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Global Health Challenges at the Point of Care: A Review of Tuberculosis Needs Assessment

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Global Health Challenges at the Point of Care: A Review of Tuberculosis Needs Assessment

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Abstract

Tuberculosis is one of the deadliest communicable diseases in the world, and consequently remains one of the biggest global healthcare challenges. Tuberculosis is treatable and curable. However, within many low resource settings, underdeveloped medical infrastructure limits the effectiveness and accuracy of existing diagnostics. These limitations severely impede the timely diagnosis of the disease, and thus contribute to the disease spreading, developing drug resistance, and killing more individuals. There is an urgent need for an inexpensive, portable, rapid, easy-to-use point of care diagnostic that can function outside of the laboratory at the community level. Currently, there is a wide range of available tuberculosis diagnostics ranging from sputum smear microscopy to nucleic acid amplification tests. Yet, none have met every standard of the ideal point of care diagnostic. Since the World Health Organization's endorsement of Xpert MTB/RIF in 2010, there has been a resurgence of interest in point of care diagnostic development. This investigation reviewed diagnostic development projects funded by the National Institutes of Health in 2008 and 2014 in order to examine the technologies being developed, how researchers in industrial and academic sectors are addressing this problem, and what challenges still need to be overcome. More projects in 2014 were expected to rely on sample types other than sputum and be funded than those in 2008. The results of this investigation confirm this hypothesis, and that the development of a point of care device is a multi-faceted challenge with numerous underlying issues that need to be addressed before such a device can be successfully implemented.

Introduction

Tuberculosis is one of the deadliest communicable diseases, afflicting 9.0 million and killing 1.5 million people in 2013 alone (WHO, 2014). While the disease is treatable and curable, it is difficult to diagnose at the point of care in low-resource settings (e.g. Central Africa and Southeast Asia) (Davies, 2003). There are several factors that hinder the development of a tuberculosis point of care diagnostic, including the challenge of working with sputum, the limitations imposed by low-resource settings, and the cost of technology (Wang *et al.*, 2013). This investigation examines what progress academic and industrial sectors have made in the development of a tuberculosis diagnostic. This was achieved by reviewing diagnostic development projects from the years 2008 and 2014 that were funded by the National Institutes of Health. The following sections will provide an overview of

tuberculosis, biotechnologies, existing tuberculosis diagnostics, needs assessment, the National Institutes of Health, and the predicted outcomes of this investigation.

I. *Biology of Tuberculosis*

Tuberculosis is caused by a bacterium called *Mycobacterium tuberculosis*. This bacterium is gram positive and possesses cell wall components, such as a high concentration of lipids and mycolic acids, that enhance its longevity and ability to trigger inflammatory responses from its host (Cole *et al.*, 1998). *M. tuberculosis* is an obligate aerobe and is commonly found in the lungs, however, complications in extrapulmonary organs may arise (WHO, 2014).

The bacterium spreads to the respiratory tract when people are exposed to droplets from infected individuals. These airborne droplets can be easily spread through coughing and sneezing, making the disease highly contagious (Silva Miranda *et al.*, 2012). The host's immune system detects and fights off the bacteria within these droplets with alveolar macrophages, type II pneumocytes, and polymorphonuclear neutrophils (Silva Miranda *et al.*, 2012). Alveolar macrophages serve as the first-line of defense against respiratory pathogens via phagocytosis and intracellular processes, including the release of cytokines (Rubins, 2003). Type II pneumocytes modify the inflammatory response and synthesize alveolar surfactant, which reduces surface tension and prevents alveolar collapse (Zhao *et al.*, 2010). Polymorphonuclear neutrophils are the most abundant blood leukocytes that serve as the first-line of defense against infection and inflammation (Carlo *et al.*, 2001). These cells, along with multinucleated giant cells and T lymphocytes, prevent the disease from spreading by forming a granuloma, a structure that contains the infection (Silva Miranda *et al.*, 2012). The granuloma is the hallmark signature of tuberculosis infection. In addition to preventing

the disease from spreading, the granuloma can also serve as a niche in which *Mycobacterium tuberculosis* can remain dormant in (Silva Miranda *et al.*, 2012).

There are two distinct forms of tuberculosis, latent and active. Latent tuberculosis occurs when individuals are infected with the bacterium but do not show symptoms because the immune system prevents bacterial proliferation (Silva Miranda *et al.*, 2012). When the immune system is compromised, for instance, by HIV infection, tuberculosis may develop into an active disease. However, the bacteria can successfully infect an individual for decades without causing disease (Silva Miranda *et al.*, 2012). About ten percent of individuals with latent tuberculosis will develop active tuberculosis (Fauci, 2008). Patients with latent tuberculosis will test positive for the disease, however, they often do not seek treatment in a timely manner due to latent infection not presenting with any symptoms (McNerney and Daley, 2011). Symptoms of tuberculosis include coughing up sputum and blood, coughing for three or more weeks, night sweats, fatigue, fever, chest pains, loss of appetite, pleurisy, and weight loss (CDC, 2014).

Latent tuberculosis is typically diagnosed with a Mantoux tuberculin skin test whereas active tuberculosis is typically diagnosed by microscopically examining sputum (a combination of saliva and mucous obtained from the respiratory tract). Other methods, such as detecting *M. tuberculosis* nucleic acids in cultures (e.g. sputum or serum), are also employed (Wang *et al.*, 2013). Treatments include a combination of antibiotics, such as isoniazid, rifampicin, ethambutol, and pyrazinamide, which are to be taken for six to twelve months (WHO, 2014). Directly Observed Therapy (DOT) is often used to ensure that patients take their medication properly (CDC, 2012). This involves health care professionals watching patients take their medication and documenting the treatment process (CDC, 2012).

The global fight against tuberculosis has been impeded by the development of drug resistant strains of the disease (Fauci, 2008). Multi-drug resistant tuberculosis (MDR-TB) is resistant to isoniazid and rifampicin, which are considered the two most common and powerful anti-tuberculosis drugs (WHO, 2014). Extensively drug-resistant tuberculosis (XDR-TB) is a subtype of MDR-TB that is also resistant to at least one fluoroquinolone and a second-line injectable drug, such as amikacin or kanamycin (Jain and Dixit, 2008). Drug resistance may evolve from infected individuals receiving erroneous concentrations of drugs or not finishing the prescribed treatment (Jain and Dixit, 2008). Different combinations of the aforementioned antibiotics are prescribed in order to combat the development of drug resistance (WHO, 2014).

