MODS and/or TLA Techniques: A Systematic Review and Meta-Analysis for Active Tuberculosis Diagnosis and an Evaluation of their Cost-Effectiveness and Feasibility

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A thesis submitted to McGill University in partial fulfilment of the requirements of the degree of Master of Science

August 2010

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Acknowledgements

I would like to take this opportunity to thank the individuals who have supported me throughout this process:

My supervisor, Dr. Dick Menzies, has been an important source of support and guidance. I feel extraordinarily fortunate to have had the opportunity to learn from him.

The members of my thesis committee, Dr. Madhukar Pai and Dr. Andrea Benedetti for providing additional advice and direction when needed. Dr. Kevin Schwartzman, for all of his advice and support that he has given me during my degree. Dr. Jessica Minion, who has also given me a lot of guidance. The professors, staff, and students from the RECRU team for teaching me so much and all of their insightful comments. The incredible professors in the Department of Epidemiology, Biostatistics and Occupational Health- people who have instilled an interest in this discipline for me.

The administration staff of the Respiratory Epidemiology and Clinical Research Unit at the McGill University Health Centre and the Department of Epidemiology, Biostatistics and Occupation Health- without whom these two departments would not function.

Lastly I would like to thank my family and friends for their continuous support and encouragement. The friends I have made and the people I have met during my MSc. have inspired me to succeed and to strive. My brother and my mother, for their constant support and for always believing in me.

This work was supported by TREAT-TB of the International Union against Tuberculosis and Lung Disease.

Abstract

OBJECTIVE: A systematic review and meta-analysis was performed to compare Microscopic-Observation Drug Sensitivity (MODS), Thin Layer Agar (TLA) and reference standards for sensitivity and specificity for tuberculosis detection and other characteristics. A questionnaire was conducted to evaluate the feasibility, costs and practical aspects of implementation of MODS/TLA.

METHODS: A random effects meta-analysis to estimate the sensitivity and specificity was performed. A self-administered questionnaire was sent to laboratories using MODS/TLA.

RESULTS: The overall sensitivity and specificity for MODS were 92% and 97% respectively and for TLA, they were 83% and 98% respectively. Equipment costs and training costs were moderate, costs for materials were low and labour costs were high.

CONCLUSION: MODS and TLA appear to offer simple, inexpensive yet rapid and accurate diagnostic tools for active TB. Overall, costs were moderate to implement MODS/TLA. Important unresolved issues for further investigation include the cost-effectiveness and optimal methods for quality assurance of these TB diagnostic tools.

Résumé

OBJECTIF: Une revue systématique et méta-analyse a été effectuée pour comparer Microscopic-Observation Drug Sensitivity (MODS), Thin Layer Agar (TLA) et normes de référence pour la sensibilité et la spécificité pour la détection de la tuberculose. Un questionnaire a été menée pour évaluer la faisabilité et les coûts de MODS/TLA.

MÉTHODES: Un méta-analyse anx effets aléatoires pour estimer la sensibilité et la spécificité a été réalisée. Le questionnaire a été envoyé aux laboratoires utilisant MODS/TLA.

RÉSULTATS: La sensibilité et la spécificité pour MODS étaient 92% et 97% respectivement et pour TLA, ils étaient 83% et 98% respectivement. Les coûts initiaux étaient modérées, les coûts des matériaux étaient faibles et les coûts salariaux étaient élevés.

CONCLUSION: MODS/TLA sont des outils diagnostiques peu coûteux, rapides et précises. Les coûts pour la mise en oeuvre étaient modérés mais le coût-efficacité et la faisabilité pour les outils diagnostiques de TB sont deux domaines importants à étudier.

List of Abbreviations

TB	Tuberculosis
WHO	World Health Organisation
DOTS	Directly Observed Treatment, Short Course
MODS	Microscopic Observation Drug Susceptibility
TLA	Thin Layer Agar
HIV	Human Immunodeficiency Virus
MDR-TB	Multidrug-resistant Tuberculosis
XDR-TB	Exensively drug-resistant TB
MTB	Mycobacterium Tuberculosis
TST	Tuberculin Skin Test
IGRA	Interferon-Gamma Release Assays
AFB	Acid-Fast Bacilli
NAAT	Nucleic Acid Amplification Test
PNB	Para-Nitrobenzoic Acid
RIF	Rifampin
INH	Izoniazid
SM	Streptomycin
PZA	Pyrazinamide
ETH	Ethambutol

Introduction

Tuberculosis is a major cause of illness and death worldwide with the majority of the cases occurring in low- and middle-income countries. The World Health Organization (WHO) estimated that 9.27 million new cases of TB occurred in 2007 (139 per 100 000 population) compared with 9.24 million new cases (140 per 100 000 population) in 2006. Of these 9.27 million new cases, an estimated 44% were new smear-positive cases[1]. The global TB mortality rate is estimated to have increased during the 1990s- a trend that was reversed around the year 2000. Now, mortality rates are in decline[1]. The WHO also estimated that, despite 15 years of efforts in expansion of national TB control programmes in almost all low and middle income countries, only 45% of all new smear positive cases were diagnosed in 2007[1]. This low rate of case detection has stimulated the search for new diagnostic tools, which are rapid, accurate and inexpensive. These could increase TB case detection, thereby reducing morbidity, mortality, and transmission of infection.

Current standard methods of diagnostics include smear microscopy, solid conventional culture and liquid conventional culture. While smear microscopy can provide results within the day, the sensitivity is low. Solid culture is more sensitive than smear microscopy but usually provides results between four to eight weeks. Liquid culture has the greatest sensitivity and provides results within two to four weeks but the equipment and materials are very costly.

New non-commercial culture techniques have been introduced that are less complex and costly than the automated liquid culture systems, but have similar accuracy, and time to results. Two of these are the Microscopic Observation Drug Susceptibility (MODS) assay and the Thin Layer Agar (TLA) method. Both techniques involve direct inoculation of clinical specimens into media - which is liquid for MODS, and solid for TLA. Microscopic examination of the cultures is performed regularly to detect microcolonies.

A systematic review and meta-analysis was performed of all published studies that reported diagnostic accuracy, time to result, contamination rates and costs of MODS or TLA assays for the detection of active tuberculosis. For detection of active TB with MODS, the overall sensitivity and specificity were 92% and 97% respectively. For TLA, the overall sensitivity and specificity were 83% and 98%, respectively. The time to result for detection of active TB was nine days with MODS and 11.5 days for TLA. This systematic review revealed that no studies reported data on feasibility issues, nor costs for implementation or recurrent labour costs for the microcolony techniques for the diagnosis of active TB. This motivated the creation of a mailed questionnaire survey of persons responsible for implementing these tests about these aspects of MODS and TLA.

