infections however, extra-pulmonary infections presents a big problem since tuberculosis often presents with non-specific signs and symptoms; hence a high index of suspicion is required from the clinician (Konstatinos, 2010). A complete medical evaluation for tuberculosis must include a Medical history, a Physical examination, Chest X-ray, microbiological smears and cultures. It may also include a tuberculin skin test, Nucleic Acid Amplification test and a Serological Test.

2.4.1 Clinical Evaluation

The patient with pulmonary tuberculosis (PTB) usually presents with a history of chronic cough of not less than 6-weeks duration, significant weight loss of not less than 20% within 6-weeks of the illness, persistent fever and night sweat.

The cough is usually productive of thick mucoid sputum which may be blood stained and frank expectoration of blood (haemoptysis) in the later stages of the illness. The bloody nature of the sputum is as a result of the invasion of the alveolar vascular bed by the tubercle, which cause damage to the vessels and subsequent bleeding into the alveolus.

There may also be a history of contact with person(s) suffering from chronic cough in the patient's household or neighbourhood.

The Medical History alluded to above appeared to be straight-forward, however there are a lot of pitfalls therein.

1. All the signs and symptoms listed above are not specific to Tuberculosis, virtually all chronic disease of the lung can produce one or combinations of similar symptoms in the patients

e.g. Pulmonary Aspergillosis can lead to chronic cough and the caseating type can actually lead to collapse and bleeding which may mimic the haemoptysis of PTB.

2. Many chronic infectious diseases can also present with persistent fever of several weeks duration e.g. Histoplasmosis, Aspergillosis, and HIV. Many non-infectious but chronic illnesses can also present with persistent fever of several weeks duration e.g. malignancy. Therefore persistent fever is not sine qua non of Tuberculosis.

3. Significant Weight loss is also a feature of several illnesses, especially chronic illnesses like Malignancy, Gastrointestinal disease like gastric outlet obstruction and diarrhoea, HIV.

From the foregoing it becomes obvious that it will be difficult to rely solely on medical history as a means of diagnosing Pulmonary Tuberculosis.

Difficult as the diagnosis of Pulmonary Tuberculosis may seem it is even more so with the Extra Pulmonary variants where disease may present with symptoms that are as varied as the organs involved e.g. Headache and/or loss of consciousness in Central Nervous System which is similar to the presentation in bacterial, non-tuberculous infection of the meninges, persistent diarrhoea in Gastro Intestinal Tuberculosis similar to presentation in other bacterial diarrhoea diseases of the Gastro Intestinal System.

2.4.2 Chest x-ray

Chest X-rays are valuable for detecting pulmonary lesions of tuberculosis, however activity of disease cannot be judged with certainty. Classic upper zone chest X-ray changes can be due to other pathology and pulmonary tuberculosis can have many other non-classic presentations with broad differential diagnoses. Unusual chest X-ray presentations (including normal chest X-ray) are more common in tuberculosis patients with immune deficiencies and other co-morbidities (Konstatinos, 2010).

X-ray is unreliable for diagnosing and monitoring treatment of tuberculosis, because;

- No chest X-ray pattern is absolutely typical of TB
- 10-15% of culture-positive TB patients not diagnosed by X-ray
- 40% of patients diagnosed as having TB on the basis of x-ray alone do not have active TB. (Toman, 1979)

2.4.3 Specimen Microscopy

Since *Mycobacterium tuberculosis* has a cell wall but lacks a phospholipids outer membrane, it is classified as a Gram-positive bacterium. However, if a Gram stain is performed, *Mycobacterium tuberculosis* either stains very weakly Gram-positive or does not retain the dye due to the high lipid and mycolic acid content of its cell wall (Southwick, 2007). However, *Mycobacterium tuberculosis* retains certain stains after pre-treatment with acidic solution; it is therefore classified as an Acid-Fast Bacillus (AFB) (Kumar *et al.*, 2007).

The most common acid-fast staining technique, the Ziehl-Neelsen stain, dyes AFB's a bright red that stands out clearly against a blue background. Other ways to visualize AFBs include an Auramine-rhodamine stain and fluorescent microscopy. (Konstatinos, 2010)

Although this technique (sample staining), along with Nucleic Acid Amplification, allows early identification it fails to detect many culture-positive cases. Nevertheless, microscopy for Acid-Fast Bacilli rapidly identifies the most infectious tuberculosis cases and a positive sputum smear is sufficient for provisional diagnosis of tuberculosis. (Konstatinos, 2010)

Ziehl-Neelsen (Z-N) Staining and Microscopy is the only Laboratory diagnostic modality approved by the World Health Organization (WHO) in the diagnosis and monitoring of TB treatment in its Directly Observed Treatment Strategy (DOTS) in the developing countries with very high disease

burden. It is cheap (highly subsidized by the WHO in the DOTS programme), can be performed in most developing countries with little resources and the result can be made available within a 48-72 hours so that treatment can commence at the earliest possible time.

However, Z-N stain and Microscopy has its own drawbacks;

- Tests are not suitable for diagnosis of Extra-pulmonary TB, smear-negative and childhood Tuberculosis infection. Some patients in these categories are not able to produce appropriate specimen.
- They can give false positive results in infection with Non-Tuberculous Mycobacteria (NTM) infection.
- They can give false negative results in pauci-bacillary TB infections.
- 3 sputum specimens need to be examined and this is not convenient for patients and drop-outs are likely, Patients ask for a test that requires a single visit (Pai *et al*., 2008).

2.4.3.1 Specimen Sample

The specimen sample in the investigation of tuberculosis depends on the organ and/or tissue involved. Collection of specimens should include three sputum specimens whatever the suspected site of disease (pulmonary or extra-pulmonary), unless chest X-ray is normal and there are no respiratory symptoms in a person with localized extra-pulmonary disease (Konstatinos, 2010). Other specimen sample may include the Cerebrospinal fluid in suspected tuberculous meningitis; gastric washing in children and stool in suspected tuberculosis of the abdomen, 24-hour urine in uro-genital tuberculosis and whole blood in miliary tuberculosis.

2.4.4 Nucleic Acid Amplification (NAA)

When smears are positive for acid-fast bacilli, nucleic acid amplification of *M. tuberculosis* DNA can be used to rule out non-Tuberculous Mycobacteriosis. This test has almost 100% specificity and

sensitivity in acid-fast bacilli positive smears, with results provided within a few days (and potentially on the same day). While a negative nucleic acid amplification test of acid-fast bacilli almost excludes tuberculosis, the test can rarely be falsely negative in pulmonary tuberculosis (Konstatinos, 2010).

Current evidence however suggests that NAA tests cannot replace conventional tests such as microscopy and culture; they should be used and interpreted in conjunction with conventional tests and clinical data (Payam *et al*., 2006).

NAA tests, in general, have high specificity and positive predictive value (Brodie, D and Schluger, N.W., 2005), these characteristics confer value in terms of their ability to rule out false results in TB. A positive test in a patient with a reasonably high pre-test probability is confirmatory of TB, particularly in patients with positive sputum smear microscopy. Therefore, NAA tests can be used to confirm that a positive sputum smear is due to *M. tuberculosis* (Brodie, D and Schluger, N.W., 2005). NAA tests, however, have relatively lower sensitivity and negative predictive value, particularly in patients with negative sputum smears and with extra-pulmonary TB. A negative test, therefore, does not rule out the diagnosis of TB. Therefore, NAA tests should not be performed if sputum smears are negative and if the index of clinical suspicion is low. A negative NAA test in a patient with a high index of clinical suspicion should not preclude continued investigation.

Because NAA tests can amplify dead mycobacteria DNA, they cannot distinguish viable from nonviable bacilli. Therefore they should not be used for monitoring in patients who are being treated (Payam *et al*., 2006)**.**

2.4.5 Specimen Culture

In nature, the bacterium can grow only within the cells of a host organism, but in the laboratory *M. tuberculosis* can be cultured in vitro (Parish, T. & Stoker, N., 1999).

The main problem with tuberculosis diagnosis using this method however is the difficulty in culturing this slow-growing organism in the laboratory (it may take 4 to 12 weeks for blood or sputum culture). *M. tuberculosis* is grown on a selective medium known as Lowenstein-Jensen medium, which has traditionally been used for this purpose. However, this method is quite slow, as this organism requires 6–8 weeks to grow, which delays reporting of results. A faster result can now be obtained using Middlebrook medium, a culture of the AFB can distinguish the various forms of Mycobacteria, although results from this may take four to eight weeks for a conclusive answer. New automated systems that are faster include the MB/BacT, BACTEC 9000, and the Mycobacterial Growth Indicator Tube (MGIT) (Niemann *et al.,* 2002).

2.4.5.1 Advantages of Specimen Culture

Mycobacterial culture and identification of *M. tuberculosis* provide a definitive diagnosis of TB, significantly increase the number of cases found (often by 30–50%) and allow earlier detection of cases (often before they become infectious). Culture also provides the necessary isolates for conventional Drug Susceptibility Testing (DST).

2.4.5.2 Disadvantages of Specimen Culture

Culture is more complex and expensive than microscopy, requiring facilities for media preparation, specimen processing and growth of organisms, specific laboratory equipment, skilled laboratory technicians and appropriate bio-safety conditions.

2.4.5.3 Limitations of Specimen Culture

Specimens must be decontaminated before culture to prevent overgrowth by other microorganisms. All decontamination methods are to some extent also harmful to Mycobacteria, and culture is therefore not 100% sensitive. Good laboratory practice maintains a delicate balance between the yield of mycobacteria and contamination by other microorganisms.