II. *Biotechnologies*

Currently, there is a wide array of tuberculosis diagnostics ranging from sputum smear microscopy to rapid assays that rely on the detection of *M. tuberculosis* bacterium, nucleic acids, or induced immune responses (Wang *et al.*, 2013). Some of these diagnostics have more potential to be developed into successful point of care tests. For instance, the urine antigen detection test has less potential than the nucleic acid amplification test due to its lower sensitivity (Wang *et al.*, 2013). Sensitive and specific technology is defined as technology that yields minimal false positives and false negatives, respectively (Yager *et al.*, 2008). The enzyme-linked immunosorbent assay (ELISA) also does not have much potential to be developed into a point of care diagnostic. This assay quantifies the host's immune response to the bacterium by measuring T cell release of interferon gamma after the T cells are mixed with antigens (Wang *et al.*, 2013). However, the ELISA does not have better sensitivity than a basic Mantoux skin test, which uses tuberculin, a protein derivative, to

detect an immune response (Wang *et al.*, 2013). Both of these tests require trained personnel to be administered, take 24 hours (ELISA) and 48 hours (Mantoux) to be read, and often yield false positive or negative results (Wang *et al.*, 2013).

Biomarkers are physiological substances or processes that can be accurately measured in order to monitor diseases and the efficacy of treatments (Strimbu and Tavel, 2010). Examples of biomarkers include the biochemical analysis of blood and tissues (Strimbu and Tavel, 2010). There is a lack of reliable biomarkers for tuberculosis because further research is needed in understanding how the bacterium interacts with its host (Wang *et al.*, 2013). A urine antigen detection test relies on biomarkers to analyze and detect tuberculosis antigens in the urine. These antigens include lipoarabinomannan, which is a component of the *M. tuberculosis* cell wall. Urine is an easy sample to collect in low resource settings, however, it yields low sensitivity in confirming infection. It has been proposed that the combination of this assay and sputum smear microscopy can improve sensitivity (Wang *et al.*, 2013).

Nucleic acid amplification tests (NAAT) are commonly used to diagnose tuberculosis, detect drug resistance, and monitor treatments (Wang *et al.*, 2013). There are several methods that can be used to amplify nucleic acids, including loop-mediated isothermal amplification, multiplex ligation-dependent probe amplification, and microarrays (Wu and Tang, 2009). Loop-mediated isothermal amplification (LAMP) relies on several primers in order to detect various regions on one gene of interest (Wu and Tang, 2009). LAMP allows for simultaneous nucleic acid amplification and gene recognition by combining and incubating samples, primers, DNA polymerase, and substrates at a constant temperature (Wu and Tang, 2009). Multiplex ligation-dependent probe amplification (MLPA), relies on probes, each composed of oligonucleotides (Wu and Tang, 2009). These

oligonucleotides work in pairs, with one containing a primer-binding site, 3' recognition

sequence, and a 5' fluorescent label, and the other containing a specified length of DNA, 3'

reverse primer-binding site, and a 5' recognition sequence (Wu and Tang, 2009). The probes

use these oligonucleotide pairs to anneal to the gene sequences of interest, after which the

probes are ligated, in order to be amplified and detected by the polymerase chain reaction

(PCR) and electrophoresis, respectively (Wu and Tang, 2009). Microarrays also rely on

oligonucleotides of specified lengths and PCR amplification in order to detect and identify

DNA sequences (Wu and Tang, 2009).

Furthermore, microfluidic technologies are used to efficiently obtain measurements

from small sample volumes (Yager *et al.*, 2006). The draw of microfluidics stems from the

fact that they can perform complex functions on a miniaturized scale, and have the potential

to be integrated into diagnostic tools that can function outside of laboratories, and without

refrigeration or electricity (Yager *et al.*, 2006). Currently, microfluidics are most commonly

incorporated into blood chemistry analysis, immunoassays, nucleic acid amplification tests,

and flow cytometry (Yager *et al.*, 2006). Blood chemistry analysis involves approximately

twelve to twenty tests being run on automated analyzers, designed to monitor physiological

functions (Yager *et al.*, 2006). Immunoassays, commonly used as lateral flow strip assays,

detect proteins with labeled antibodies in numerous samples, including blood, serum, and

urine (Yager *et al.*, 2006). Flow cytometry is used to count cells with specific physical and

chemical compositions (Yager *et al.*, 2006).

The evolution of nanotechnology and microfluidics has lead to the development of *M.*

tuberculosis biosensors (Wang *et al.*, 2013). Essentially, these biosensors work in

conjunction with an analyzer device by responding to physiological and chemical changes in

a specific area (Wang *et al.*, 2013). Biosensors contain bioreceptors that detect specific analytes or molecules, thus enhancing the specificity of the biosensor (Wu and Tang, 2009). The detected analytes are then transduced into electrical signals that can be detected by electrochemical and optical platforms (Wu and Tang, 2009). There are different types of biosensors that are based on antibody-antigen interactions, the bacterium itself, or nucleic acids obtained from the bacterium (Wang *et al.*, 2013). For instance, an optical biosensor may work by exciting samples with a laser light, and then passing a beam of monochromatic light through the sample in order to spectrographically detect *M. tuberculosis* strains (Wang *et al.*, 2013). Although biosensors tend to have high specificity, they are expensive and rely on complex and not readily accessibly equipment, such as lasers and fluorescence microscopes (Wang *et al.*, 2013).