1 Background

1.1 Epidemiology of TB

1.1.1 Epidemiology of TB worldwide

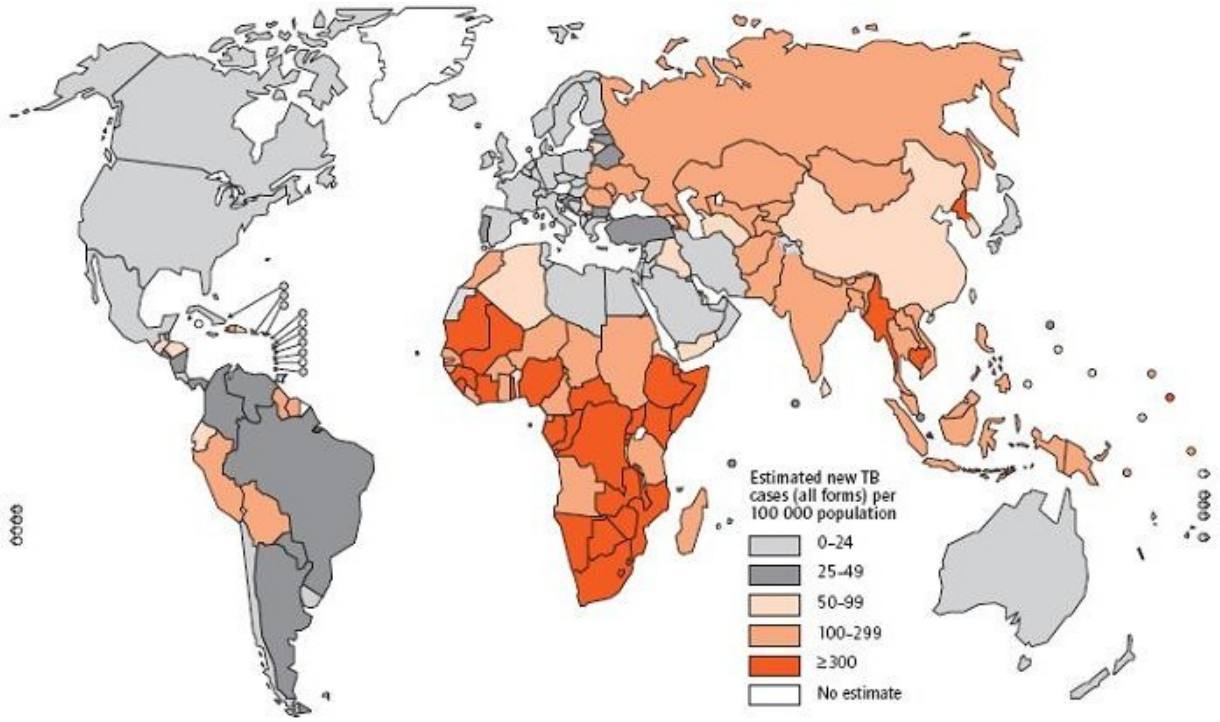
Almost one third of the world population is infected with tuberculosis[1]. It is the fifth most important cause of death worldwide, causing 1.6 million deaths annually. In 2008, there were an estimated 9.4 million incident cases of TB globally, an increase from the 9.3 million new TB cases estimated to have occurred in 2007. An estimated 1.32 million HIV-negative people (19.7 per 100 000 population) died from TB in 2007 with an additional 456 000 TB deaths among HIV-positive people.

In high-income countries, the number of cases of TB is declining and most new cases are among immigrants from low-income countries[2]. Transmission rates are low, and have fallen steadily over the last 50 years. As a result among the native born population the cases have shifted to older adults and a high proportion of these cases are attributable to reactivation of latent TB[3].

1.1.2 Epidemiology of TB in Low- and Middle-Income Countries

TB is a leading cause of adult mortality in low-and middle-income countries, ranking third after HIV and ischaemic heart disease as a cause of death amongst those aged 15-59 years[4-5]. There are 22 high-burden countries that account for 80% of the TB cases in the world, of which five rank highest in terms of total number of incident cases: India, China, South Africa, Nigeria, and Indonesia. The South East Asia and Western Pacific regions account for 55% of global cases and the African Region accounts for 31%. Among the 15 countries with highest TB incidence rates, 13 of them are African countries[6]. Much of the increase in TB incidence in Africa since 1980 is also attributable to the spread of HIV[5-6]. While mortality rates have been declining in some parts of the world, they increased in the African and Eastern European regions substantially in the 1990s.

Figure 1. Estimated Number of New TB Cases, by Country, 2007[1]

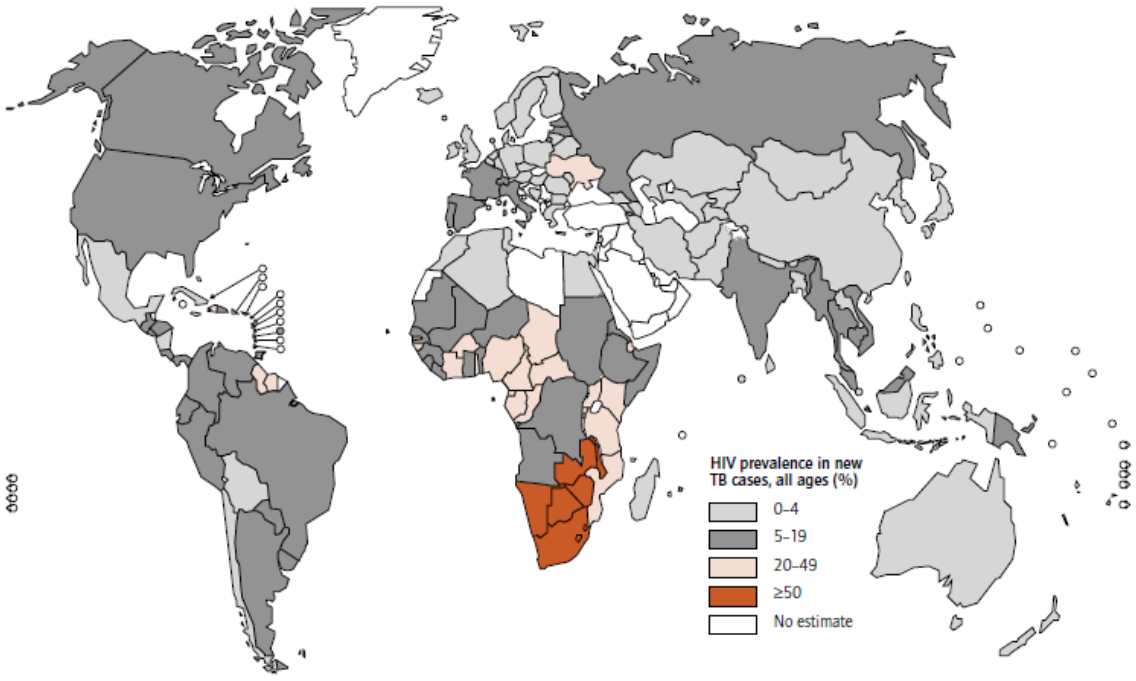


1.1.3 Factors Contributing to the Increase in Global TB Worldwide

Some factors contributing to the increase of the global TB incidence are the HIV epidemic, the emergence of drug resistance and the low case detection rate.

HIV infection has been identified as a potent risk factor for developing TB. The global number of incident HIV-positive TB cases is estimated to have peaked in 2005, at 1.39 million. In 2007, the African Region accounted for the majority (79%) of the HIV-positive TB cases. Thirty-one percent of these cases were found in South Africa alone. The South-East Asia Region followed, with 11% of total cases- the majority of the cases were found in India[1].

Figure 2. Estimated HIV Prevalence in New TB Cases, 2007[1]



The growing prevalence of drug-resistant disease has also been an important problem which has limited proper TB control. Patients who are infected with drug-resistant strains

of TB need a modified treatment regimen which incorporates second line drugs and lasts longer[7]. However, even when patients with drug-resistant strains are treated properly, they often have worse outcomes and tend to be infectious for a longer time [8-9].

Multidrug-resistant TB (MDR-TB) refers to active disease caused by strains that are resistant to two of the most potent first-line antimicrobial drugs used to treat TB: rifampin and isoniazid. In 2008, of all new TB cases globally, 3.6% were estimated to have MDR-TB – for a total of 440,000 cases, of which almost 50% of MDR-TB cases were estimated to have occurred in China and India. Over 10% of the TB cases in Eastern Europe and Russia were MDR-TB in 2008[10]. In 2008, MDR-TB alone caused an estimated 150,000 deaths[11].

Extensively drug-resistant TB (XDR-TB) indicates resistance to isoniazid, rifampin, as well as a fluoroquinolone, and to one or more of the following injectable drugs: amikacin, capreomycin or kanamycin[12]. By November 2009, there were 58 countries and territories that have reported at least one case of XDR-TB. There were 5.4% of MDR TB cases that were found to have XDR-TB[12-13].