Solid and liquid culture methods are suitable for central reference laboratories (or regional laboratories in large countries). Usually, one culture laboratory is adequate to cover a population of 500 000–1 million. Solid culture methods are less expensive than liquid systems, but the results are invariably delayed because of the slow growth of mycobacteria. Several culture methods are recommended (WHO, 1998). Liquid culture increases the case yield by 10% over solid media, and automated systems reduce the diagnostic delay to days rather than weeks (WHO, 2007). Liquid systems are, however, more prone to contamination, and the manipulation of large volumes of infectious material mandates appropriate, adequate bio-safety measures.

Positive cultures must be identified to differentiate *M. tuberculosis* from Non Tuberculous Mycobacteria, which are more common in HIV-infected patients, with a prevalence that varies from country to country. Diseases due to Non-Tuberculous Mycobacteria are treated entirely differently from drug-resistant TB. As a minimum, laboratories performing DST must differentiate *M. tuberculosis* from non-tuberculous mycobacteria; further speciation is not recommended at programme level.

Confirmation is usually done from the biological characteristics of the culture growth and with selected molecular or biochemical tests (which invariably delay the final result) (WHO, 1998). Rapid immuno-chromatographic assays (so-called strip speciation tests) for species identification on culture isolates provide a definitive identification of *M. tuberculosis* in 15 min and are recommended (WHO, 2007). Molecular tests, biochemical methods and strip speciation assays are suitable for laboratories where culture and DST are performed.

2.4.6 The Tuberculin Skin Test

The tuberculin skin test is based on the observation that infection with *M. tuberculosis* caused cutaneous reactivity to tuberculin, a concentrated filtrate from cultures of *M. bovis* that had been

heat-killed (Menzies, 2002). The standard tuberculin test consists of 0.1 ml (5 tuberculin units) of PPD administered intracutaneously, usually in the volar surface of the forearm. The reaction is read 48 to 72 hours after injection, although a reading obtained up to one week later is accurate (Slutkin *et al.,* 1986)

The size of the reaction is determined by measurement of the indurations across the forearm at the site of the injection. The criterion for interpreting the reaction as positive (indicating the presence of tuberculosis infection) varies depending on certain characteristics of the person being tested (Robert *et al.,* 2002). The general principle is that if a smaller reaction is defined as indicating infection, the sensitivity of the test is increased in those who are at greatest risk for the development of tuberculosis. Sensitization to tuberculin can also be induced by infection with Non-Tuberculous Mycobacterium, including BCG, which is used in many parts of the world for active Immunization.

The interpretation of the tuberculin skin test depends upon the person's risk factors for infection and progression to tuberculosis disease, such as exposure to other cases of tuberculosis or immune suppression (CDC, 2000).

2.4.6.1 Centre for Disease Control (CDC) classification of tuberculin reaction

Indurations (palpable raised hardened area of skin) of more than 5–15 mm (depending upon the person's risk factors) to 5 Mantoux units is considered a positive result, indicating TB infection (CDC, 2005).

- **5 mm or more is positive in**

    a. HIV-positive person

    b. Recent contacts of TB case

c. Persons with nodular or fibrotic changes on CXR consistent with old healed TB

d. Patients with organ transplants and other immune-suppressed patients

- **10 mm or more is positive in**

a. Recent arrivals (less than 5 years) from high-prevalent countries

b. Injection drug users

c. Residents and employees of high-risk congregate settings (e.g., prisons, nursing homes, hospitals, homeless shelters, etc.)

d. Mycobacteriology lab personnel

e. Persons with clinical conditions that place them at high risk (e.g., diabetes, prolonged corticosteroid therapy, leukaemia, end-stage renal disease, chronic mal-absorption syndromes, low body weight, etc)

f. Children less than 4 years of age, or children and adolescents exposed to adults in high-risk categories

- **15 mm or more is positive in**

a. Persons with no known risk factors for TB

A tuberculin test conversion is defined as an increase of 10 mm or more within a 2-year period, regardless of age.

2.4.6.2 The Drawbacks of Tuberculous Skin Test (TST)

Over time, delayed hypersensitivity resulting from Mycobacterial infection may wane in some persons, resulting in a nonreactive tuberculin skin test despite the presence of true infection (anergy) (Robert *et al.*, 2002).

The stimulus of this negative tuberculin test may "boost" or increase the size of the reaction to a second test administered later, resulting in a positive tuberculin test and misleadingly suggesting tuberculin conversion (the "booster"phenomenon) (Brodie, D and Schluger, N.W.,2005).

The test is highly technical and therefore prone to errors, injection must be intra dermal, if given into the muscle there will be no reaction even if subject is infected with TB.

It is very prone to operator error especially in the reading and Patients also find it burdensome coming back for reading 48-72 hours later for reading.

2.4.7 Immunological In-Vitro Testing

New immune tests for in vitro diagnosis of tuberculosis infection, so-called interferon-γ release assays (IGRAs) and Immuno-chromatographic test with nitrocellulose paper have recently been developed:

- The QuantiFERON-TB Gold In-Tube (Cellestis, Australia),
- A whole blood enzyme-linked immunosorbent assay (ELISA),
- T-SPOT.TB (Oxford Immunotech,UK), an enzyme-linked immunospot assay (ELISPOT) using peripheral blood mononuclear cells (PBMCs).

In those assays, interferon-γ release by sensitized T lymphocytes is measured after overnight stimulation with antigens highly specific for M. tuberculosis (Early Secreted Antigen Target 6 kilodalton [ESAT-6], Culture Filtrate Protein 10 kilodalton [CFP-10], in the Quantiferon TB Gold In-Tube, additionally TB 7.7). These antigens are expressed by all mycobacteria from the M.

tuberculosis complex but not by common NTM (e.g. *M. avium*) as well as BCG vaccination strains. Cross reactivity occurs with some NTM such as *M. kansasii, M. marinum, M. szulgai,* and *M. flavescens,* which are rare causes of mycobacteria infections in children in central Europe. Like the TST, IGRAs cannot differentiate clearly between LTBI and active disease. In active tuberculosis, IGRAs have shown to be at least as sensitive as the TST (80–97%) with a higher specificity (90–100%) (Pathan *et al.,* 2001). In the diagnosis of LTBI, IGRAs correlate better with exposure to active tuberculosis than TST (Liebeschuetz *et al.,* 2004). There is rising evidence that IGRAs are more sensitive than TST in high-risk populations such as immune-compromised patients (Rose*et al.,* 2001). Data in children is still limited, but IGRAs seem to be more sensitive than TST in young as well as immune-compromised children (Control and prevention of tuberculosis in the United Kingdom, 2000). They show a high correlation with a gradient of exposure, are highly specific, and can therefore help to discriminate between infections with *M. tuberculosis* and NTM in TST positive children (Soysal *et al.,* 2005).

The newer Interferon Gamma Release Assays (IGRA) overcomes many of these problems. IGRAs are in vitro blood tests that are more specific than the skin test. IGRAs detect the release of interferon gamma in response to Mycobacterial proteins such as ESAT-6, (Ewer *et al.,* 2003). These are not affected by immunization or environmental Mycobacteria, so generate fewer false positive results, (Pai *et al.,* 2008) There is also evidence that the T-SPOT.TB, IGRA is more sensitive than the skin test, (Lalvani *et al*., 2005).

The enzyme-linked immunospot assay (ELISPOT) is another blood test available in the UK that may replace the skin test for diagnosis, (Ewer *et al.,* 2003).T-SPOT.TB, a type of ELISPOT assay, (Meier *et. al.*, 2005) counts the number of activated T lymphocytes that secrete interferon gamma (IFN-γ).

For diagnosing latent TB, three systematic reviews of IGRAs concluded that the tests shows excellent specificity in distinguish latent TB from prior vaccination, (Liu *et al*., 2004).

According to a study from Korea, where there is a high prevalence of LTBI, QuantiFERON-TB Gold and T-SPOT.TB have good sensitivity but reduced specificity for diagnosing *active* TB, due to their ability to detect latent TB, (Mazurek *et al.*, 2005).

There is a new serological method which employs the use of Antigens from Bacteria of the MTC's impregnated onto a nitrocellulose paper at a designated spot; this paper is dipped for a short while (about ten seconds) at one end into a serum collected from a patient suspected of having tuberculosis disease, the serum migrate upwards by capillary action. The Antibody from serum of the infected individual will react with the antigen on the nitrocellulose paper at the designated point. The site of Antigen-Antibody reaction will show some indentation that will indicate positivity of the patient to the test.

Immunological methods use the specific humoral or cellular responses of the host to infer the presence of infection or disease, (Sudha *et al.*, 2000) They do not require a specimen from the site of infection, therefore diagnosis of extra-pulmonary Tuberculosis will be a lot easier than hitherto using this method.

This test though costlier and scarce is simple, fast and easy to use especially in the developing economy where the disease is most prevalent. It has however generated a lot of controversy.

2.4.7.1 Advantages of Immunological In-Vitro Testing

- They can be developed into point of care test
- They provide rapid results, so Clinician can take decision faster

- For some patients especially children, blood specimen are easier to get than sputum
- For extra-pulmonary TB serology if accurate, could replace more invasive test.