An additional advance in the diagnostics field is multiplexed technology. An ideal point of care device would rely on multiplexed techniques due to their efficiency and accuracy (Zumla *et al.*, 2014). These techniques have the ability to assay multiple substances (e.g. pathogens) simultaneously. This generates more results at a faster rate, such as the concurrent detection of *M. tuberculosis* nucleic acids and drug resistant strains of the bacteria (Zumla *et al.*, 2014). One example of a multiplexed technique is a PCR test that relies on numerous primers within one PCR mixture in order to amplify and identify multiple nucleic acid fragments from different diseases at the same time (Zumla *et al.*, 2014). In the case of MLPA, multiplexing is conducted by altering the specified lengths of DNA in the oligonucleotides in different probe pairs (Wu and Tang, 2009). This may especially be beneficial in diagnosing individuals who are infected with multiple diseases, such as in the case of tuberculosis and HIV co-infection.

III. *Existing Tuberculosis Diagnostics*

In 2010, WHO endorsed the assay, Xpert MTB/RIF, created by Cepheid Inc. in Sunnyvale, California (Denkinger and Pai, 2012). This endorsement subsequently lead to an increased interest in tuberculosis diagnostic development in the research community. Xpert MTB/RIF is a cartridge-based nucleic acid amplification test that detects M. tuberculosis and rifampicin resistance from the sputum (Wang *et al.*, 2013).

This device essentially works by using five probes, each of which is a fragment of DNA oligonucleotides and complimentary to the rifampicin resistance domain of the *M. tuberculosis rpoB* gene (Van Rie *et al.*, 2010). Sputum is extracted from infected individuals and treated for fifteen minutes with a reagent containing sodium hydroxide and isopropanol (Van Rie *et al.*, 2010). The nucleic acids in the sputum are amplified by PCR, which causes the probes to undergo a conformational change that can be detected by fluorescence (Wang *et al.*, 2013). This fluorescence allows detection of bacterial DNA and mutations in the DNA that lead to drug resistance (Wang *et al.*, 2013).

Although PCR amplification techniques have been available and widely used in the diagnosis of tuberculosis, Xpert MTB/RIF integrated sample processing, DNA extraction, and PCR amplification in a disposable cartridge (Wang *et al.*, 2013). This was a significant development because it greatly increased the specificity and sensitivity of the test (Wang *et al.*, 2013). However, Xpert MTB/RIF is not efficient in low resource settings due to its cost (<ten dollars per cartridge), maintenance requirements, and reliance on electricity (Niemz *et al.*, 2011).

Another example of an existing technology is the Biological System for Molecular Antibiotic Resistance Testing (B-SMART). B-SMART is also a NAAT, and relies on

bacteriophages to detect the metabolic activity of bacteria that have been exposed to antibiotics (Sequella Incorporated, 2014). These bacteriophages normally synthesize a nucleic acid, however, this synthesis is impaired when the phages are exposed to effective antibiotics (Sequella Incorporated, 2014). Consequently, the nucleic acid serves as a biomarker for the detection of bacteria and drug resistance. If the bacteria are resistant to the antibiotic, the antibiotic will have no effect on the bacteria's metabolism, and the nucleic acid will be synthesized normally. If the bacteria are not resistant to the antibiotic, the antibiotic will impair the bacteria's metabolism, and subsequently, the synthesis of the nucleic acid. Ultimately, B-SMART assesses how well bacteria respond to the presence or absence of various antibiotics by amplifying nucleic acids (Sequella Incorporated, 2014).

IV. *Needs Assessment*

Various organizations, such as the Foundation for Innovative Diagnostics (FIND) and World Health Organization (WHO), are devoted to combatting prevailing illnesses, such as tuberculosis, by focusing on needs assessment and diagnostic solutions at the point of care. This is achieved by surveying patients' needs in afflicted countries, building partnerships with other organizations, including government agencies and industries, and developing technologies. A point of care diagnostic is defined as a test that is accurate, sensitive, specific, and inexpensive, does not require sophisticated equipment or highly trained personnel to operate, and can be read immediately and used at the community level (Denkinger and Pai, 2012).

According to the WHO, tuberculosis can be diagnosed at four levels: community health clinics, microscopy centers, referral laboratories, and national reference laboratories (WHO, 2006). Approximately 60% and 25% of patients are seen at the community health

clinics and microscopy centers, respectively (WHO, 2006). Approximately 10% of patients

are seen at the referral laboratories, where the primary testing methods are culture assays

(WHO, 2006). At the microscopy centers, the primary testing method involves observing

sputum samples under a microscope, otherwise known as sputum smear microscopy. This

method is usually combined with a Ziehl-Neelsen stain, and is inexpensive and fast (Wang *et

al.*, 2013). However, sputum smear microscopy requires trained personnel to detect the

bacteria, which can only be detected at approximately 10,000 colony forming units per

milliliter (Hobby *et al.*, 1973). This makes it difficult to detect the disease in its early stages,

when there is less bacteria present in sputum (Wang *et al.*, 2013). Furthermore, sputum

smear microscopy has low sensitivity (Denkinger and Pai, 2012). These factors are

particularly problematic in low-resource settings, where there is a shortage of trained lab

technicians who can use sputum smear microscopy to accurately diagnose tuberculosis. At

the community health clinics, there is currently no diagnostic test, and patients are screened

based on their presenting symptoms (WHO, 2006). This means that the majority (60%) of

patients do not have access to diagnostic tests at the point of care (WHO, 2006).

There is no contention about the fact that a point of care device is necessary at the

community level in order to combat the global health burden of tuberculosis. There has been

progress in the development of efficient, rapid, and sensitive technologies that can operate in

the laboratory, such as Cepheid's Gene Xpert MTB/RIF. However, the reliance on a lab

greatly minimizes public access to these technologies in impoverished areas (McNerney and

Daley, 2011). Several factors are responsible for the lack of an accurate, easy-to-use

diagnostic that can provide results within the same day the individual is tested (McNerney

and Daley, 2011). These factors include underdeveloped medical infrastructure, limited

access to public health services, the difficulty of obtaining and working with sputum, and the

high cost of technology. Accurately diagnosing tuberculosis has substantial benefits. This

includes the patient receiving appropriate treatment before spreading the disease to others,

health care providers preventing the disease from progressing, and public health

organizations having up to date information for more efficient public policy development

(Mahony, 2010). Additionally, timely diagnosis of the disease may prevent drug resistant

strains from evolving (McNerney and Daley, 2011).

Current diagnostics have poor detection rates and may take weeks to provide results.