Finally, a low case detection rate also contributes to the increase of global TB, through continued transmission – since undiagnosed patients will remain untreated and therefore contagious for long periods. For an untreated smear-positive TB patient, the median survival time is two years. During this time, patient can infect up to 20 other people (ten new infections per year). This will result in the creation of an ever-increasing pool of infected persons, from which active cases will later arise, causing further transmission.

1.2 Clinical Aspects of TB

1.2.1 The Causative Organism and Transmission of TB

Mycobacterium tuberculosis (MTB) is the etiologic agent of tuberculosis in humans. It is a slender, rod-shaped bacterium about two to four micrometres in length and 0.2-0.3 um

in width. MTB is a very complex bacterium which can survive under very adverse conditions for long periods of time. The bacterium is an obligate aerobe and a facultative intracellular parasite with a slow generation time, which is a characteristic that contributes to the insidious nature of the disease[14].

Humans are the only reservoir for the bacterium and it is spread through aerosol droplets. Airborne transmission through the respiratory tract is by far the most common method of transmitting TB. Simply put, what is necessary for TB infection is contact with someone with active disease. The likelihood of having contact with someone carrying active TB is determined by the underlying disease burden of the community- hence people living in high TB-prevalence areas are at greater risk of exposure[15]. If exposed, the key determinants of infection with TB are: the concentration of microorganisms in the air, the degree of susceptibility of the exposed person, and the duration of exposure.

Once a patient has active pulmonary TB they are able to spread TB to others. Transmission occurs from expulsion of droplets containing mycobacteria through coughing, sneezing or speaking. These droplets can stay in the air for hours and if the patient breathes in these droplets, the TB germs will get into the lungs[16].

1.2.2 Pathogenesis of TB

1.2.2.1 Primary Infection

Primary infection requires the inhalation of particles that are small enough to be deposited into the lower respiratory tract. If the tuberculosis bacteria reach the lower respiratory tract, primary infection can result.

The TB bacilli will induce an early inflammatory response which will isolate and phagocytise the bacilli but their destruction depends upon innate immune mechanisms. In many persons, the innate system is not sufficient to destroy the initial few bacteria,

allowing them to replicate. Once the bacterial population is large enough, cell-mediated immunity and delayed-type hypersensitivity are stimulated[17]. Cell-mediated immunity involves CD4 receptor lymphocytes which secrete cytokines (such as IFN-γ) which enhance the capacity of macrophages to ingest and kill the mycobacterium. Delayed-type hypersensitivity is thought to involve T-lymphocytes and can be protective or harmful to the host[17]. At the site of implantation, if cell mediated immunity develops, the primary infection is usually contained and tubercles begin to form. If cell mediated immunity does not develop then primary TB can progress directly to active disease.

In immunocompetent hosts, alveolar macrophages ingest the MTB organisms but resistance mechanisms of the bacteria may destroy these macrophages[17]. New macrophages attracted to the site can engulf these bacilli and the cycle continues. The bacilli can spread from the initial lesion to other parts of the body through the circulatory system, but usually the growth of the bacilli is contained within a single lesion in the lung, known as the primary lesion - typically a small, circumscribed nodule in the lung.
This is accompanied by a lesion in the hilar lymph node draining that part of the lung. Through-out all this immunologic activity the infected individual remains asymptomatic.

1.2.2.2 Latency

In 95-99% of persons, primary infections will be controlled by the development of adequate cell mediated immunity and the infection will enter the latent phase. There are usually no signs or symptoms of latent TB[18].

1.2.2.3 Reactivation

In about 10% of persons the latent infection reactivates into active disease. In most cases, TB will reactivate within the first two years but it can reactivate decades later as well. Some of the risk factors include those that impair the host defence against infection and break-down to disease such as HIV infection, malnutrition, tobacco smoke, indoor air

pollution caused by burning of solid fuels, alcohol abuse, diabetes, malignancies and immunosuppressive treatment[15]. Prince et al. also showed that depression and stress can have negative effects on the cell-mediated immune system, which could also theoretically increase TB risk[19].

While active disease can involve virtually any organ of the body, in 80% of individuals with disease the lungs are affected[1]. The symptoms of active disease are often non-specific. Pulmonary disease symptoms can include productive cough, fatigue, anorexia, weight loss, fever, sweating or chills and chest pain[20-21]. Extra-pulmonary TB can also cause anorexia and weight loss as well as fatigue and night sweats, while other symptoms specifically related to the affected organ will be prominent[20].

1.3 Treatment of active TB

Effective treatment is a key element of TB control. Effective treatment prevents mortality, and further morbidity in the individual infected with TB and also renders the patient non-infectious.

The first line drugs that are used for tuberculosis treatment are Rifampin (RIF), Isoniazid (INH), Streptomycin (SM), Pyrazinamide (PZA) and Ethambutol (EMB).

The initial treatment consists of:

- Two months of RIF, INH, PZA and EMB
- Followed by four months of RIF and INH.

If the patient is resistant to any of these drugs and receives standard treatment (due to undiagnosed resistance) then the risk of failure/relapse is much higher. If a patient fails, relapses or defaults from the initial treatment, they receive retreatment. The WHO-recommended retreatment consists of:

- 2 months of SM, INH, RIF, PZA and EMB

- One month of RIF, INH, PZA and EMB
- Followed by 5 months of INH, RIF and EMB.

If the patient has MDR-TB or XDR-TB, they will require treatment with second line drugs which are more costly, less available, more toxic and less effective. These drugs include:

- Aminoglycosides (Kanamycin, Amikacin)
- Polypeptides (Capreomycin)
- Floroquinolones (Ofloxacin, Levofloxacin)
- Thiomides (Ethionamide, Prothionamide)
- Cycloserine
- p-aminosalicylic acid

1.4 Global Strategies to Stop TB

Directly Observed Treatment, Short-Course (DOTS) is a programmatic strategy to improve access to high quality diagnosis and treatment for TB patients. The WHO has promoted this strategy since 1993, and established targets for country programs to detect 70% of smear-positive TB cases, and cure 85% of those detected.

1.4.1 Success of DOTS

A lot of progress has been made since WHO first began promoting the implementation of DOTS in 1993. Within a decade, 182 countries had implemented DOTS. Seventy-seven percent of the world's population lived in countries or regions covered by DOTS. On average, 82% of newly identified cases completed the treatment.

Many patients with active TB now have access to treatment when they did not before. By 2004, more than 20 million patients had been treated by DOTS and more than 16 million

had been cured through DOTS. A key concept of DOTS is decentralized diagnostic and treatment services to improve access for TB patients.

DOTS is also one of the most cost-effective health programmes in the developing world[25].

1.4.2 Limitations of DOTS

However, there are some limitations with DOTS. While case detection rates have improved in some areas, WHO estimated that in 2008 only about 45% of new cases were identified globally rather than the 70% WHO target. Moreover, the total number of incident cases of TB is increasing, even in countries that have achieved the WHO DOTS targets[22]. Because of these limitations, the DOTS Expansion Working Group Strategy 2006-2015 was created to increase and improve access to DOTS. Specific goals were to enhance access to new TB tests that were expected to become available and to contribute to the 2015 global targets for TB control that were part of the Millennium Development Goals. These goals include the objective to halt and reverse the incidence of TB by 2015 and to halve the TB prevalence and death rate by 2015, as compared with 1990 levels[24]. The expanded DOTS programme has added components to the basic DOTS programme in order to achieve their goals.