2.4.7.2 The Disadvantages of Immunological In-Vitro Testing

- They are costlier than existing methods
- They are not standardized
- They cannot distinguish between old and new infections.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Inclusion Criteria

The study was designed to compare the performance of the two tests to diagnose Tuberculosis; therefore patients chosen for this test must meet a certain criteria that will enhance the attainment of this objective. It was therefore decided that:

- Only patient whose TB status was yet to be determined at the time of presentation were recruited for the study.
- Only patients who completed the submission of three (3) sputum samples were included in the study.
- Patients whose sera were viscous such that migrations of the sera on the nitrocellulose strips were impeded were also excluded.

3.2 Methods

All the patients were investigated for Tuberculosis using Two (2) different modalities concurrently; these were;

1. Three (3) Sputum samples were obtained from the patient at different times and stained for Acid Fast Bacilli, using the Ziehl-Neelsen (Z-N) stain and Microscopy.
2. Detection of interferon gamma using strips impregnated with antibody to interferon gamma on the serum of the patients adhering strictly to the manufacturers' instructions.

3.3 Sample Collection

3.3.1 Sputum for AFB Staining

Three sputum samples were collected from each patients, a spot sample was collected on the day of presentation (Spot 1), the subject was then given a specimen bottle to collect the early morning sputum of the next day (E/Morn), a third sample was collected at the clinic on submission of sample 2 (Spot 2). Sputum collection was done in open air or well-ventilated room. Specimens were collected in a plastic, wide mouth, leak proof container with a screw top lid. The appearance of specimens was noted as purulent, muco-purulent, or blood stained.

The three samples were then stained for Acid Fast Bacilli using the Ziehl-Neelsen staining method. A total of 756 samples (3 per patient) were collected and stained, Smear results were considered positive if any of the three (3) samples were positive for Acid Fast Bacilli as espoused by Mark *et al.* (2003)

3.3.2 Blood for Serology

This involved the collection of whole blood from the Patients, extraction of the serum and demonstration of Antigen-Antibody reaction using commercial TB immuno-chromatographic test strips. 5ml of whole blood was collected from each patient on the first day of presentation. The collection site was the ante-cubital fossa of the forearm after cleaning with methylated spirit.

A total of 300 blood samples were collected but only 252 samples were eventually used in the study, due to some factors that include;

- Three (3) Patients who passed away before the completion of investigation

- In six subjects (6), the kits did not yield readable results, either because of operator error or because of viscous serum that failed to migrate on the nitrocellulose test strip.
- The remaining thirty-nine (39) patients could not complete the test for unknown reasons, the inability to complete the sputum sample collection means that they were not able to meet the inclusion criteria; hence they have to be excluded from the study.

3.4 Methods/Procedure

3.4.1 Ziehl-Neelsen AFB staining

3.4.1.1 Slide Preparation

A concentrated smear is made by picking up the sediment using a sterile disposable 10µ loop (Broom head was used as an alternative at sometimes) and spread in the middle of the slide 2 x 1 cm and allowed to dry.

The cap of the specimen container was replaced firmly on the plastic tube.

The concentrated smear is then placed on a specimen rack and heat with a naked flame for 3 min for fixing.

3.4.1.2 Slide Processing

The fixed slide was then flooded with carbol fuchsin and heated using an open flame underneath the slide until steam is seen, for five minutes. The specimen was not allowed to boil or dry.

It was then rinsed with water. Acetone was then added for two minutes to decolorize the smear. The slide was then rinsed again with water, Counter-staining was then done with methylene blue for one minute. The slide was thereafter rinsed with water and allowed to dry. The back of the slides were wiped clean and the slides were placed in a draining rack for the smears to dry.

The slide was thereafter examined under oil immersion with a 100x objective and eyepiece of moderate magnification (8x –10x).100 fields were scanned before considering the stain negative. Acid fast organisms stain red against a Blue background and may appear slightly beaded.

Results are recorded according to the normal convention; if there were more than 10 Acid Fast Bacilli (AFB) per oil immersion field with 20 fields examined the result is considered as positive and graded 3+, if there were more than 10 Acid Fast Bacilli (AFB) per oil immersion field with 50 fields examined the result is considered as positive and graded 2+, if there were 10-99 Acid Fast Bacilli (AFB) per 100 oil immersion fields with 100 fields examined the result is considered as positive and graded 1+, if there were 1-9 Acid Fast Bacilli (AFB) per 100 oil immersion fields with 100 fields examined the result is considered as scanty and the exact number is recorded, however if no AFB is seen per 100 Oil immersion fields and having examined 100 fields the result is considered negative and graded 0. For the purpose of this study no distinction was made between 1+, 2+, 3+ or Scanty, all were recorded as positive.

3.4.2 Serology

The blood collected is allowed to settle over time separating them into the packed cell volume (PCV) and the serum. Alternatively the specimen was separated into serum and PCV by centrifuge at 30 ppm over 5 minutes. Both methods were used in the study as the occasion demands, centrifuge were used when available, otherwise the samples were collected and allowed to settle down naturally under gravity, separating the PCV from the Serum.

The separated serum was then harvested by pouring the serum which is the straw-coloured layer on top of the mixture in a separate test-tube; the test strip was then immersed into the serum for 10 seconds at the designated end following the manufacturer’s recommendation.

The test strip is then read within ten minutes of removal. The presence of 2 clear lines on the test strips is indicative of reactivity of the patient's serum to the antigen on the test strip (that is, the test line and the control line).If only one line shows on the test strip after ten minutes, then the result is negative or invalid (that is, if only the control line is shown, the test is negative conversely if the test line shows and the control line does not show, the test is deemed to be invalid.)

If no line shows after the stipulated time of ten minutes, then the test is invalid. The result was then recorded in a result slip designed for the purpose.

3.5 Principles of the Test

3.5.1 Ziehl-Neelsen Staining Technique

The lipoid capsule of the mycobacterium is of such high molecular weight that it is waxy at room temperature and successful penetration by the aqueous-based staining solutions (such as Gram's stain) is prevented.

The lipoid capsule of the acid-fast organism however, takes up carbol-fuchsin and resists decolourization with a dilute acid rinse.

3.5.2 Serological Test.

The species of mycobacterium in the *Mycobacterium tuberculosis* Complex (MTC) are the causative agents of Tuberculosis. They contain a unique group of antigens which are located in the region of Difference 1 (RD-1) in their genome. Two of these antigens namely Culture Filtrate Protein 10 (CFP-10) and Early Secreted Antigen Target 6 (ESAT-6) are used to identify subjects that have active TB infection. Patients infected with MTC are expected to produce Antibody to these Antigens, such that when their serum containing one or more of these Antibodies comes in contact with the Test Strips impregnated with one or more of these Antigens, an Antigen-Antibody reaction occurs which is demonstrable on the Test Strip according the manufacturer's specification.

3.6 QUESTIONAIRE

Questionnaire was administered on each subject to gather biometric data including the age and sex and the clinical status especially the HIV status of the patient to aid in the analysis of results later on.

3.7 STATISTICAL ANALYSIS

Statistical tests of agreement were performed on the data set to estimate how well the two methods agree in the diagnosis of tuberculosis. This was done by calculating the Raw Agreement Index, Cohen's Kappa Coefficient, the Kendal Tau's Test and the Mcnemar's test.

Statistical Test of Agreement was chosen because; in clinical measurement comparison of a new measurement technique with an established one is often needed to see whether they agree sufficiently for the new to replace the old, (Altman, D. G. and Bland, J.M. 1986).

While some of the tests are to be calculated manually, Computer Statistical software packages; Statistical Package for Social Science (SPSS®), MedCalc® and Analyse-it® was used to analyze some of the results.

Also, the Sensitivity, Specificity, Positive predictive and Negative predictive value of the test strip was measured using the result of the Sputum Z-N stain technique as the Gold standard.

3.7.1 **Raw Agreement Index**: The measure of raw agreement indicates the number of instances in which raters (methods) agree exactly, relative to the total number of judgements (tests), (von Eye *et al*., 2007).

It is calculated mathematically by;

$$P = \underline{\sum \pi_{ij}}$$

n

Where, P is the raw agreement index

$\pi_{ij,}$ is the sum of the diagonal agreements, both positive and negative

n is the total number of participants in the study.

The raw agreement index is easy to calculate but has very limited diagnostic value.

Related measures of agreements are the Negative Agreement and the Positive Agreement.

a. **Positive Agreement** = $\frac{\text{Number of Positive Agreement}}{\text{Total number of all observations}}$

b. **Negative Agreement** = $\frac{\text{Number of Negative Agreement}}{\text{Total number of all observation}}$

3.7.2 The Kendall tau test, $T^2 = \chi^2/(C-1)n$

Where; χ^2 is the chi-square,

C is the number of columns,

n is the total number of participants in the study.

3.7.3 The Cohen's Kappa test. $\kappa = (p_o-p_e)/ (n-p_e)$

Where; p_o = probability or proportion expected by chance;

p_e = probability or proportion observed.

n = total observed

The Cohen's Kappa Coefficient is interpreted as elucidated in Table 3.3.

3.7.4 **McNemar's test** for marginal symmetry: McNemar's test is a non-parametric method used on nominal data. It is applied to 2 × 2 contingency tables with a dichotomous trait, with matched pairs of subjects, to determine whether the row and column marginal frequencies are equal ("marginal homogeneity").

$H_0, \pi ij = \pi ji$

$H_{1,} \pi ij \neq \pi ji$

Where, H_0 is the null hypothesis

H_1 is the alternative hypothesis

πij is the total row marginal frequency

πji is the total column marginal frequency

It is calculated by finding the χ^2 of the data and comparing the result with the tabulated χ^2 at 0.05 and (C-1) degree of freedom. If the tabulated figure is higher than the calculated figure then the Ho is accepted otherwise, it is rejected.