M. tuberculosis is a big challenge to work with because it grows slowly, taking

approximately 4-8 weeks to grow on solid culture and 10-14 days to grow in liquid cultures

(Wang *et al.*, 2013). Also, the bacterium's tendency to lay dormant in granulomas leads to

infected individuals not seeking treatment in a timely manner. This is not only detrimental to

the individual's health, but also increases the risk of the disease spreading to others.

Moreover, sputum is hard to work with. In order to obtain accurate and consistent results,

sputum must be properly collected, stored, and transported, which may be difficult in low

resource setting (e.g. where there may be no electricity) (Wang *et al.*, 2013). Sputum is also

hard to collect from immunocompromised individuals, such as children and those co-infected

with HIV (Leonard *et al.*, 2005). Children account for 6-10% of tuberculosis cases

worldwide and 40% of cases in countries with a high burden of tuberculosis (e.g. Central

Africa and Southeast Asia) (CDC, 2014). Approximately 33% of individuals that are HIV-

infected develop latent tuberculosis (WHO, 2014). Sputum from these individuals is

compromised because not only can a limited amount be collected, but it is also usually mixed

with blood, which could lead to inaccuracies in the results by inhibiting PCR (Abu Al-Soud

and Rådström, 2000). Studies have also show that up to three sputum specimens should be collected in order to obtain a sensitive and accurate diagnosis (Leonard *et al.*, 2005). Ultimately, problems with diagnosing tuberculosis at the point of care arise from three limitations: low specificity of the diagnostic, unavailability of appropriate diagnostic technology in developing countries, and the inability to observe and treat infected individuals for the entire course of treatment (Wang *et al.*, 2013). There is an urgent need for an easy-to-use, inexpensive, portable, and fast diagnostic device for tuberculosis that can be used at the point of care and relies on sample types other than sputum (Wang *et al.*, 2013).

V. *The NIH and Predicted Research Outcomes*

The National Institutes of Health (NIH), an agency of the United States Department of Health and Human Services, is composed of twenty-seven institutes and centers with specific research foci (NIH, 2014). For instance, the National Heart, Lung, and Blood Institute and National Institute of Allergy and Infectious Diseases are considered to be two of the twenty-seven institutes comprising the NIH, and fund many of the tuberculosis diagnostic projects (NIH, 2014). Most of these institutes and centers receive funding from Congress, and then they allocate these funds to biomedical and health-related research (NIH, 2014). The NIH offers several research grants, including the NIH Research Project Grant Program and Small Business Innovative Research grant (NIH, 2014). The NIH Research Project Grant Program, for instance, is usually awarded for three to five years and has no dollar limit (NIH, 2014). The amount of funding allocated to research is correlated with various measures of the burden of disease, including measures that take into account age of infected individuals, mortality rates, and the extent of disability (Gross *et al.*, 1999). However, the NIH has been criticized for not taking into account other measures, such as disease

prevalence (total number of cases at a given time) and incidence (number of new cases at a given time), when allocating funds (Gross *et al.*, 1999).

The National Institutes of Health Research Portfolio Online Reporting Tools Expenditures and Results (NIH RePORTer) publicly reports information about publications and projects funded by the NIH. Examining the projects undertaken by both academic and industrial sectors may reveal whether or not the development of a point of care tuberculosis diagnostic is feasible and if so, what underlying factors, besides technological issues, are delaying its development. In 2012, UNITAID, a global health organization devoted to making health care products more accessible and affordable for developing countries, released the "TB Diagnostic Landscape Report". The report outlined existing tuberculosis diagnostics and addressed what the diagnostics market was lacking (UNITAID, 2012). Consequently, this report, in addition to WHO's endorsement of Xpert MTB/RIF, generated an increased interest in the field of point of care diagnostics, specifically tuberculosis. This leads the investigators of this project to hypothesize that there will be more tuberculosis point of care diagnostic projects in 2014 than in 2008. Due to the challenges of working with sputum, another hypothesized difference between 2008 and 2014 projects is that more diagnostics will rely on other sample types.

Methods

This investigation sought out to compare data from diagnostic development projects from 2008, 2011, and 2014. This study is an extension of previous research conducted by Dr. Steven Casper at Keck Graduate Institute in Claremont, California. Dr. Casper had already obtained data from 2011, and his research methods served as a reference for acquiring data from 2008 and 2014. Initially, data sets from each year were planned on being

used in this analysis. However, due to inconsistencies in data collection between the

previously obtained 2011 data, and the 2008 and 2014 data specifically collected for this

study, only the 2008 and 2014 data were used. The NIH RePORTer was used to search for

an extensive range of diagnostic development projects. Search criteria included "point of

care diagnostics", " multiplex diagnostics", "tuberculosis point of care", and "device

development". Additionally, there was a search tool to view similar projects, so when one

device development project was found, others were searched for using this option.

The goal was to find all of the diagnostic projects being funded by the NIH. These

projects were not limited to tuberculosis device development, and included all point of care

device development. Projects were searched for until the data were saturated, meaning there

were no more point of care diagnostic development projects or the remaining projects were

repeats of the same project receiving a different type of grant. The data were then

categorized by which company or university was responsible for the project, the amount of

money being funded to the project, the type of NIH grant awarded, whether or not the device

being developed was multiplex, the type of technology being developed and its purpose, how

much the proposed device would cost (cost aim), the sensitivity of the device, the amount of

time the device would take to provide results, the size of the device, the power of the device,

the sample type that could be tested, whether or not tuberculosis was the primary market,

whether or not a device was being developed (e.g. rather than a kit), and the markets the

device would serve. The principle investigators (PIs) of the project were considered when

classifying projects as part of the industrial or academic sector.

This information was gathered primarily from the "details" and "descriptions"

subsections on the NIH RePORTer's project pages. More information on the projects, such

as other collaborators, or the size and sensitivity of the device, was gathered by researching

the device further outside of the NIH RePORTer. The data gathered were then analyzed by

calculating the total, average, and median funding of tuberculosis, non-tuberculosis, and all

projects in 2008 and 2014. The various markets for the device developments were totaled

and compared by generating a bar graph. The sample types were also compared by

generating pie charts.