DOTS (1993-2005)	Expanded DOTS (2006-2015)
1. Political commitment with increased and sustained financing	1. **Sustained political commitment** to increase human and financial resources and make TB control a nation-wide activity integral to national health system
2. Case detection through quality-assured bacteriology	2. **Access to quality-assured TB sputum microscopy** for case detection among persons presenting with, or found through screening to have, symptoms of TB (most importantly prolonged cough). Special attention is necessary for case detection among HIV-infected people and other high-risk groups, e.g. people in institutions
3. Standardized treatment, with supervision and patient support	3. **Standardized short-course chemotherapy to all cases of TB under proper case-management conditions including direct observation of treatment-** proper case management conditions imply technically sound and socially supportive treatment services
4. An effective drug supply and management system	4. **Uninterrupted supply of quality-assured drugs** with reliable drug procurement and distribution systems
5. Monitoring and evaluation system, and impact measurement	5. **Recording and reporting system enabling outcome assessment** of each and every patient and assessment of the overall programme performance.

The Stop TB Partnership, established in 2000, aims to eliminate TB as a public health problem and achieve a world free of TB. The main activities focus on raising awareness about TB and advocating more commitment to TB prevention, treatment and research[23].

1.5 Diagnosis of Active TB Disease

1.5.1 Clinical Examination and Chest X-Rays

Clinical examination and chest radiography are usually the first diagnostic measures. Limitations of chest X-rays include: low sensitivity, low specificity and problems in inter-reader variability. Sensitivity ranges from 70-80%, and specificity ranges from 60-70%[24]. With chest X-rays, the interpretation is variable depending on the reader which can be problematic in determining likelihood of active disease[24]. For these reasons, chest X-rays are not considered the gold standard for diagnosis of pulmonary TB.

1.5.2 Serologic Diagnosis

There are many commercial serological antibody detection tests available for pulmonary and extra-pulmonary TB detection. These tests require collection of a blood sample which is generally much easier than collection of a specimen from the site of disease and are particularly attractive for extra-pulmonary TB[25]. However, these tests are neither sensitive nor specific, and as a result WHO has recently issued a statement discouraging their use for active TB diagnosis [26].

1.5.3 Immune-Based Diagnostics (TST/IGRA)

Some of the more familiar tools available to diagnose latent TB are immune-based diagnostics- the tuberculin skin test (TST) and T-cell based Interferon-Gamma Release Assays (IGRA). The TST consists of the injection of 0.1ml of purified protein derived

from the MTB bacteria. For someone who has pre-existing cell-mediated immunity to these tuberculin antigens, a delayed hypersensitivity reaction will occur within 48-72 hours, causing localised swelling[27]. False-positive results can occur; some causes for this include errors in administering the TST, prior vaccination with the BCG vaccine and prior sensitisation with non-tuberculous mycobacteria[28].

IGRAs are *in-vitro* T-cell based assays such that measure the IFN-gamma response of peripheral blood lymphocytes in response to MTB-specific antigens. These assays operate on the basis that T-cells previously sensitised to TB antigens produce high levels of IFN-gamma when re-exposed to the same mycobacterial antigens[29]. Two tests are available: the Quantiferon-TB Gold In-Tube (Cellestis) and the T-SPOT.*TB* (Oxford Immunotec). However, both the TST and the IGRA play a limited role in diagnosis of active TB because they cannot distinguish between latent TB and active TB.

1.5.4 Microbiologic Diagnosis (The Gold Standard)

Microbiologic tests are considered the gold standard to confirm the diagnosis of active disease and are also useful to perform drug susceptibility tests.

1.5.4.1 Smear Microscopy

The most widely used rapid test is direct microscopy of a smear of sputum (or other clinical sample) which has been stained for acid-fast bacilli (AFB smear). AFB smear microscopy is the main diagnostic method currently used in most low income countries because it is simple, inexpensive, widely applicable and specific for TB. It is also useful because it has the ability to identify the sickest and most infectious patients[30].

The main disadvantage to smear microscopy is that its overall sensitivity ranges from 20% to 80%, depending on the type of specimen, the patient population, and the technician's performance. Moreover, the sensitivity of smear microscopy is limited in

paediatric TB, as well as in HIV-infected patients. Because direct microscopy cannot distinguish between *mycobacterium tuberculosis* **(**MTB**)** and nontuberculous mycobacteria (NTM), specificity is also of concern, although this is not a serious concern in countries with high TB incidence. AFB smear is also labour intensive, so in countries with high cost of labour this will not be as cheap.

1.5.4.2 Cultures

Mycobacterial culture is more sensitive and specific for the diagnosis of TB than AFB smear and is considered the gold standard in diagnostics for TB. While a single positive culture for MTB is considered to define active disease, for pulmonary TB the sensitivity of three sputum cultures is usually more than 90% and with six specimens it is possible to achieve 100% sensitivity[24]. However, two sputum cultures are currently recommended because this represents the best balance between maximal sensitivity and lower costs. The specificity of culture is also usually greater than 90%[28].

Mycobacteria can be cultured on solid media or on liquid media. The length of time before a culture will show a positive result depends on which culture media is used, as well as the number of mycobacteria in the original specimen. Some types of solid cultures that are used are the Lowenstein-Jensen or Middlebrook medium. Solid cultures are only moderately expensive as materials and equipment are relatively cheap compared to liquid culture. However solid cultures take four to eight weeks before growth of mycobacteria is detectable. Two types of liquid culture currently used are MGIT and BACTEC. The major advantage of liquid culture is that results are available more quickly - in as little as 11 days, although usually positive cultures are detectable only after two to four weeks. The major disadvantage is that the equipment and materials needed are costly and complex.

1.5.4.3 Nucleic acid amplification tests (NAAT)

Nucleic acid amplification tests amplify target sequences of DNA or RNA from the MTB organisms. These tests have been introduced into clinical use in the last two decades. NAATs were considered to be a major breakthrough in TB diagnosis when they were first introduced but have not replaced any previously recommended tests. These tests are complex and expensive but have advantages[31], including high specificity, greater sensitivity than smear microscopy, with an average of about 80% compared to culture. They are also very quick as they can provide results within one day[31].

However, sensitivity is modest in smear-negative pulmonary TB, and in extra-pulmonary disease averages 50-60%. False positives can also be very frequent, because without meticulous laboratory procedures and techniques, cross-contamination is frequent, which can lead to all specimens being positive.

1.5.4.4 Drug Sensitivity Testing

In addition to increasing case detection, a second major priority is to find a cheap, fast and reliable method of drug susceptibility testing to detect drug resistant strains, given the rising prevalence of drug resistance, as discussed earlier.

Delayed diagnosis of drug resistance can lead to extended ineffective treatment of TB. This will result in increased morbidity and mortality, continued transmission, and the promotion of multidrug-resistant TB[32-33]. Thus, it is important to find a way to ensure timely identification of drug resistant cases.

1.5.5 Why New Diagnostic Tests Are Needed

Because only 45% of all new cases were detected in 2008 one of the main objectives in developing new TB diagnostics is to expand case finding. This in turn, will lead to overall better TB control and reduced TB transmission in the future. Smear microscopy is not

sensitive enough, solid culture is not quick enough and liquid culture is too expensive using complex technology and still takes two to four weeks.

The ideal new diagnostic test would detect both smear-positive and smear-negative cases accurately, within a few days – from the time of specimen receipt to the time to result. The new test should be affordable for low- and middle-income countries, where the majority of TB cases are found. And finally, the ideal test would also use simple technology so that it is easy to implement and learn.

Palomino et al. emphasized that if a "magic bullet" is created for TB diagnostics, this new method would need to be feasible in every possible setting. The test should be evaluated in well-designed and controlled clinical trials, in high-incidence, low-resource settings where the implementation and use of these methods are most needed [34]. Foulds et al. stated that an ideal test would address four areas of need in TB diagnostics: 1) replace or facilitate AFB microscopy for the identification of smear-positive cases, 2) improve the diagnosis of AFB smear-negative cases, 3) determine drug susceptibility in cases where standard treatment fails and 4) identify persons with latent TB infection[35].