That is, $$\chi^2 = \frac{\sum (\pi ij - \pi ji)^2}{\pi ij + \pi ji}$$

3.7.5 **Sensitivity:** Ability to test positive in a known case of the disease =

$$\frac{\text{True Positives}}{\text{True positives +False Negatives}}$$

3.7.6 **Specificity**: Ability to test negative in a subject known not to have the disease. =

$$\frac{\text{True Negatives}}{\text{True Negatives + False Positives}}$$

3.7.7 **Positive Predictive Value**: Ability to correctly detect a disease =

$$\frac{\text{True Positives}}{\text{True Positives + False Positives}}$$

3.7.8 **Negative Predictive Value:** Ability to correctly exclude the disease =

$$\frac{\text{True Negatives}}{\text{True Negatives + False Negatives}}$$

TABLE 3.1: Interpretation of Cohen's Kappa Coefficient

Value of κ	Strength of Agreement
≤0.20	Poor
0.21 - 0.40	Fair
0.41-0.60	Moderate
0.61-0.80	Good
0.81-1.00	Very good

CHAPTER FOUR

4.0 RESULTSAND DISCUSSION

4.1 Results

A total of 300 patients referred to the Directly Observed Treatment (DOTS) clinic in the General Hospital, Minna, were investigated for Tuberculosis, using commercially sourced TB Immuno-chromatological Test Strip on samples of their serum and 3 sputum specimens collected at different times for AFB staining and Microscopy. Only 252 patients completed the test, out of which 116 (46%) were females and 136 (54 %) were Males. The age range of the patients was between 7 and 73 years of age, (Table 4.3). Forty-eight (48) patients were not able to complete the test for various including;

Three (3) Patients who passed away before the completion of investigation, in six (6) subjects, the kits did not yield readable results, either because of operator error or because of viscous serum that failed to migrate on the nitrocellulose test strip. The remaining thirty-nine (39) patients could not complete the test for unknown reasons.

Of the 252 patients evaluated only 19(7.54%) were tested positive for Tuberculosis by the serological method using Immuno-chromatographic test strip (ICT), of which 11 (57.89%) were Males and 8 (42.11%) were females, while 51(20.23%) sputum AFB positive by sputum microscopy of which 34 (66.67%) were Males and 17 (33.33%) were Females, (Table 4.1).

The highest percentage of patients that participated in the study was from the 16-30 years group. Incidentally the Group also recorded the highest positive case for Z-N stain at 29

cases or 56.86% (Table 4.3); the group also recorded the highest positive rate for serology at 10 or 52.63% (Table 4.4).

Table 4.1 Percentage of patients positive for TB at different stages of the tests.

Number of Patients		Tests carried out				
		Serology		Sputum Z-N stain		
Sex	Number of patients (%)	Number (+)	Number (-)	Spot 1	E/Morn	Spot 2
Male	136 (54)	11	125	32	34	33
Female	116 (46)	8	106`	13	17	18

TABLE 4.2: AGE DISTRIBUTION OF PATIENTS IN THE STUDY

Age Group (years)	Male	Female	Total (%)
0-15	6	5	11(4.37)
16-30	53	62	115(45.63)
31-45	32	27	59(23.41)
46-60	33	12	45(17.86)
61-75	7	1	8(3.17)
Indeterminate (AD)	7	7	14(5.56)
Total	138	113	252(100)

TABLE 4.3: AGE DISTRIBUTION OF PATIENTS POSITIVE FOR Z-N STAIN

Age Group (years)	Male	Female	Total (%)
0-15	1	0	1(1.96)
16-30	16	13	29(56.86)
31-45	10	2	12(23.53)
46-60	4	0	4(7.84)
61-75	1	0	1(1.96)
Indeterminate (AD)	2	2	4(7.84)
Total	34	17	51(100)

TABLE 4.4: AGE DISTRIBUTION OF PATIENTS FOR POSITIVE SEROLOGY

Age Group (years)	Male	Female	Total (%)
0-15	1	0	1(5.26)
16-30	5	5	10(52.63)
31-45	2	1	3(15.79)
46-60	2	1	3(15.79)
61-75	1	0	1(5.26)
Indeterminate (AD)	0	1	1(5.26)
Total	11	8	19(100)

The Raw Agreement between the two (2) methods for this study was an average of 76% (range of 75-77%), (Table 4.5). The average percentage of Positive Agreement was 1.4% (range of 1.2-1.6%) while the average percentage of Negative Agreement was 75% (range of 74-76%), (See Table 4.5).

The Kendal Tau's Coefficient of Agreement ranged between 0.006 and 0.015(range of κ is 0.006-0.014; range of T is 0.0056-0.0154), (Table 4.5).

The range of the χ^2 for the study was 14.9, Range 10.78-16.52, these are all higher than the tabulated $\chi^2_{1,\,0.05}$ which is 3.84(Table 4.5).

The Sensitivity of the Test Strip in the study was 12.76%, while the Specificity was 92.42%. It has a Positive Predictive value of 16.22%, while the negative predictive value was 80.53%, (Table 4.5).

TABLE 4.5: Summary of the results of statistical tests for the study

STATISTICAL TEST	SCORES
RAW AGREEMENT INDEX	75%
NEGATIVE AGREEMENT	74.67%
POSITIVE AGREEMENT	1.47%
COHEN'S KAPPA	0.009
KENDAL TAU	0.009
SENSITIVITY	12.76%
SPECIFICITY	92.42%
POSITIVE PREDICTIVE VALUE	16.22%
NEGATIVE PREDICTIVE VALUE	80.53%

4.2 Discussions

The need for improved TB diagnostics has long been recognized. Local diagnostic needs may vary markedly, and it is unlikely that a single diagnostic test 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 the peripheral level, and decrease diagnostic confusion and delay, these was according to Perkins *et al*., (2003).

Recognizing these needs, serologic approaches to TB diagnosis have been tried on and off since the sero-agglutination work of Arloing in 1898. Early TB serology tests used PPD or other crude antigens and showed relatively poor specificity, as many of the dominant antibody responses are to shared antigens (Perkins *et al*., 2003).

It is in the light of the foregoing that the introduction of the Immuno-chromatographic test strip into the local market met the local Medical Community in Minna, Niger State, with excitement. However the discordant results observed in some patients who were investigated for TB using this Test Strip, prompted this study.

The choice of Sputum Z-N stain as the Gold standard in this study was made because that was the method most favoured by the World Health Organization in its Directly Observed Treatment Strategy programme for control of Tuberculosis, even though the method itself is not without its flaws as earlier explained.

The Raw Agreement index, which is a measure of the percentage of concordance of result between the two methods was 75% in this study (the highest value of 100% shows total agreement), this is a high value that however has very little diagnostic value. That is because it measure where the two methods gives similar result, whether positive or negative (Cicchetti, D.V. & Feinstein, A.R., 1990).

The Positive Agreement index however was 1.47%, which shows a very negligible concordance in positive identification of MTB by the two methods, which means that the two methods cannot substitute one for the other in the clinical diagnosis of Tuberculosis. The Negative Agreement Index was 74.67% (Table 4.2), that is, there is a high level of agreement between both in ruling out Tuberculosis disease.

The Coefficient of Agreement between the Sputum Z-N stain and the TB Immuno-chromatograhic Test Strip in this study was 0.009(range of κ is 0.006-0.014; range of T is 0.0056-0.0154), (Table 4.2) this shows a very low level of Agreement (concordance) which makes it less suitable as a replacement to the widely used Sputum Z-N stain and Microscopy in this study.

The sensitivity of this test was 12.76%.Conversely a similar work done by Mark *et al.,* (2003), which recorded a sensitivity of 27.6%, though the higher figure of Mark's study might not be unconnected with the use of multiple Antigens instead of the single antigen that was used in this study. Both are however in accordance with the pooled sensitivity of (0% to 100%) in a Meta-analysis of results of similar studies in immunological diagnosis of TB that was carried out in a study organized by the WHO study group, (Steingart *et al.*, 2011).

The high specificity of 92.42% for TB Immuno-chromatograhic Test Strip does not completely rule out the issue of false negative which is also the bane of Sputum AFB; it is also in accordance with Steingart *et al.,* (2011), where it was found that specificity of similar studies was in the region of (31% to 100%).

The Positive Predictive value of 16.22% is very low, it is an indicative of it poor ability to detect the disease in a sufferer, it therefore does not help the clinician to repose much confidence in the method.

The very good Negative Predictive value of 80.53% still needs to be compared against Tuberculin Skin Test (TST) and/or a placebo to determine it suitability as a screening test.

With all these, little wonder then that the World Health Organization (WHO) in 2011, for the first time in its history had to come out to issue a negative policy on the use of serological test in the Diagnosis of Tuberculosis. This arose because of the finding in 2008 of a panel that carried out an assessment of 19 commercial assays by Tropical Disease Research (TDR), the UN special programme for research and training in tropical diseases found that none of the assays were good enough to replace sputum microscopy or as an add-on test to rule out tuberculosis, (Kelly, 2011).