Results

All of the diagnostic development projects in 2008 (n=61) and 2014 (n=68) were researched on the NIH RePORTer. These projects catered to approximately twenty-seven and twenty-four markets in 2008 and 2014, respectively, including tuberculosis (Figure 1-2). For both years, tuberculosis was the leading market, followed by HIV/AIDS in 2014 (Figure 2), and influenza and other respiratory viruses in 2008 (Figure 1).
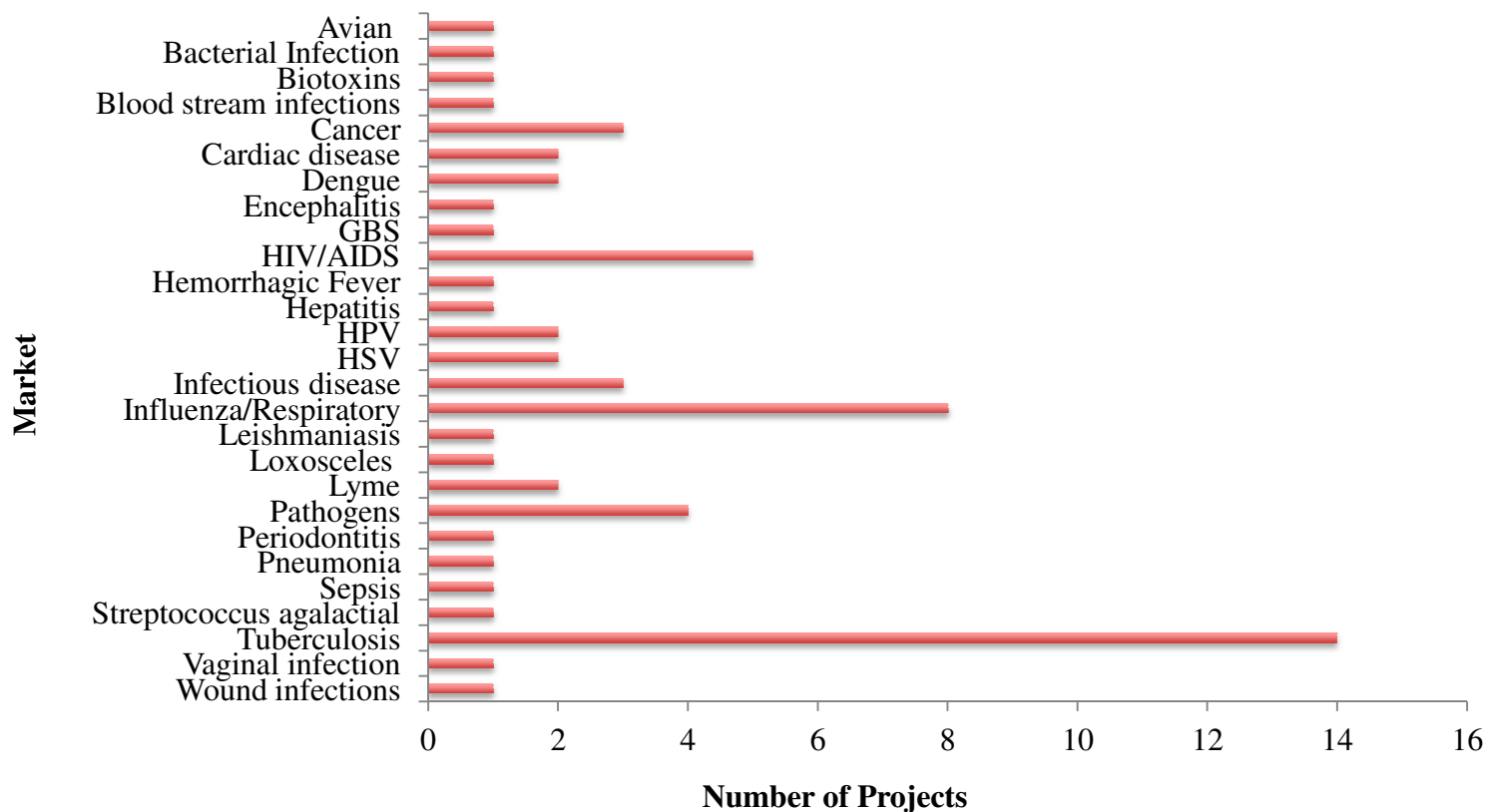