1.5.6 Microcolony-Based Culture Methods

The Microscopic-Observation Drug Sensitivity (MODS) and Thin Layer Agar (TLA) are two inexpensive and rapid microcolony-based culture methods.

1.5.6.1 MODS

Microscopic-Observation Drug Sensitivity (MODS) involves direct inoculation of patient specimens to liquid media. Microscopic examination of the cultures is performed regularly to detect early growth of microcolonies. This method makes use of two properties of MTB: growth is faster in liquid than solid media, and growth in liquid media produces an easily recognisable and characteristic microscopic cording appearance[36]. This

method is simple, and requires very little specialized equipment except an inverted light microscope[36], and all the materials needed to perform MODS are inexpensive and readily available from laboratory suppliers. This enhances the feasibility of this test in resource limited settings.[36].

1.5.6.2 TLA

Thin Layer Agar (TLA) involves direct inoculation of patient specimens onto solid media. Microscopic examination of the cultures is performed regularly to detect early growth of microcolonies. TLA allows early identification of MTB based on colony morphology, visualized microscopically and by incorporation of para-nitrobenzoic acid (PNB) in the medium for species identification.

These two techniques, MODS and TLA, are considered together because they are both based on the microscopic detection of early mycobacterial growth on culture. The major difference is the type of media being used- MODS uses liquid media while TLA uses solid media.

1.6 Background to Methods Used in the Two Studies

1.6.1 Conducting a Systematic Review and a Meta-Analysis

Systematic reviews and meta-analyses are considered the best sources of evidence for evidence-based medicine. Systematic reviews synthesize data from existing primary research and can also demonstrate areas where the available evidence is insufficient [37-38].

A systematic review is a clearly formulated question that uses systematic and explicit methods to identify, select and critically appraise relevant research and to collect and analyse data from the studies that are included in the review. A meta-analysis is the

statistical pooling of data across studies to generate summary estimate of effects and is usually the final step in a systematic review [37].

There are several steps involved when performing a systematic review. The formulation of a focused review question is the first step. By having a focused review question, it will help in the conduct of more specific searches in the databases and in the creation of the criteria for study selection. A comprehensive and exhaustive search for the primary studies is then required. In searching for primary studies, an exhaustive search might include using both general and subject-specific databases, screening of bibliographies of included studies, hand searching relevant journal and contacts with authors and experts in order to identify as many studies as possible. The next step is to assess the quality of the included studies- the quality criteria used will depend on the study design. Data extraction using data extraction forms then follows; the information extracted can include study characteristics, methodology, population and outcomes. The synthesis of the study results begins by analyzing the study characteristics (study design, study quality) and results. Forest plots can be used to display effect estimates and provide a visual summary of the data. Pooling of effect measures across the studies is also performed. The last step is to interpret the results and discuss the limitations[37].

Health care providers, researchers and policy makers are often inundated with an abundance of information. Systematic reviews can efficiently integrate existing information, provide data for decision making, and establish consistency in scientific findings which can allow for generalizations across settings and population variations. They can limit bias and improve reliability and accuracy of conclusions[39]. However, a limitation to systematic reviews is that if researchers combine different kinds of studies in order to estimate a summary effect – this may not be a valid overall estimate as it ignores important differences between studies[40].

1.6.2 Systematic Review and Meta-Analysis for TB Diagnostics

High quality evidence on TB diagnostics is critical to develop better evidence-based policies on TB diagnosis. It is only recently that systematic reviews of various TB tests have been performed. The most important difference to note is that most systematic reviews are reviews of randomized trials which is not the case for systematic reviews of diagnostic studies.

1.7 Assessing the Feasibility of Implementing a New Diagnostic Test

There are important limitations of the current available evidence for diagnostic tests in TB. To date all systematic reviews and meta-analyses of TB diagnostic tests have emphasized accuracy estimates such as sensitivity and specificity. This is because almost all the primary research studies have not assessed other characteristics such as the feasibility of implementing tools. However this is a very important issue to consider because many of the countries which need to implement better diagnostic tests have very limited resources- hence, they cannot afford to implement a test that will not deliver accurate results under field conditions.

1.7.1 Definition of Feasibility

The Merriam-Webster Dictionary definition of "feasible" is: capable of being done or carried out (a feasible plan); or, capable of being used or dealt with successfully[41].

1.7.2 Issues to Consider when Assessing Feasibility

For the assessment of implementation of a diagnostic test, several issues related to test accuracy and feasibility should be considered, as reviewed by Foulds et al.[35],

Test accuracy considerations:

- *Sensitivity and specificity:* The new test will need to accurately diagnose active TB.

- *Monitoring treatment results:* The new diagnostic test should be able to diagnose and follow up TB patients because AFB smear is also used to follow treatment progress of smear-positive cases.

- *Speed:* a highly desirable test would be able to provide results within one day.

Feasibility Considerations:

- *Ease of use:* the new test should be used easily at peripheral health centres and not require specialised training or equipment. The availability of facilities and trained personnel is particularly limited in remote areas in low and middle-income countries.

- *Sturdiness:* In many health facilities, electricity for required equipment such as a refrigerator is unavailable or unreliable. Therefore, the reagents and materials required for performance of a test must have a long shelf life and not require refrigeration for storage. The equipment used to perform the test must also be sturdy, able to withstand power outages or surges, high temperature, dust or sand and require little maintenance.

- *Safety:* TB is the sixth most common lab-acquired infections so tests that do not pose a biosafety risk are most wanted.

- *Applicability:* the ideal test should function well on all types of specimens, from different sites of disease, and from patients of all ages.

1.7.3 Examples of Studies that Assessed the Accuracy of New Diagnostic Tests for TB

Monitoring treatment results: In assessing the ability for a diagnostic tool to monitor treatment results, Whitfield and colleagues used IsoScreen, a rapid point-of-care test that would examine the patient's urine for the presence of drug metabolites. The test was developed and used to assess adherence to treatment in a TB clinic in South London. IsoScreen was positive in 93.2% of the patients, suggesting that 6.8% were poorly adhering to treatment. IsoScreen was shown to be useful in monitoring treatment adherence of all patients under routine conditions; therefore it is likely that it should prove easy to implement more widely.

Speed: In assessing the speed to result, Srisuwanvilai and colleagues evaluated the performance of the liquid culture BACTEC MGIT 960 compared to solid culture and showed that the median time for growth with MGIT was 11 days compared to 27 days with solid culture. They showed that MGIT improved the speed of MTB isolation compared to the standard technique, in a resource-limited setting[42], suggesting that the enhanced speed should be achieved in other resource limited settings.

7.4 Previous Studies that have Assessed Feasibility of a New Diagnostic TB Test

Ease of use: In assessing the ease of use of loop-mediated isothermal amplification (LAMP), Boehme et al investigated the operational performance which included factors such as hands-on time and inter-reader variability. The evaluation was conducted by sending out questionnaires to teams at all sites using the diagnostic test, who were asked to comment on the ease of implementation. The sample-processing steps of the LAMP test were less complex than for culture. The interpretation of results of LAMP was also considered easier and faster than culture and microscopy [43]. The study demonstrated that technicians without training in molecular biology techniques were capable of performing

the test with high reproducibility using a simple laboratory space without specialised equipment. As a result, LAMP was deemed easy to use, which favours implementation.

Applicability: No publications were found that assessed the applicability of TB diagnostic tools.

Sturdiness: No publications were assessed the sturdiness of TB diagnostic tools.

Safety: No publications were found that assessed the bio-safety of TB diagnostic tools.

1.8 Economic studies

Economic studies are increasing in importance and visibility in the health care field. They are important in the context of TB control as there are many issues that are cost-related such as how much to invest in TB control interventions, how TB affects a patient's socioeconomic status and the cost that TB imposes on the local, national and global economy[28].