In the word of the WHO, 'Commercial serological tests provide inconsistent and imprecise findings resulting in highly variable values for sensitivity and specificity. There is no evidence that existing commercial serological assays improve patient-important outcomes, and high proportions of false-positive and false-negative results adversely impact patient safety. Overall data quality was graded as very low and it is strongly recommended that these tests not be used for the diagnosis of pulmonary and extra-pulmonary TB', (Kelly, 2011).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In this study, the performance of Commercial Chromatographic TB Test Strip using serum in the diagnosis of Pulmonary Tuberculosis was poor as indicated by the low sensitivity of 12.76%. (Table 4.5)

The Positive Agreement of 1.47% between the Commercial Chromatographic TB Test Strip using serum and sputum Z-N staining and Microscopy in the Diagnosis of Pulmonary Tuberculosis was too low to allow the Test strip to replace Z-N stain in the diagnosis of Tuberculosis. (Table 4.5)

Therefore, available Commercial Chromatographic TB Test Strip cannot replace sputum Z-N staining and Microscopy in the Diagnosis of Pulmonary Tuberculosis for now.

Commercial Chromatographic TB Test Strip can also not be recommended as a screening test to replace Tuberculin Skin test (TST).

5.2 Recommendations

The ultimate means of diagnosing an infectious disease remains isolation of the infective agent(s) from the sufferer of the disease, the elusiveness of Mycobacteria agents is still a matter for concern especially with regards to getting appropriate specimen sample from the site of infection, cumbersomeness of the staining technique, long time require for culturing with the attendant low yield and the poor specificity of the clinical sign and symptoms of the disease.

There is therefore, a future for immunological test methods that does not depend on the isolation of the agent in the diagnosis of TB; however more work still needed to be done to achieve that. It may

be worthwhile to use multiple antigens instead of a single Antigen; this may improve the sensitivity of the test.

Also, any diagnostic method to be introduced into the market must pass through appropriate regulatory agency before being sold in the open market, it is quite unfortunate that the developing countries where the disease burden is highest are the ones with ineffective regulatory bodies, little wonder then that this discredited test method sand similar ones which were not allowed to be used in the developed world where it was manufactured, are being allowed in the poor developing world.

Effort must also be made to evolve a standardized method and manufacturers should be made to disclose fully the antigen(s) being used in their test strip.

For now, diagnosis of Tuberculosis should be multi-pronged, combining a strong index of suspicion with good clinical evaluation, Specimen smear Z-N staining and Microscopy including fluorescence and possibly Polymerase Chain Reaction (PCR) when available, to confirm smear positive TB.

Most importantly, routine immunization with Bacille Calmette Guerin (BCG) at infancy, still remain the best method to prevent Tuberculosis. Effort should therefore be made to strengthen routine immunization.

Vigorous case detection, including screening of members of the household of confirmed Tuberculosis sufferers and rigorous treatment are still the best strategy to control the menace of Tuberculosis.

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APPENDICES

Appendix I: Raw Data from the Field

	PATIENT'S INFO			SEROLGY		ZIEHL-NEELSEN STAINING		
S/N	PIN	AGE	SEX	RESULTS		SPOT 1	E/MORN	SPOT 2
1	408	40	F	-ve	+ve	-ve	-ve	-ve
2	410	22	F	-ve	+ve	-ve	-ve	-ve
3	414	40	F	-ve	+ve	-ve	-ve	-ve
4	419	14	M	-ve	+ve	+ve	+ve	+ve
5	425	45	F	-ve	+ve	-ve	+ve	+ve
6	426	32	M	-ve	+ve	-ve	-ve	-ve
7	427	38	M	-ve	+ve	-ve	-ve	-ve
8	429	8	F	-ve	+ve	-ve	-ve	-ve
9	430	28	F	-ve	+ve	-ve	-ve	-ve
10	436	55	M	-ve	+ve	-ve	-ve	-ve
11	529	31	F	-ve	+ve	-ve	-ve	-ve
12	579	AD	M	-ve	+ve	-ve	-ve	-ve
13	582	40	F	-ve	+ve	-ve	-ve	-ve
14	583	AD	F	-ve	+ve	-ve	-ve	-ve
15	584	AD	M	-ve	+ve	-ve	-ve	-ve
16	588	22	M	-ve	+ve	-ve	-ve	-ve
17	598	AD	M	-ve	+ve	+ve	+ve	+ve
18	608	70	M	-ve	+ve	-ve	-ve	-ve
19	609	35	F	-ve	+ve	-ve	-ve	-ve
20	611	50	M	-ve	+ve	-ve	-ve	-ve
21	612	18	F	-ve	+ve	+ve	+ve	+ve
22	615	43	M	-ve	+ve	-ve	-ve	-ve
23	619	42	M	-ve	+ve	+ve	+ve	+ve
24	623	15	F	-ve	+ve	-ve	-ve	-ve
25	625	14	M	+ve	-ve	-ve	-ve	-ve
26	626	20	M	-ve	+ve	-ve	-ve	-ve
27	631	18	M	+ve	-ve	-ve	-ve	-ve
28	633	28	M	-ve	+ve	+ve	+ve	+ve
29	636	19	F	-ve	+ve	-ve	-ve	-ve
30	639	50	M	-ve	+ve	-ve	-ve	-ve
31	641	22	M	+ve	-ve	-ve	-ve	-ve
32	642	30	F	-ve	+ve	-ve	-ve	-ve
33	645	18	M	-ve	+ve	-ve	-ve	-ve
34	646	30	F	-ve	+ve	-ve	-ve	-ve
35	647	22	F	-ve	+ve	+ve	+ve	+ve
36	649	30	M	-ve	+ve	-ve	-ve	-ve

37	650	25	F	-ve	+ve	-ve	-ve	-ve
38	651	50	F	-ve	+ve	-ve	-ve	-ve
39	653	51	F	-ve	+ve	-ve	-ve	-ve
40	655	45	F	-ve	+ve	-ve	-ve	-ve
41	656	22	M	-ve	+ve	-ve	-ve	-ve
42	660	23	F	-ve	+ve	-ve	-ve	-ve
43	661	52	M	-ve	+ve	+ve	+ve	+ve
44	662	50	M	-ve	+ve	-ve	-ve	-ve
45	663	23	M	-ve	+ve	-ve	-ve	-ve
46	664	32	F	-ve	+ve	-ve	-ve	-ve
47	667	41	M	-ve	+ve	+ve	+ve	+ve
48	668	45	M	-ve	+ve	-ve	-ve	-ve

	PATIENT'S INFO			SEROLGY		ZIEHL-NEELSEN STAINING		
S/N	**PIN**	**AGE**	**SEX**	**RESULTS**		**SPOT 1**	**E/MORN**	**SPOT 2**
49	669	31	F	-ve	+ve	-ve	-ve	-ve
50	671	50	M	-ve	+ve	-ve	-ve	-ve
51	672	30	F	-ve	+ve	+ve	-ve	+ve
52	675	35	M	-ve	+ve	+ve	+ve	+ve
53	677	31	M	+ve	-ve	-ve	-ve	-ve
54	678	55	M	-ve	+ve	-ve	-ve	-ve
55	679	30	F	-ve	+ve	+ve	+ve	+ve
56	683	8	F	-ve	+ve	-ve	-ve	-ve
57	685	28	M	-ve	+ve	-ve	-ve	-ve
58	686	30	M	-ve	+ve	-ve	-ve	-ve
59	688	40	F	-ve	+ve	-ve	-ve	-ve
60	689	30	F	-ve	+ve	-ve	-ve	-ve
61	690	28	F	+ve	-ve	-ve	-ve	-ve
62	691	27	F	-ve	+ve	-ve	-ve	-ve
63	692	36	M	-ve	+ve	-ve	-ve	-ve
64	693	22	M	-ve	+ve	+ve	+ve	+ve
65	697	25	M	+ve	-ve	-ve	-ve	-ve
66	698	45	F	-ve	+ve	-ve	-ve	-ve
67	699	25	M	-ve	+ve	-ve	-ve	-ve
68	701	50	F	-ve	+ve	-ve	-ve	-ve
69	703	55	M	-ve	+ve	-ve	-ve	-ve
70	705	50	M	-ve	+ve	-ve	-ve	-ve
71	706	28	M	-ve	+ve	-ve	+ve	+ve
72	707	AD	F	-ve	+ve	-ve	+ve	+ve
73	712	35	F	-ve	+ve	-ve	-ve	-ve
74	713	29	M	-ve	+ve	+ve	+ve	+ve
75	714	55	F	-ve	+ve	-ve	-ve	-ve

76	717	30	M	-ve	+ve	-ve	-ve	-ve
77	718	50	F	-ve	+ve	-ve	-ve	-ve
78	720	37	M	-ve	+ve	-ve	-ve	-ve
79	721	27	M	-ve	+ve	-ve	-ve	-ve
80	722	50	M	-ve	+ve	-ve	-ve	-ve
81	723	56	M	+ve	-ve	-ve	-ve	-ve
82	724	25	F	+ve	-ve	-ve	-ve	-ve
83	725	52	M	-ve	+ve	-ve	-ve	-ve
84	729	40	F	-ve	+ve	-ve	-ve	-ve
85	731	22	F	+ve	-ve	-ve	-ve	-ve
86	733	55	F	+ve	-ve	-ve	-ve	-ve
87	734	50	F	-ve	+ve	-ve	-ve	-ve
88	736	6	M	-ve	+ve	-ve	-ve	-ve
89	741	65	M	-ve	+ve	-ve	-ve	-ve
90	744	30	F	-ve	+ve	-ve	-ve	-ve
91	745	35	F	-ve	+ve	-ve	-ve	-ve
92	746	55	M	-ve	+ve	-ve	-ve	-ve
93	747	52	M	+ve	-ve	+ve	+ve	+ve
94	748	30	M	-ve	+ve	-ve	-ve	-ve
95	749	65	M	-ve	+ve	-ve	-ve	-ve
96	750	57	M	-ve	+ve	-ve	-ve	-ve