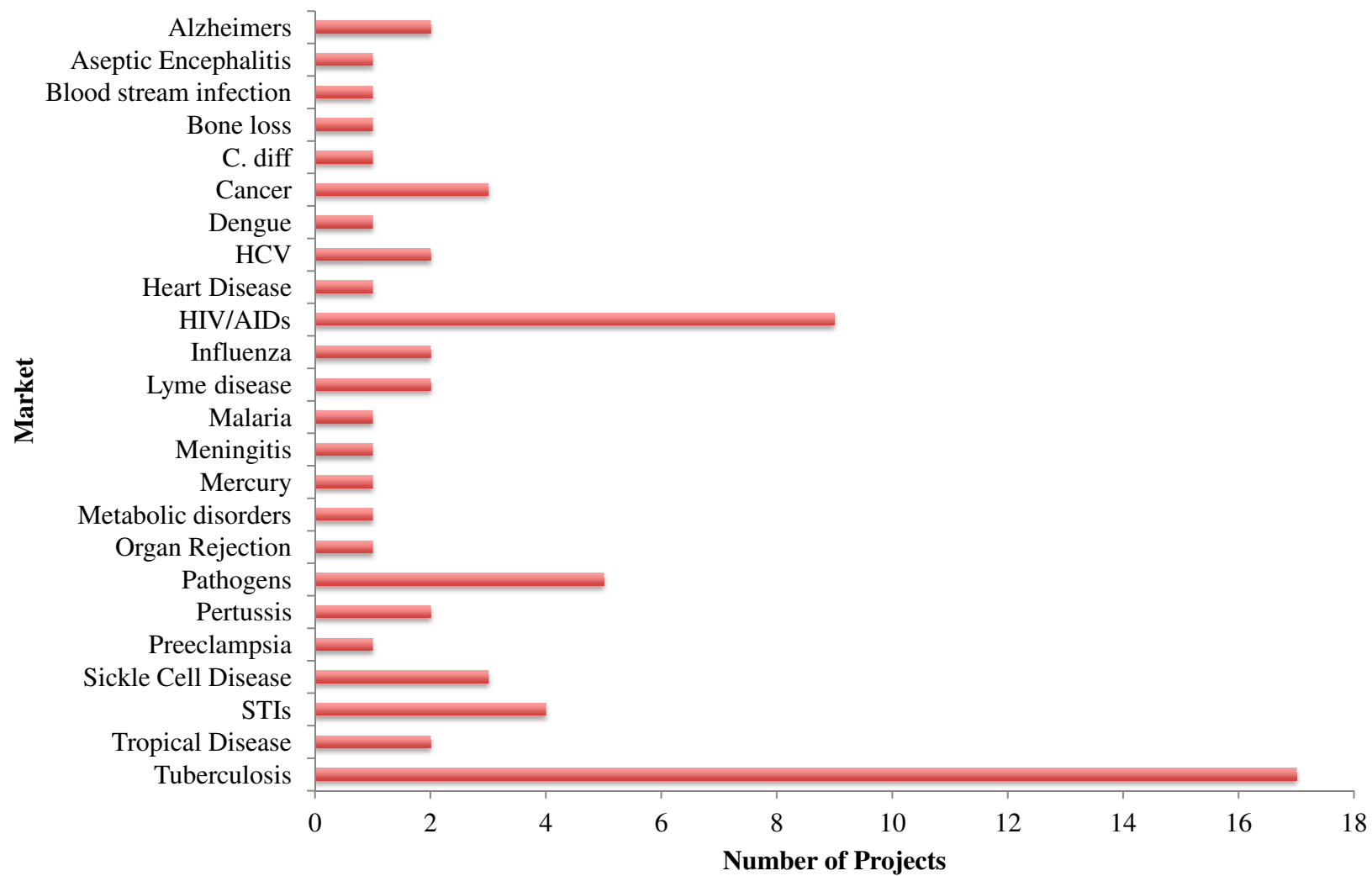**Figure 1.** All diagnostic development projects in 2008.

Figure 2. All diagnostic development projects in 2014.

In 2008, there were ten industrial and four academic tuberculosis diagnostic development projects funded by the NIH**.** The technologies being developed and sample types varied, however, all of the projects were multiplex (Tablea 1-2).

Table 1. Tuberculosis diagnostic projects in the industrial sector in 2008.

Company	Funding (USD)	Technology	Sample	Multiplex
Howard Shapirio Lab	299,229	Flow cytometry assay	Sputum	Yes
Investigen, Inc.	293,209	NAAT	Sputum	Yes
Lynntech, Inc.	145,242	NAAT	Sputum	Yes
Biosense Technologies, Inc.	100,000	Blood-based assay	Blood	Yes
Chembio Diagnostic Systems, Inc.	296,406	Blood-based assay	Blood	Yes
Pulsar Clinical Technologies, Inc	120,209	Immunoassay	Serum	Yes
Sequella, Inc	244,346	NAAT	Sputum	Yes
Investigen, Inc.	130,141	NAAT	Sputum	Yes
Cepheid Inc.	1,160,774	NAAT	Sputum	Yes
Akonni Biosystems, Inc.	296,316	Immunoassay	Nasal swabs, Sputum	Yes

Table 2. Tuberculosis diagnostic development projects in the academic sector in 2008.

University	Funding (USD)	Technology	Sample	Multiplex
UCSF	122,288	NAAT	Sputum	Yes
UCD	84,450	Immunoassay	Serum	Yes
University of Cape Town	132,435	Microscope development	Sputum	Yes
Beth Israel Deaconess Medical Center w/ Harvard Medical School of Public Health	126,630	Antigen detection test	Urine	Yes

In 2014, there were seven industrial and ten academic tuberculosis diagnostic development projects funded by the NIH. Similarly to 2008, the technologies being developed and sample types varied, however, all of the projects were multiplex (Tables 3-4).

Table 3. Tuberculosis diagnostic development projects in the industrial sector in 2014.

Company	Funding (USD)	Technology	Sample	Multiplex
Akonni Biosystems	399,604	NAAT	Sputum	Yes
Wave 80 Biosciences, Inc.	207,164	Microfluidic bioassay	Sputum	Yes
Akonni Biosystems	1,364,708	NAAT	Sputum	Yes
Sequella, Inc.	231,000	NAAT	Sputum	Yes
ImmunoMycologies, Inc.	299,640	Immunoassay	Serum, Urine	Yes
TB Biosciences, Inc.	515,802	Immunoassay	Serum	Yes
Viti, Inc.	996,814	Blood-based assay	Blood	Yes

Table 4. Tuberculosis diagnostic development projects in the academic sector in 2014.

University	Funding (USD)	Technology	Sample	Multiplex
Harvard Medical School	2,916,905	NAAT	Sputum, Urine	Yes
Keck Graduate Institute of Applied Life Sciences	607, 711	NAAT	Sputum	Yes
RBHS New Jersey Medical School	749,849	Flow cytometry assay	Blood	Yes
UCSF	1,167,864	Biomarker assay	Blood	Yes
Albert Einstein College of Medicine	208,750	Biomarker assay	Serum	Yes
RBHS New Jersey Medical School	1,123,270	Blood based assay	Blood	Yes
Stellenbosch University Tygerberg Campus	132, 468	NAAT	Sputum	Yes
RBHS New Jersey Medical School	1,548,810	NAAT	Sputum	Yes
University of Pennsylvania	186,767	Biosensor	Sputum	Yes
Michigan State Universty	202,234	Biomarker assay	Sputum	Yes