1.8.1 Cost

The countries that most need diagnostic tests for TB are also those with the least resources; this imposes the challenge of balancing low cost with accuracy. As several new diagnostic tools are being developed and evaluated for TB, it is vital that these tests are introduced for widespread use only after assessment of cost-effectiveness[44].

Palomino et al. described the various methods that are available for TB detection but the high cost of most of these techniques and the requirement for sophisticated equipment or highly skilled personnel were barriers to their implementation in low-income countries[34, 45].

1.8.2 Costs related to TB diagnosis

There are many issues to consider when estimating costs associated with diagnosis of TB. These include the perspectives to be considered (societal vs. health system), the types of costs (such as direct vs. indirect costs), whether initial or recurrent costs are to be considered, and discounting.

Health system costs include costs for buildings (facilities), personnel, equipment, materials and supplies, transport and maintenance. Costs of TB from a societal perspective include the same health system costs, plus costs for patients – including income lost by the patient or family for lost time from work, disability and death, as well as direct-out-of-pocket spending.

Start-up or initial costs include training, purchase of equipment and construction costs for lab space. Ongoing or recurrent costs that should be considered include cost of materials and reagents, labour, supervision, quality assurance/quality control, and overhead.

Labour cost is extremely important to consider. For example, even though AFB smear is a very cheap test, it is very labour-intensive. Thus, a test that can detect smear-positive TB cases with similar accuracy but requires less labour time will be less expensive. Ultimately, an ideal diagnostic test would require less technician time than AFB smear if labour costs are high.

Direct costs for patients include out of pocket spending for consultation fees, investigations (tests), travel, food, as well as expenses incurred for persons accompanying the patient. Indirect costs for patients involve loss of wages or decreased earning ability due to illness, time spent seeking care including hospitalisation, or long-term disability that required a change in work[46]. According to Rajeswari who performed a study on the socio-economic impact of TB in India in 1999, the average number of work days lost was 83 days (82 for females, 85 for males) with 48 days before treatment and 35 days during treatment[46]. This was related to age, literacy, personal income and type of occupation.

Approximately 67% of rural patients and 75% of urban patients borrowed money on account of their disease. The proportion of the various costs in relation to the patient's annual family income was 13% for direct costs and 26% for indirect costs. In fact the indirect costs were twice as much as the direct costs, indicating that more costs were incurred from income lost due to the illness than direct out-of-pocket spending[46]. Mesfin et al studied similar features in Ethiopia. The indirect costs for the patients included income lost from the time lost from work for consultations and the travel for consultations; the mean was $33 USD per patient. The direct costs included costs for transportation, lodging, extra drugs not provided free of charge, consultations, investigations and hospital admissions and averaged $26 USD per patient[47]. In Ethiopia, the gross national income per capita is $280 USD[48]. This example emphasises that more is lost in indirect costs than in direct costs, and that patient costs from TB can have a huge economic impact, since the total cost of $59 USD represented 21% of the average annual income. Mesfin et al. concluded that these costs incurred by the patients were comparable to those estimated in other African studies.

Discounting: The timing of programme costs and benefits must also be taken into account. The primary benefits of a particular programme can be immediate while the benefits of another programme can occur well in the future. In the latter situation, future costs and benefits are reduced or "discounted" to reflect the fact that dollars spent or saved in the future should not weigh as heavily in programme decisions as dollars spent or saved today[49].

1.9 Summary of Background and Rationale for Study

Tuberculosis is a global health problem. As the number of TB cases continues to increase worldwide, this stimulates the search for new diagnostic tests to improve current global control efforts for this disease.

While smear microscopy is the most widely used test, the overall sensitivity is low. Liquid culture such as BACTEC provides results quickly but is costly. Solid culture such

as LJ is inexpensive but requires four to eight weeks for results. Immune-based tests such as TSTs and IGRAs cannot distinguish between latent and active tuberculosis. Newer tests such as NAATs have variable sensitivity, and may not be practical in resource-limited settings. Thus, there is a need for new diagnostic tests because none of the currently available methods are rapid, accurate and inexpensive. MODS and TLA are two plausible options for TB diagnostic tests because they appear to be simple, should be low cost and applicable in many settings, especially in low- and middle-income countries.

Systematic reviews and meta-analyses are standard methods that can be used to assess MODS and TLA for accuracy and other test characteristics (cost, time to result, contamination rates). However, this will not be sufficient to conclude that these can be implemented successfully in low- and middle-income countries. Additional information on feasibility will also be necessary

2 Objectives

2.1 General Objective

To compare the accuracy, feasibility and costs of implementation of two new microbiologic tests for the diagnosis of active TB - the Microscopic-Observation Drug Susceptibility (MODS) and the thin layer agar (TLA) with the currently recommended culture techniques.

2.2 Primary Objectives

Objective 1: To conduct a systematic review and meta-analysis to compare the accuracy, speed, contamination proportion and cost of two microcolony techniques (MODS and TLA) for the diagnosis of active TB, with traditional culture methods (LJ or Middlebrook media for solid cultures and MGIT or BACTEC for liquid cultures).

Objective 2: To conduct a questionnaire survey to evaluate the implementation of two microcolony techniques (MODS and TLA) for the diagnosis of active TB. This includes training, recurrent costs (including materials and labour), and other issues of feasibility of implementation.

3 Methods

Methods of the Systematic Review and Meta-Analysis (Objective 1)

A standard protocol for systematic reviews and meta-analyses was used[37] as well as methods recommended by the Cochrane Diagnostic Test Accuracy Working Group[50].

3.1 Search Strategy

The literature was systematically searched for all articles published from January 1990 (earliest records) until February 2009 in English, French or Spanish, using three databases: PubMed, EMBASE and BIOSIS. The search was updated in March 2010. All electronic searches were performed by two persons including an experienced librarian. Reference lists from included studies were also hand searched.

The key words used for the electronic search were: TB or Tuberculosis, AND diagnosis or detection or screening or diagnostic tests or case-finding or case detection, AND, Microscopic-Observation Drug Sensitivity or MODS or Thin Layer Agar AND, sensitivity or specificity or positive or negative predictive values or yield or utility or feasibility or feasibility or accessibility or implementation or training or operational effectiveness or costs or cost-effectiveness.

3.2 Eligibility Criteria

All studies that reported an evaluation of MODS or TLA for diagnosis of active TB disease were included. For the primary analysis, the predetermined eligibility criteria were: evaluation of MODS or TLA for detection of tuberculosis and use of an accepted reference standard. Accepted reference standards included direct or indirect culture using solid media such as Lowenstein-Jenson or Middlebrook agar, or liquid cultures such as

BACTEC® or BACTEC MGIT® (Becton Dickinson Diagnostics, Towson, Maryland, USA). All specimens from all sites of disease were considered.

Studies reporting insufficient data for the estimation of sensitivity were excluded. Studies were included if sensitivity could be calculated, even if they incorporated the index tests as part of the reference standard (meaning that the diagnosis of active TB was considered confirmed if any culture, including MODS or TLA, was positive). In these studies specificity could not be estimated. This is a form of incorporation bias and can potentially lead to an overestimation of diagnostic accuracy.

All study designs were considered. Editorials, letters to the editor and conference abstracts were excluded.

MODS and TLA can also be used for rapid detection of drug resistance[51-52]. However, in this systematic review, studies that evaluated only results of MODS or TLA for drug susceptibility testing (DST) were excluded – a separate systematic review was done to assess the studies using MODS or TLA for drug susceptibility testing

3.3 Study Selection

All selection steps were performed by two independent reviewers. Titles and abstracts were screened to select studies and any citations identified as eligible by either reviewer were selected for full text review. Articles retrieved for full text review along with reasons for exclusion are available from the authors. Disagreements were resolved through a third reviewer[12].