	PATIENT'S INFO			SEROLGY		ZIEHL-NEELSEN STAINING		
S/N	**PIN**	**AGE**	**SEX**	**RESULTS**		**SPOT 1**	**E/MORN**	**SPOT 2**
97	752	10	M	-ve	+ve	-ve	-ve	-ve
98	753	32	F	-ve	+ve	-ve	-ve	-ve
99	755	52	M	-ve	+ve	-ve	-ve	-ve
100	756	25	M	-ve	+ve	-ve	-ve	-ve
101	757	16	M	-ve	+ve	-ve	-ve	-ve
102	761	56	M	-ve	+ve	-ve	-ve	-ve
103	762	29	F	-ve	+ve	+ve	+ve	+ve
104	763	45	F	-ve	+ve	-ve	-ve	-ve
105	766	56	F	-ve	+ve	-ve	-ve	-ve
106	768	36	M	-ve	+ve	-ve	-ve	-ve
107	769	28	M	-ve	+ve	-ve	-ve	-ve
108	772	30	M	-ve	+ve	+ve	+ve	+ve
109	773	39	F	+ve	-ve	-ve	+ve	+ve
110	775	20	F	-ve	+ve	-ve	-ve	-ve
111	781	28	F	-ve	+ve	-ve	+ve	+ve
112	783	42	M	-ve	+ve	-ve	-ve	-ve
113	785	35	M	-ve	+ve	-ve	-ve	-ve
114	788	28	F	-ve	+ve	-ve	-ve	-ve

115	791	AD	F	-ve	+ve	-ve	-ve	-ve
116	792	AD	F	-ve	+ve	-ve	-ve	-ve
117	793	25	F	-ve	+ve	-ve	-ve	-ve
118	794	25	M	-ve	+ve	-ve	-ve	-ve
119	796	60	M	-ve	+ve	-ve	-ve	-ve
120	799	45	M	-ve	+ve	+ve	+ve	+ve
121	801	25	F	-ve	+ve	-ve	-ve	-ve
122	802	30	F	-ve	+ve	-ve	-ve	-ve
123	804	28	F	-ve	+ve	-ve	-ve	-ve
124	806	22	F	-ve	+ve	-ve	-ve	-ve
125	807	48	M	-ve	+ve	-ve	-ve	-ve
126	810	30	M	-ve	+ve	-ve	-ve	-ve
127	814	40	F	-ve	+ve	-ve	-ve	-ve
128	815	30	F	-ve	+ve	-ve	-ve	-ve
129	819	25	F	-ve	+ve	-ve	-ve	-ve
130	820	50	M	-ve	+ve	-ve	-ve	-ve
131	821	15	F	-ve	+ve	-ve	-ve	-ve
132	822	45	M	-ve	+ve	-ve	-ve	-ve
133	824	60	M	-ve	+ve	-ve	-ve	-ve
134	825	42	M	-ve	+ve	-ve	-ve	-ve
135	828	35	F	-ve	+ve	-ve	-ve	-ve
136	829	29	F	-ve	+ve	-ve	-ve	-ve
137	830	20	M	-ve	+ve	-ve	-ve	-ve
138	831	26	M	-ve	+ve	-ve	-ve	-ve
139	832	30	M	-ve	+ve	-ve	-ve	-ve
140	834	33	M	-ve	+ve	-ve	-ve	-ve
141	835	35	F	-ve	+ve	-ve	-ve	-ve
142	838	30	M	-ve	+ve	+ve	+ve	+ve
143	839	45	M	-ve	+ve	-ve	-ve	-ve
144	840	20	F	-ve	+ve	-ve	-ve	-ve

	PATIENT'S INFO			SEROLGY		ZIEHL-NEELSEN STAINING		
S/N	**PIN**	**AGE**	**SEX**	**RESULTS**		**SPOT 1**	**E/MORN**	**SPOT 2**
145	843	24	M	-ve	+ve	+ve	+ve	-ve
146	844	20	M	-ve	+ve	-ve	-ve	-ve
147	845	62	M	+ve	-ve	-ve	-ve	-ve
148	848	AD	M	-ve	+ve	-ve	+ve	+ve
149	849	50	M	-ve	+ve	-ve	-ve	-ve
150	850	39	M	-ve	+ve	-ve	-ve	-ve
151	851	18	M	-ve	+ve	-ve	-ve	-ve
152	852	23	M	-ve	+ve	-ve	-ve	-ve

153	853	34	M	-ve	+ve	-ve	-ve	-ve
154	854	17	F	+ve	-ve	-ve	-ve	-ve
155	884	50	M	-ve	+ve	-ve	+ve	+ve
156	892	35	M	-ve	+ve	-ve	-ve	-ve
157	893	45	M	-ve	+ve	+ve	+ve	+ve
158	894	AD	F	-ve	+ve	+ve	+ve	+ve
159	895	27	M	-ve	+ve	-ve	-ve	-ve
160	896	50	F	-ve	+ve	-ve	-ve	-ve
161	898	26	M	-ve	+ve	-ve	-ve	-ve
162	899	48	M	-ve	+ve	-ve	-ve	-ve
163	901	35	F	-ve	+ve	-ve	-ve	-ve
164	902	73	M	-ve	+ve	+ve	+ve	+ve
165	903	27	M	-ve	+ve	-ve	-ve	-ve
166	905	70	F	-ve	+ve	-ve	-ve	-ve
167	906	48	F	-ve	+ve	-ve	-ve	-ve
168	910	22	F	-ve	+ve	+ve	+ve	+ve
169	915	58	M	-ve	+ve	-ve	-ve	-ve
170	918	30	F	-ve	+ve	+ve	+ve	+ve
171	919	28	F	-ve	+ve	-ve	-ve	-ve
172	920	25	F	-ve	+ve	-ve	-ve	-ve
173	921	30	F	-ve	+ve	-ve	-ve	-ve
174	922	53	M	-ve	+ve	-ve	-ve	-ve
175	923	55	M	-ve	+ve	-ve	-ve	-ve
176	924	28	M	-ve	+ve	-ve	-ve	-ve
177	925	25	F	-ve	+ve	-ve	-ve	-ve
178	926	20	M	+ve	-ve	+ve	+ve	+ve
179	928	20	F	-ve	+ve	+ve	+ve	+ve
180	929	40	M	-ve	+ve	-ve	-ve	-ve
181	930	15	M	-ve	+ve	-ve	-ve	-ve
182	931	32	F	-ve	+ve	-ve	-ve	-ve
183	932	22	M	-ve	+ve	-ve	-ve	-ve
184	935	30	F	-ve	+ve	-ve	-ve	-ve
185	937	35	M	-ve	+ve	+ve	+ve	+ve
186	939	30	F	-ve	+ve	-ve	-ve	-ve
187	940	19	F	-ve	+ve	+ve	+ve	+ve
188	942	65	F	-ve	+ve	-ve	-ve	-ve
189	943	38	M	-ve	+ve	-ve	-ve	-ve
190	944	22	M	-ve	+ve	-ve	-ve	-ve
191	945	60	M	-ve	+ve	-ve	-ve	-ve
192	948	18	F	-ve	+ve	-ve	-ve	-ve
	PATIENT'S INFO			**SEROLGY**		**ZIEHL-NEELSEN STAINING**		
S/N	**PIN**	**AGE**	**SEX**	**RESULTS**		**SPOT**	**E/MORN**	**SPOT**

						1		2
193	949	18	M	-ve	+ve	+ve	+ve	+ve
194	950	40	F	-ve	+ve	-ve	-ve	-ve
195	951	18	F	-ve	+ve	-ve	-ve	-ve
196	952	18	F	+ve	-ve	-ve	-ve	-ve
197	953	22	M	-ve	+ve	-ve	-ve	-ve
198	954	40	M	-ve	+ve	+ve	+ve	+ve
199	955	22	M	-ve	+ve	+ve	+ve	+ve
200	957	25	M	-ve	+ve	+ve	+ve	+ve
201	958	18	F	-ve	+ve	+ve	+ve	+ve
202	959	55	M	-ve	+ve	+ve	+ve	+ve
203	960	26	F	-ve	+ve	+ve	+ve	+ve
204	961	27	M	-ve	+ve	+ve	+ve	+ve
205	963	20	M	-ve	+ve	-ve	-ve	-ve
206	966	30	M	-ve	+ve	+ve	+ve	+ve
207	967	41	M	-ve	+ve	+ve	+ve	+ve
208	974	19	F	-ve	+ve	-ve	-ve	-ve
209	975	59	F	-ve	+ve	-ve	-ve	-ve
210	976	20	M	-ve	+ve	+ve	+ve	+ve
211	977	35	M	-ve	+ve	-ve	-ve	-ve
212	978	43	M	+ve	-ve	+ve	+ve	+ve
213	981	50	F	-ve	+ve	-ve	-ve	-ve
214	984	AD	M	-ve	+ve	-ve	-ve	-ve
215	985	AD	F	-ve	+ve	-ve	-ve	-ve
216	987	AD	M	-ve	+ve	-ve	-ve	-ve
217	989	AD	M	-ve	+ve	-ve	-ve	-ve
218	990	27	F	-ve	+ve	-ve	-ve	-ve
219	994	30	F	-ve	+ve	-ve	-ve	-ve
220	996	22	F	-ve	+ve	-ve	-ve	-ve
221	997	37	F	-ve	+ve	-ve	-ve	-ve
222	999	69	M	-ve	+ve	-ve	-ve	-ve
223	1000	30	F	-ve	+ve	-ve	-ve	-ve
224	1001	20	M	-ve	+ve	-ve	-ve	-ve
225	1002	25	F	-ve	+ve	-ve	-ve	-ve
226	1003	20	M	-ve	+ve	-ve	-ve	-ve
227	1006	50	M	-ve	+ve	-ve	-ve	-ve
228	1007	25	F	-ve	+ve	-ve	-ve	-ve
229	1010	35	F	-ve	+ve	-ve	-ve	-ve
230	1012	35	M	-ve	+ve	+ve	+ve	+ve
231	1014	33	M	-ve	+ve	-ve	-ve	-ve
232	1042	28	M	-ve	+ve	-ve	-ve	-ve
233	1043	21	F	-ve	+ve	-ve	-ve	-ve
234	1045	50	F	-ve	+ve	-ve	-ve	-ve