In both 2008 and 2014, the majority of the tuberculosis diagnostics relied on sputum samples (Figures 3-4). However, in 2014, there was an increase in the use of serum and blood as sample types (Figure 4).
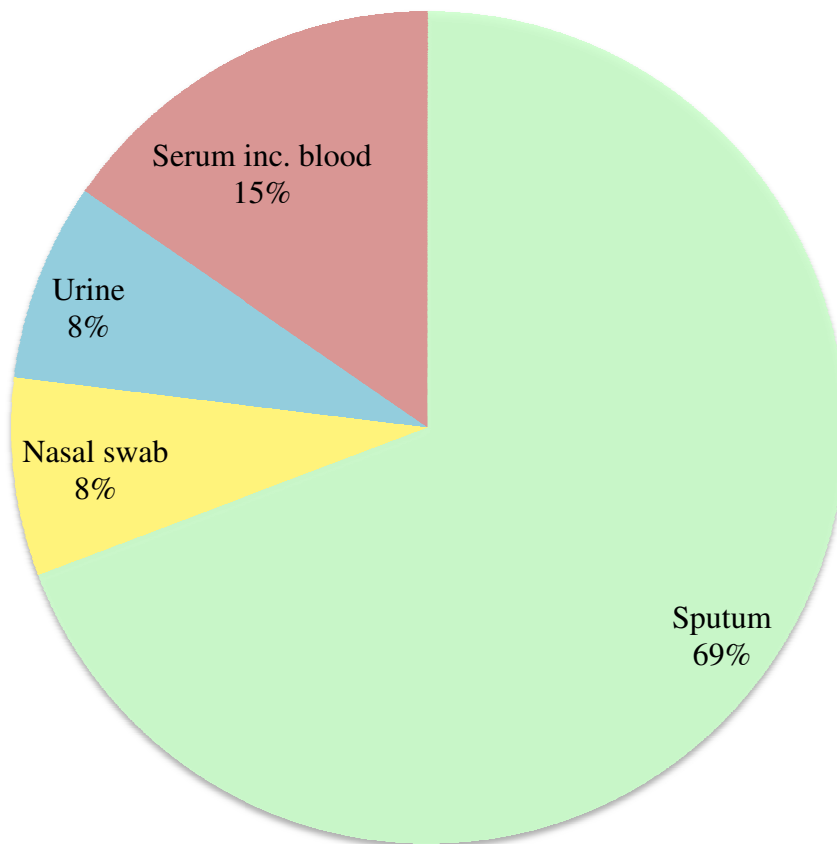

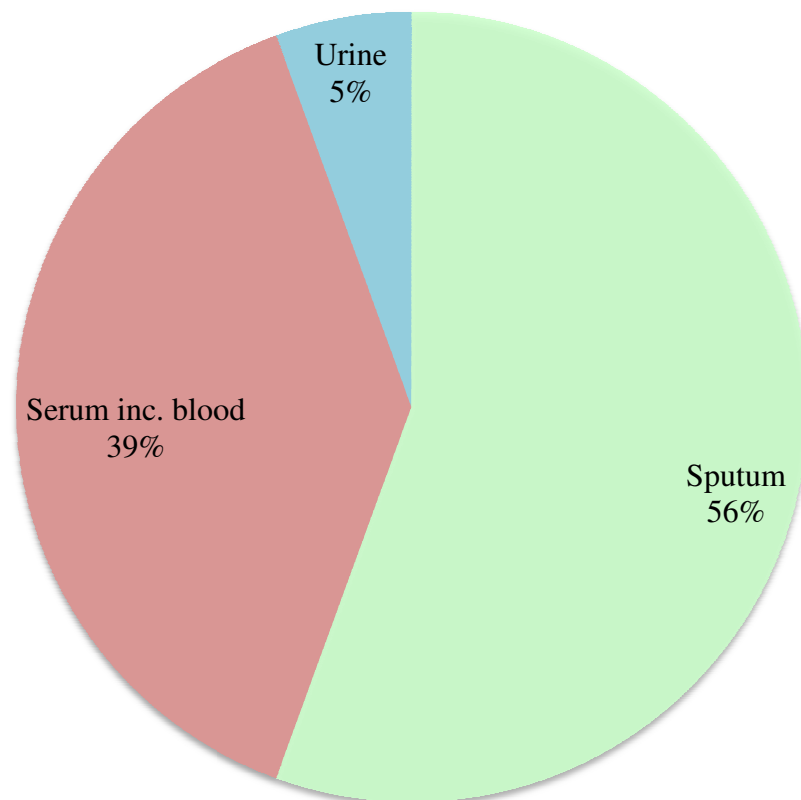**Figure 3.** Sample types used by proposed 2008 diagnostics.

Figure 4. Sample types used by proposed 2014 diagnostics.

A comparison was conducted between all diagnostic development projects, including tuberculosis, and just the tuberculosis diagnostic development projects being funded by the NIH. The comparison revealed that in 2008, approximately 15% of the total funds were allocated to tuberculosis diagnostics projects whereas in 2014, approximately 39% of the total funds were allocated to tuberculosis diagnostics projects (Table 5-6). In 2014, there were fewer projects in the industrial sector than in 2008, however, more funding was allocated to the industrial sector in 2014 (Tables 5-6). Even though there were only three more tuberculosis diagnostic projects in 2014 than in 2008, 8,567,506 USD more was allocated to projects in 2014 (Tables 5-6).

Table 5. Diagnostic development funding by the NIH in 2008.

	All Diagnostic Development (USD)	Tuberculosis Diagnostic Development (USD)
Total Funding	23,733,784	3,551,675
Median Funding	299,978	138,839
Average Funding	387,280	253,691
Academic Grants	10,935,577	465,803
Industrial Grants	12,798,207	3,085,872

Table 6. Diagnostic development funding by the NIH in 2014.

	All Diagnostic Development (USD)	Tuberculosis Diagnostic Development (USD)
Total Funding	31,417,193	12,119,181
Median Funding	329,390	515,802
Average Funding	541,676	807,945
Academic Grants	15,058,463	8,104,449
Industrial Grants	16,358,730	4,014,732

Discussion

Thorough research has been conducted in the field of tuberculosis treatment, and the disease has proven to be highly treatable and curable (Fox *et al.*, 1999). However, the problem of diagnosing the disease rapidly and effectively in low-resource settings, where unfortunately, tuberculosis is most prevalent, has yet to be resolved (WHO, 2014). A considerable amount of attention has been directed at developing diagnostic technologies for tuberculosis. In fact, in both 2008 and 2014, tuberculosis was the leading market for diagnostic development projects (Figures 1-2).