3.4 Data Extraction

A data extraction form was created and piloted with a subset of eligible studies. Data from the studies selected for inclusion were extracted independently by two reviewers

using a standardized data extraction form (see Appendix); any disagreements were resolved by consensus. Data were extracted on the following variables: type of index test, type of reference standard, blinding, whether all specimens were verified with an accepted reference standard, type of specimen (pulmonary vs. extra-pulmonary, smear positive vs. smear negative), age of patient, HIV sero-status, study design including directionality and method of selection of the specimen/patient, all costs, interval from reception until availability of result within the lab, and rate of contamination due to bacterial or fungal overgrowth.

3.5 Assessment of Study Quality

Four quality criteria were considered relevant to assess the quality of diagnostic studies for this particular review: (i) whether or not the technicians performing the reference and index tests were blinded to results of the other tests; (ii) whether or not the diagnosis was verified in all patients using the diagnostic reference standard; (iii) whether patients/specimens were selected consecutively or randomly, or using some other method; and (iv) whether the study design was cross-sectional versus case-control.

3.6 Meta-Analysis Methods

Data were analysed using a random effects meta-analysis to estimate the overall pooled estimates and 95% confidence intervals (CI) of sensitivity and specificity using the "Proc Nlmixed" program in SAS (SAS Institute, Carey NC, USA)[53]. An exact binomial likelihood approach which uses a binomial distribution to approximate the distribution of the outcome of interest was used[53]. This approach accounts for study size, has been demonstrated to produce less bias estimates of the pooled effect and includes a random effect to account for inter-study heterogeneity [53].

Forest plots visually displaying sensitivity and specificity estimates and their 95% confidence intervals (CI), using exact methods for proportions, from each study were constructed using MetaDiSc software[54].

Some of the studies included in the meta-analysis contributed both sensitivity (true-positive rate) and specificity (one minus false-positive rate). Because these measures tend to be correlated and vary with the thresholds used across individual studies, a summary receiver operating characteristic curve analysis was performed to explore the effect of thresholds on results[37, 55]. This curve displays the sensitivity and specificity estimates from each study within the receiver operating characteristic space and a regression curve is fitted through the distribution of pairs of sensitivity and specificity. A shoulder-like curve indicates that the heterogeneity between studies can be due to a threshold effect (e.g. different cut-offs across the studies)[55-57]. A non-shoulder-like curve indicates that sensitivity and specificity are not correlated. Moreover, the area under the curve (AUC)[12] can estimate the overall diagnostic accuracy. An AUC of 50% indicates that the test has a poor ability to discriminate between diseased samples and non-diseased samples while an AUC of 100% denotes that the test discriminates perfectly between samples from persons with and without disease[55-57].

3.7 Subgroup Analysis

Heterogeneity refers to the degree of variability in estimates across studies [58], and can be due to variability in thresholds or different reference standards used, as well as differences in the study populations or in study quality. The heterogeneity of outcomes of interest was assessed by estimating the I-squared statistic and associated 95% confidence intervals [59]. The I-squared statistic is a description of the percentage of variation across studies that is due to heterogeneity rather than chance alone; it expresses the inconsistency of studies' results. Sub-group analyses were performed to minimize heterogeneity within strata defined on the basis of type of reference standard, whether the specimen was smear positive or smear negative, type of clinical specimen, and blinding status of the technicians reading results. All stratified analyses were performed for studies without incorporation bias, which assessed the MODS assay only.

3.8 Outcome Measures

Results from each study were classified as true positive (TP), false positive (FP), false negative (FN), and true negative (TN) values. True positives were defined as specimens (or patients) that were culture positive for TB using the reference standard, and were also detected by MODS or TLA. False positives were defined as specimens that were culture positive with MODS or TLA but were negative using the reference culture method. False negatives were defined as specimens which provided negative MODS or TLA results, but the reference method was culture positive. True negatives were defined as specimens which were culture negative using MODS or TLA and the reference method. From these data the sensitivity and specificity for each study was calculated.

Other outcomes extracted included the time from specimen receipt to availability of culture results within the laboratory, contamination rate, and costs. A contaminated culture was defined as a culture with fungal or bacterial overgrowth on the first inoculation [60]. Costs, expressed in 2007 US dollars, included all health system costs for reagents, supplies, equipment, labour, overhead and other related costs. Where costs were expressed in a foreign currency, they were converted to US dollars using purchasing power parity rates for the year that costs were reported in the study [61-62]. Costs were then adjusted to 2007 using the consumer price index[63].

Methods for the Questionnaire Survey (Objective 2)

The studies used in the above brief literature review and meta-analysis had not reported any data on feasibility issues (information about implementation, recurrent costs, labour costs) for the two thin layer culture techniques. This motivated the creation of a questionnaire to address these topics.

For this thesis, a brief literature search was performed to locate publications describing feasibility studies of new diagnostic tests in TB. Two databases were searched for relevant publication. PubMed and EMBASE were search for relevant publications in English or French from January 2000-February 2010 inclusive. The key words used for the electronic search were: TB or Tuberculosis or infectious disease*, AND diagnosis or detection or screening or diagnostics or diagnostic tests or case-finding or case detection, AND utility or feasibility or accessibility or implementation or training or operational effectiveness or costs or cost-effectiveness.

However, no study could be identified that had assessed the feasibility of implementing a new diagnostic test for TB in a low-income country. One reason that there is so little published evidence regarding feasibility of implementation of new diagnostics may be that the methodology for gathering this type of evidence is not well-established.
The finding that there was very little published information on feasibility motivated the creation of a survey about these implementation and feasibility aspects.

3.9 Questionnaire Development

A questionnaire was created with items considered relevant to the assessment of implementation of a new diagnostic test for active tuberculosis. The questionnaire included items regarding general information about the facility, such as services offered, lab protocols, recurrent labour costs, start up costs, bio-safety measures, training procedures and problems faced during the introduction of MODS or TLA.

The topics addressed and variables examined can be categorized as (see Appendix B for the questionnaire):

- General Facility Information
    - Type of laboratory, and health sector (private or public)
    - Service Capacity for TB
    - TB laboratory protocols
    - Types of specimens received (how many were smear positive, culture positive, drug resistant)
    - Turnaround time for results
    - Quality control used (if any)
    - Biosafety levels of the laboratory

- Recurrent Costs (Labour)
    - Number of tests performed
    - Personnel involved -, how many were required, how much time spent on MODS/TLA, and salary scales

- Start Up Costs
    - Cost needed to construct or renovate space dedicated for MODS/TLA
    - Equipment purchased

- Training
    - Technician's previous lab experience
    - How many underwent formal training and with whom
    - Other methods used to learn technique (MODS website, other publications)
    - Where did the trainees come from
    - Time needed per training session
    - People needed per training session
    - Follow-up training for quality assurance

- Other issues/ miscellaneous problems experienced with MODS/TLA

The questionnaire was created and sent to three collaborators and co-investigators with content area knowledge for feedback, corrections and clarifications. After revisions based on their input, all three reviewed the questionnaire again. Then it was reviewed by a fourth person, who did not have content area knowledge, for readability.

3.10 Identification of Potential Respondents for the Questionnaire Survey

The questionnaire was sent to all authors who had published papers on MODS and TLA that were identified through the systematic review. This included all authors of papers published on use of thin layer culture techniques for diagnosis of active TB, and also authors of studies evaluating these techniques only for drug sensitivity testing.

In addition, Dr. David Moore was contacted, who has performed substantial work in the development and evaluation of MODS, including training many others to use these techniques. He supervised and trained many health professionals in the use of MODS. Dr. Moore provided a list of all his previous trainees to whom the questionnaire was also sent.

The questionnaire was first mailed in July 2009. The questionnaire was sent a second time to the non-respondents, in September 2009, and was sent out a third time in November 2009 to all who had still not replied. For some respondents who returned the survey with questions unanswered, the questions were resent to the respondent in their first language (which was Spanish, French or Portuguese).