235	1046	28	F	-ve	+ve	-ve	-ve	-ve
236	1047	30	F	-ve	+ve	-ve	-ve	-ve
237	1054	7	M	-ve	+ve	-ve	-ve	-ve
238	1055	45	F	-ve	+ve	-ve	-ve	-ve
239	1056	56	F	-ve	+ve	o	o	o
240	1058	20	F	-ve	+ve	+ve	+ve	+ve

	PATIENT'S INFO			SEROLGY		ZIEHL-NEELSEN STAINING		
S/N	**PIN**	**AGE**	**SEX**	**RESULTS**		**SPOT 1**	**E/MORN**	**SPOT 2**
241	1059	30	M	-ve	+ve	+ve	+ve	+ve
242	1060	30	F	-ve	+ve	+ve	+ve	+ve
243	1061	45	F	-ve	+ve	-ve	-ve	-ve
244	1062	52	M	-ve	+ve	-ve	-ve	-ve
245	1063	8	F	-ve	+ve	-ve	-ve	-ve
246	1065	AD	F	+ve	-ve	-ve	-ve	-ve
247	1066	20	F	-ve	+ve	-ve	-ve	-ve
248	1067	35	M	-ve	+ve	-ve	-ve	-ve
249	1068	25	M	+ve	-ve	-ve	-ve	-ve
250	1071	26	F	-ve	+ve	-ve	-ve	-ve
251	1072	50	M	-ve	+ve	+ve	+ve	+ve
252	1073	24	F	-ve	+ve	-ve	-ve	-ve

Key :

+ve = Positive

-ve = Negative

APPENDIX B

Appendix II: ANALYSIS OF DATA WITH SPSS® SOFT WARE;

Table IIa: Case Processing Summary

	Cases					
	Valid		Missing		Total	
	NO OF SAMPLES	Percent	NO OF SAMPLES	Percent	NO OF SAMPLES	Percent
SEROLOGY VS SPUTUM AFB SPOT 1	252	100.0%	0	.0%	252	100.0%
SEROLOGY VS SPUTUM AFB EARLY MORNING	252	100.0%	0	.0%	252	100.0%
SEROLOGY VS SPUTUM AFB SPOT2	252	100.0%	0	.0%	252	100.0%

Table IIb: COMPARING THE RESULT OF SEROLOGY TEST AND THE FIRST SPUTUM SAMPLE (SPOT1) IN 2X2 CONTINGENCY TABLE

			Sputum AFB SPOT1		
			-ve	+ve	Total
SEROLOGY IMMUNO-CHROMATOGRAPHIC TEST	-ve	Count	191	42	233
		Expected Count	191.4	41.6	233.0
	+ve	Count	16	3	19
		Expected Count	15.6	3.4	19.0
Total		Count	207	45	252
		Expected Count	207.0	45.0	252.0

Table IIc: CHI-SQUARE TEST FOR THE RESULT OF SEROLOGY TEST AND THE FIRST SPUTUM SAMPLE (SPOT1)

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.060[a]	1	.807		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.062	1	.804		
Fisher's Exact Test				1.000	.550
Linear-by-Linear Association	.060	1	.807		
McNemar Test				.001[c]	
N of Valid Cases	252				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.39.

b. Computed only for a 2x2 table

c. Binomial distribution used.

Table IId: SYMMETRIC MEASURES FOR THE RESULT OF SEROLOGY TEST AND THE FIRST SPUTUM SAMPLE (SPOT1)

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	-.015	.060	-.256	.798
Measure of Agreement	Kappa	-.014	.054	-.245	.807
N of Valid Cases		252			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Table IIe: COMPARING THE RESULT OF SEROLOGY TEST AND THE EARLY MORNING SPUTUM SAMPLE (E/MORN) IN A 2X2 CONTINGENCY TABLE

			Sputum AFB E/MORN		Total
			-ve	+ve	
SEROLOGY IMMUNO-CHROMATOGRAPHIC TEST	-ve	Count	186	47	233
		Expected Count	185.8	47.2	233.0
	+ve	Count	15	4	19
		Expected Count	15.2	3.8	19.0
Total		Count	201	51	252
		Expected Count	201.0	51.0	252.0

Table IIf: CHI-SQUARE TEST FOR THE RESULT OF SEROLOGY TEST AND THE EARLY MORNING SPUTUM SAMPLE (E/MORN)

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.008[a]	1	.927		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.008	1	.927		
Fisher's Exact Test				1.000	.561
Linear-by-Linear Association	.008	1	.927		
McNemar Test				.000[c]	
N of Valid Cases	252				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.85.

b. Computed only for a 2x2 table

c. Binomial distribution used.

Table IIg: SYMMETRIC MEASURES FOR THE RESULT OF SEROLOGY TEST AND THE EARLY MORNING SPUTUM SAMPLE (E/MORN)

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	.006	.064	.091	.928
Measure of Agreement	Kappa	.005	.055	.092	.927
N of Valid Cases		252			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Table IIh: COMPARING THE RESULT OF SEROLOGY TEST AND THE THIRD SPUTUM SAMPLE (SPOT 2) IN A 2X2 CONTINGENCY TABLE

			Sputum AFB SPOT2		
			_-ve	+ve	Total
SEROLOGY IMMUNO-CHROMATOGRAPHIC TEST	-ve	Count	186	47	233
		Expected Count	185.8	47.2	233.0
	+ve	Count	15	4	19
		Expected Count	15.2	3.8	19.0
Total		Count	201	51	252
		Expected Count	201.0	51.0	252.0

Table IIi: CHI-SQUARE TEST FOR THE RESULT OF SEROLOGY TEST AND THE THIRD SPUTUM SAMPLE (SPOT 2)

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.008[a]	1	.927		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.008	1	.927		
Fisher's Exact Test				1.000	.561
Linear-by-Linear Association	.008	1	.927		
McNemar Test				.000[c]	
N of Valid Cases	252				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.85.

b. Computed only for a 2x2 table

c. Binomial distribution used.

Table IIj: SYMMETRIC MEASURES FOR THE RESULT OF SEROLOGY TEST AND THE THIRD SPUTUM SAMPLE (SPOT2)

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	.006	.064	.091	.928
Measure of Agreement	Kappa	.005	.055	.092	.927
N of Valid Cases		252			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Appendix III:RESULT OF ANALYSIS OF DATA WITH MedCalc® SOFTWARE.

Table IIIa: McNemar's test for the result of serology compared with First Sputum sample (Spot 1)

Classification A	serology1		
Classification B	Spot1		
	Classification A		
Classification B	-	+	
-	191	16	207(82.1%)
+	42	3	45(17.9%)
	233 (92.5%)	19 (7.5%)	252
Difference			10.32%
95% CI			4.19 to 15.35

Table IIIb: Chi-square test with continuity correction for the result of serology compare with First Sputum sample (Spot 1)

Chi-square	10.7759
DF	1
Significance	P = 0.0010

Table IIIc: McNemar test for the result of serology compared with Second Sputum sample (E/MORN)

Classification A	serology1		
Classification B	E/Morn		
	Classification A		
Classification B	-	+	
-	186	15	201(79.8%)
+	47	4	51(20.2%)
	233 (92.5%)	19 (7.5%)	252
Difference			12.70%
95% CI			6.52 to 17.61

Table IIId: Chi-square test with continuity correction result of serology compared with Second Sputum sample (E/MORN)

Chi-square	15.5000
DF	1
Significance	P = 0.0001

Table IIIe: McNemar's test for the result of serology compared with second Sputum sample (Spot 2)

Classification A	serology1		
Classification B	1spot2		
	Classification A		
Classification B	-	+	
-	186	15	201(79.8%)
+	47	4	51(20.2%)
	233 (92.5%)	19 (7.5%)	252
Difference			12.70%
95% CI			6.52 to 17.61

Table IIIf: Chi-square test with continuity correction for the result of serology compared with Second Sputum sample (Spot 2)

Chi-square	15.5000
DF	1
Significance	P = 0.0001

Table IIIg: Frequency table & Chi-square test for Serology vs Spot 1

Codes X	Serology		
Codes Y	Spot1		
	Codes X		
Codes Y	-	+	
-	191	16	207(82.1%)
+	42	3	45(17.9%)
	233 (92.5%)	19 (7.5%)	252
Chi-square			0.004
DF			1
Significance level			P = 0.9468
Contingency coefficient			0.004