The review of proposed technologies revealed that a wide range of technology types was funded, that all technologies were multiplexed, a progression to the use of more serum and blood sample types in 2014 compared to 2008, and that there were more academic projects in 2014 (Figures 3-4, Tables 1-4). There was no notable difference between the technology types being developed in 2008 and 2014 or between academic and industrial sectors (Tables 1-4). It is interesing, yet not surprising, that all of the technologies are multiplexed, since multiplexed technology is more efficient and accurate (Zumla *et al.*, 2014). It is also economically savvy to develop multiplex technology because it can cater to numerous diagnostic markets. For example, if a point of care multiplex device does not generate a signigicant profit from the tuberculosis market in developing countries, it may generate a profit by being incorporated into a different market in developed countries. The use of more serum and blood samples supports the initial hypothesis that sputum would be relied on less as more point of care diagnostics are developed. Sputum is not only difficult to extract, but the necessity of obtaining, storing, and transporting more than one specimen to laboratories without having effective medical record systems in place, makes an accurate and

timely tuberculosis diagnosis almost impossible in developing countries (Wilson *et al.*,

2011). The development of a device that can effectively operate independent of those

variables can bypass the complicated logistical problems of health-care systems (Wilson *et

al.*, 2011).

In 2008, approximately 16% and 27% of the total academic and industrial grants,

respectively, were given to tuberculosis projects. In 2014, approximately 32% and 19% of

the total academic and industrial grants, respectively, were given to tuberculosis projects

(Tables 1-6). It is important to note, however, that this investigation was able to find seven

more NIH-funded projects in 2014 than in 2008. Most of the tuberculosis projects being

funded by the NIH in 2008 were focused on researching the pathogenesis of the disease.

Additionally, more of the total NIH funds were granted to tuberculosis-related projects in

2014 compared to 2008 (39% in 2014 compared to 15% in 2008) (Tables 5-6). These

findings are in alignment with the hypothesis that there would be a larger focus on point of

care devices in 2014 due to the WHO endorsement of Xpert MTB/RIF in 2010 and

UNITAID's 2012 report. The endorsement and the report instigated the resurgence of

interest in the tuberculosis diagnostic development, which explains why there were more

projects in 2014 (Wang *et al.*, 2013).

If point of care diagnostics are to be successfully implemented in developing

countries, industrial and academic sectors need to take into consideration the constraints

imposed by low-resource settings. First, developing countries have fewer well-funded

centralized testing centers compared to developed countries. Centralized testing centers

employ highly trained personnel, and have sophisticated infrastructure and resources (Chin *et

al.*, 2007). However, the centralized testing centers in developing countries lack in resources

compared to centers in developed countries (Chin *et al.*, 2007). Centralized testing centers can have moderate to high priced (e.g. more than 10,000 USD) technology if the price of disposables, such as the Xpert MTB/RIF cartridges is kept to a minimum (e.g. pennies) (Chin *et al.*, 2007). This is a major criticism of Xpert MTB/RIF because currently the cartridges do not meet this cost requirement. In contrast, point of care diagnostics requires minimal costs of both technology and disposables (Chin *et al.*, 2007). These economic and technological constraints severely hinder the development of an ideal point of care device, but they may also explain the greater interest in diagnostic development in the academic sector in 2014 (Table 4).

Research in the industrial sector is focused more on developing profitable technologies whereas research in the academic sector is focused more on solving scientific problems (Sauermann and Stephan, 2010). Therefore, there may not be as many incentives for industries to focus on or fund projects that cannot generate a significant profit, such as the cheap point of care diagnostics needed in developing countries. This would support why, in 2014, there was more money granted to industrial projects compared to academic projects, even though there were three more academic than industrial projects. The greater number of academic projects may stem from the incentive of finding a solution (potentially award-winning) to the tuberculosis global health challenge. The greater level of funding given to fewer industrial projects may stem from the economic and policy incentives of developing effective and profitable technologies for either centralized testing centers or health care systems in developed countries. It is important to consider that private industries also fund approximately twice as many research projects as the NIH, which means that the NIH fund allocation only paints a small portion of the bigger picture (Gross *et al.*, 1999). More focus

on the point of care diagnostic dilemma in the academic sector may be beneficial due to the greater motivation towards finding an effective solution, rather than a marketable one. However, more research needs to be focused on finding a non-sputum based diagnostic.

Inaccuracies in the obtained data may have stemmed from double counting projects and not finding all of the diagnostic development projects. Double counting may have occurred because some industries will collaborate with academic sectors to work on projects, and consequently receive separate grants from the NIH. For instance, in 2008, Cepheid Incorporated collaborated with the New Jersey Medical School to develop a NAAT cartridge (Table 1). To avoid this problem, all of the projects were thoroughly researched outside of the NIH RePORTer, however, some projects had no publications describing them. Most of the projects did not have information about the cost, size, sensitivity, time, or power supply of the proposed diagnostic. This made it difficult to establish how successful these projects would be at the point of care in low-resource settings. Future investigations should conduct a more thorough review of collaborations between academic and industrial sectors. More years, in addition to 2008 and 2014, should be reviewed in order to establish if there has been a trend in diagnostic development in the past decade. Furthermore, it would be interesting to examine what diagnostic projects in 2008 were finalized or successfully implemented in health care systems.

Conclusion

In summation, progress has been made towards developing a tuberculosis diagnostic device that can function effectively at the point of care. There are more diagnostics being developed and many of them are non-sputum based. However, developing a tuberculosis diagnostic is a multi-faceted problem. The high cost of technology and underdeveloped

medical infrastructure within low-resource settings are major factors that need to be addressed before a point of care device can be actualized. For instance, it is much easier to implement diagnostics in healthcare systems that provide access to well-funded laboratories and highly trained lab technicians (Wilson *et al.*, 2011). Hopefully, the academic sector's focus on diagnostic development will persist and result in an effective solution that can be implemented at the community level. With more research come more solutions that can combat the global burden of tuberculosis.

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Resources

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