Table IIIh: Frequency table & Chi-square test for Serology vs E/Morn

Codes X	Serology		
Codes Y	E/MORN		
	Codes X		
Codes Y	-	+	
-	186	15	201(79.8%)
+	47	4	51(20.2%)
	233 (92.5%)	19 (7.5%)	252
Chi-square			0.042
DF			1
Significance level			P = 0.8376
Contingency coefficient			0.013

Table IIIi: Frequency table & Chi-square test for Serology vs Spot 2

Codes X	Serology		
Codes Y	Spot2		
	Codes X		
Codes Y	-	+	
-	186	15	201(79.8%)
+	47	4	51(20.2%)
	233 (92.5%)	19 (7.5%)	252
Chi-square			0.042
DF			1
Significance level			P = 0.8376
Contingency coefficient			0.013

Table IIIj: Inter-rater agreement (kappa) for Serology vs Spot 1

Observer A	Serology		
Observer B	Spot1		
	Observer A		
Observer B	-	+	
-	191	16	207(82.1%)
+	42	3	45(17.9%)
	233 (92.5%)	19 (7.5%)	252
Weighted Kappa			-0.014
Standard error			0.054
95% CI			-0.119 to 0.0915

Table IIIk: Inter-rater agreement (kappa) for Serology vs E/Morn

Observer A	Serology		
Observer B	E/MORN		
	Observer A		
Observer B	-	+	
-	186	15	201(79.8%)
+	47	4	51(20.2%)
	233 (92.5%)	19 (7.5%)	252
Weighted Kappa			0.005
Standard error			0.055
95% CI			-0.102 to 0.112

Table IIIl: Inter-rater agreement (kappa) for Serology vs Spot 2

Observer A	Serology		
Observer B	Spot 2		
	Observer A		
Observer B	-	+	
-	186	15	201(79.8%)
+	47	4	51(20.2%)
	233 (92.5%)	19 (7.5%)	252
Weighted Kappa			0.005
Standard error			0.055
95% CI			-0.102 to 0.112

Appendix IV: Statistical Test and Calculations

1. Raw Agreement Indices

a. For Serology vs Spot 1

$$P = \frac{\sum \pi_{ij}}{n} = 191 + 3 = 194/252 = 0.77 \infty 77\%$$

b. For Serology vs E/Morn

$$P = \frac{\sum \pi_{ij}}{n} = 186 + 4 = 190/252 = 0.75 \infty 75\%$$

c. For Serology vs Spot 2

$$P = \frac{\sum \pi_{ij}}{n} = 186 + 4 = 190/252 = 0.75 \infty 75\%$$

Range (75% - 77%)

1.1 The percentage of Positive Agreement

a. For Serology vs Spot 1

3/252 = 0.012 ∞ 1.2%

b. For Serology vs E/Morn

4/252 = 0.016∞ 1.6%

c. For Serology vs Spot 2

4/252 = 0.016∞ 1.6%

Range (1.2% - 1.6%)

1.2 The percentage of Negative Agreement

a. For Serology vs Spot 1

191/252 = 0.76 ∞ 76%

b. For Serology vs E/Morn

186/252 = 0.74 ∞ 74%

c. For Serology vs Spot 2

186/252 = 0.74 ∞ 74%

Range (74% -76%)

2. Kendal Tau Test

$T = \sqrt{\chi^2}/ (C-1)\ n$

a. For Serology vs Spot 1

$T = \sqrt{\chi^2}/ (C-1)\ n$

$= \sqrt{0.06}/(2-1)252$

$= \sqrt{0.0002381}$

$= 0.0154$

b. For Serology vs E/Morn

$T = \sqrt{\chi^2}/ (C-1)\ n$

$= \sqrt{.008}/ (2-1)\ 252$

$= 0.0056$

c. For Serology vs Spot 2

$T = \sqrt{\chi^2}/ (C-1)\ n$

$= \sqrt{.008}/ (2-1)\ 252$

$= 0.0056$

Range (0.0154 – 0.0056)

3. The Kappa test.

$\kappa = (po-pe)/ (n-pe)$

a. For Serology vs Spot 1

$$\kappa = \frac{(191.4 + 3.4) - (191 + 3)}{252 - (191.4 + 3.4)} = \frac{194.8 - 194}{252 - 194.8} == \frac{0.8}{57.2} = 0.014$$

b. For Serology vs E/Morn

$$\kappa = \frac{(185.8 + 3.8) - (186 + 4)}{252 - (185.8 + 3.8)} = \frac{(189.6) - (190)}{252 - 189.6} = \frac{-.4}{62.4} = 0.0064$$

c. For Serology vs Spot 2

$$\kappa = \frac{(185.8 + 3.8) - (186 + 4)}{252 - (185.8 + 3.8)} = \frac{(189.6) - (190)}{252 - 189.6} = \frac{-.4}{62.4} = 0.0064$$

Range (0.014 – 0.0064)

4. McNemar's test for Marginal Symmetry

$$\chi^2 = \frac{\sum (\pi ij - \pi ji)^2}{\pi ij + \pi ji}$$

a. For Serology vs Spot 1, therefore

$$\chi^2 = \frac{(42 - 16)^2}{42 + 16} = \frac{26^2}{58} = \frac{676}{58} = 11.66$$

But tabulated $\chi^2_{1,\,0.05} = 3.84$, therefore H_0 is rejected and H_1 is accepted.

b. For Serology Vs E/Morning)

$$\chi^2 = \frac{(47 - 15)^2}{47 + 15} = \frac{32^2}{62} = \frac{1024}{62} = 16.52$$

c. For Serology Vs Spot 2

$$\chi^2 = \frac{(47 - 15)^2}{47 + 15} = \frac{32^2}{62} = \frac{1024}{62} = 16.52$$

But tabulated $\chi^2_{1,\,0.05} = 3.84$, therefore H_0 is rejected and H_1 is accepted.

Appendix V: Questionaire

My names are Uthman Taofeek, I am a postgraduate student of Medical Microbiology with the Federal University of Technology, Minna. I am doing a study on the effectiveness of serological test in the diagnosis of Tuberculosis and I am hoping that you will be able to assist me in this regard by agreeing to partake in the experiment. I promised that any information provided by you will be treated with utmost confidentiality and that it would not be traceable to you or used against you in whatever form. If this is acceptable to you kindly attest by appending your signature (or left thumbprint) in the space provided below.

…….

1) AGE: 0-5yrs old ☐ 5-10yrs old ☐ 11-20yrs old ☐ 21-30yrs old ☐
   31-40yrs old ☐ 41-50yrs ☐ >50yrs ☐
2) SEX: Female ☐ Male ☐
3) MARITAL STATUS: Married ☐ Single ☐
4) OCCUPATION (please specify): …….
5) EDUCATIONAL QUALIFICATION: None ☐ FSLC ☐ SSSC ☐
   Post Secondary ☐
6) RELIGIOUS AFFILIATION: Christianity ☐ Islam ☐
   others ☐
7) Do you have any of the signs and symptom listed below, please tick as appropriate?

Sign/symptom			**Duration**	
	Yes	No	< 6months	> 6months
8) Persistent Cough	☐	☐	☐	☐
9) Weight loss	☐	☐	☐	☐
10) Night sweat	☐	☐	☐	☐
11) Fever	☐	☐	☐	☐

12) Any other signs and/or symptoms (please specify) ……

13) Have you had any vaccination against Tuberculosis (BCG) Yes? ☐ No? ☐ If Yes, when? <15 yrs ago ☐ 15yrs ago ☐ >15yrs ago ☐

14) Have you ever been investigated for Tuberculosis before now? Yes ☐ No ☐

15) If yes, what mode of investigations? Chest-x-ray ☐ Sputum for AFB ☐ Mantoux/ Heaf test ☐ serology (use of blood) ☐

16) Have you ever been diagnosed of having Tuberculosis? Yes? ☐ No? ☐

17) If yes, how? Laboratory test ☐ symptomatically ☐

18) Have you undergone any treatment Yes ☐ No ☐

19) If yes, have you done any investigations after the treatment? Yes ☐ No ☐

20) If yes, what was mode of investigations? Chest-x-ray ☐ Sputum for AFB ☐ Mantoux/ Heaf test ☐ serology (use of blood) ☐

21) What was the result? Positive ☐ Negative ☐

22) Are you suffering from any of these illnesses (please tick as appropriate) Cancer Di☐es mellitus HIV ☐sthma☐ Chronic☐nal Failure ☐ers (please specify) …….

23) Are you currently on any of these medications? anti cancer drugs ☐ Prednisolone/Dexamethasone or any other steroids☐ any other drugs (please specify) …….

Thank you for participating and God Bless. Amen.

Uthman, Taofeek A.

M.Tech/SSSE/2008/1958

Appendix VI: Data Collecting Tool

RESULTS OF TEST

TESTRESULTS (PLEASE TICK AS APPROPRIATE)

1. SPUTUM AFB *3

DAY 1	
+	–

DAY 2	
+	–

DAY 3	
+	–

2. MANTOUX TEST

0-5mm	6-10mm	> 11mm

3. SEROLOGY

POSITIVE (+)	NEGATIVE (-)

TABLE 3.2: REPORTING OF SPUTUM AFB MICROSCOPY

Examination	Result	Grading	No. of fields examined
More than 10 AFB per oil immersion fields	Positive	3+	20
More than 10 AFB per oil immersion fields	Positive	2+	50
10-99 AFB per 100 oil immersion fields	Positive	1+	100
1-9 AFB per 100 oil immersion fields	scanty	Record exact number seen	100
No AFB per 100 oil immersion fields	Negative	0	100