3.11 Study Variables

Dependent Variable:

The dependent variable in assessing implementation was the overall cost to implement MODS/TLA.

Independent Variables:

The independent variables for the cost per sample of MODS/TLA included:

Recurrent labour costs were calculated from the average weekly salary reported by the respondents for:

  - Technician
  - Supervision
  - Administration
  - Cleaning
- Materials, supplies and reagents (taken from the results from the systematic review).

The independent variables for start-up costs included:

- Construction cost for new space
- Cost for equipment
- Initial training for the technicians

The questionnaire included a list of all equipment needed to perform MODS or TLA, including standard laboratory items such as a refrigerator, a vortex, a centrifuge, an incubator, an autoclave, and a balance. Respondents indicated if they had to purchase these items, and if so, how many units, or if this equipment was already in place. It was assumed that all labs needed to purchase an inverted microscope as this is essential equipment for the performance of MODS– and is not used for other purposes. Performance of TLA does not require an inverted microscope - a normal microscope can be used. The cost to purchase all the equipment for MODS/TLA for a laboratory/centre that was already established was then calculated. The probability that that each laboratory needed to purchase a particular piece of equipment was calculated as the number of laboratories that reported needing to purchase this, divided by the total number of respondents. Information on the price of most of the equipment was obtained from The Fisher Scientific laboratory catalogue, except for the inverted microscope, which was provided by Dr. Moore. This price was then multiplied times the probability that each

laboratory/centre would need to purchase this - to provide an average cost a laboratory/centre that already had equipment used for other purposes.

Training sessions for technicians were included as start up costs as this was considered a one-time start-up activity. The independent variables for training a new trainee included:

- Average duration per course
- Number of technicians per course
- Number of trainers per course
- How long after initial training until full proficiency for the technician
- Average cost for a trainer, per session
- Average salary for an attendee, per session
- Average salary for the quality assurance training session

The methods of quality assurance reported by each respondent were reviewed and judged as acceptable or not by Dr. Jessica Minion. Methods that were deemed acceptable were: (I) if the centre sent samples to another lab for confirmation (either by culture or another method) or (II) another culture method was used in the same lab.

3.12 Analysis

Descriptive analysis of the various cost expenditures was performed. Costs from the health system perspective were calculated to quantify the total expenditures necessary to initiate MODS or TLA as a laboratory service. Start-up costs included construction of new space, purchase of new equipment, and initial training for the technicians. Recurrent costs included labour, materials and supplies and overhead. Recurrent labour costs were calculated for the technicians, supervisors, administration and cleaning/housekeeping. Overhead costs could not be calculated- as there was insufficient information provided by the respondents.

Responses are from many different countries using various currencies; so the purchasing power parity method was used to convert all currency to the standard US dollar. The year 2009 was chosen because it was the year all responses were collected.

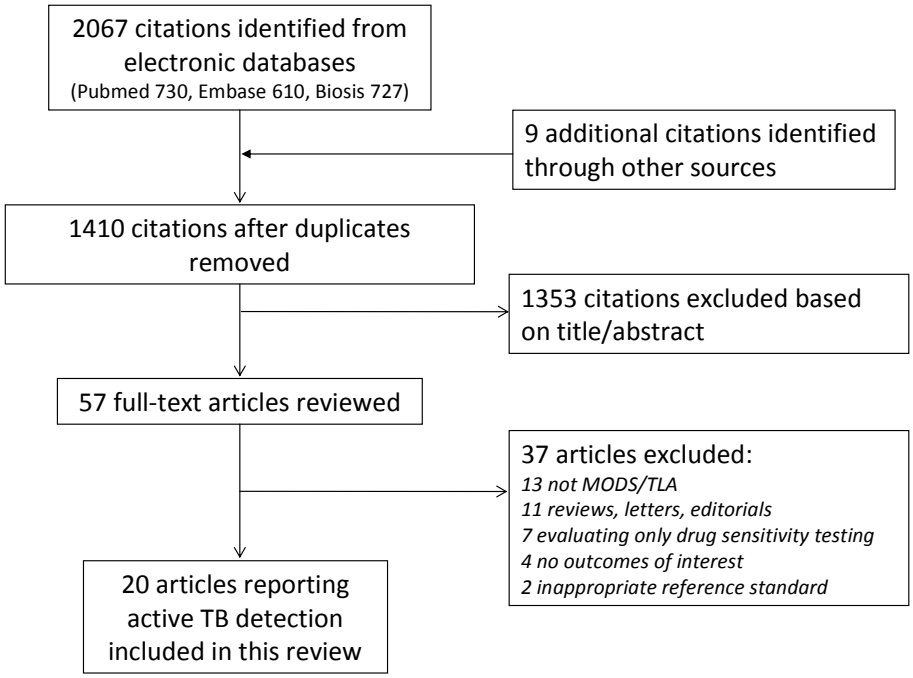
4 Results

Results for Objective 1

4.1 Characteristics of Included Studies

As seen in Figure 3, 2067 citations were identified from the initial electronic searches and an additional nine citations were identified from the update. After excluding the duplicate citations, 1410 unique citations were left. From these 57 potentially relevant articles were retrieved for full text review, of which 20 were considered eligible for this review. These are summarized in Table 1.

Figure 3: Included Studies



The following table describes the various characteristics of the studies included in the review .

Table 1. Characteristics of Studies Included in the Systematic Review

Author Year (Reference)	Countries	Sample Size (Total *n*)	Index test	Reference test for Diagnosis	Samples that were Smear Positive (%)	Time to Result measured	Blinding Status†
Arias et al. 2007[64]	USA	1639	MODS	LJ	36	Yes	Single
Caws et al. 2007[65]	Vietnam	150	MODS	LJ and MGIT	23	Yes	Single
Giacomazzi et al. 2010 [66]	Ecuador	507	MODS	LJ	10	Yes	Unclear
Ha et al. 2009 [67]	Vietnam	217	MODS	LJ	21	Yes	Double
Michael et al. 2010 [68]	India	38	MODS	LJ and BACTEC	Unknown	Yes	Double
Moore et al. 2004 [69]	Peru	406	MODS	LJ	Unknown	Yes	Unclear
Moore et al. 2006 [70]	Peru	4213	MODS	LJ and MBBacT	6	Yes	Double
Oberhelman et al. 2006 [71]	Peru	38	MODS	LJ	2	Yes	Single
Reddy et al. 2010 [72]	India	889	MODS	LJ	4.9	Yes	Double
Shiferaw et al. 2007 [73]	Ethiopia	262	MODS	LJ	100	Yes	Unknown
Tovar et al. 2008 [74]	Peru	111	MODS	LJ	Unknown	Yes	Unclear
Idigoras et al. 1995 [75]	Spain	1997	TLA	LJ	2	Yes	Unknown
Martin et al. 2009 [76]	Belgium	210	TLA	MGIT	95	Yes	Unknown
Martin et al. 2009 [77]	Kenya	298	TLA	LJ	0	Yes	Unclear
Mejia et al. 1999 [78]	Colombia	84	TLA	LJ	10	Yes	Unclear
Mejia et al. 2004 [79]	Colombia	1809	TLA	LJ	Unknown	Yes	Unclear
Robledo et al. 2006 [80]	Colombia	1118	TLA	LJ	13	Yes	Unclear
Welch et al. 1993 [81]	USA	103	TLA	LJ	62	Yes	Unclear
Caviedes et al. 2000 [82]	Peru	97	BOTH	LJ and MGIT	44	Yes	Double
Irfan et al. 2006 [83]	Pakistan	200	BOTH	LJ and BACTEC	30	Yes	Unclear

†Single blinded studies are studies whereby only the reader of the index test was reported as blinded. Double blinded studies are studies whereby the reader for both the index test and the reference test were reported as blinded to the other result. Studies labelled as "unclear" are studies whereby the blinding status was not reported at all.

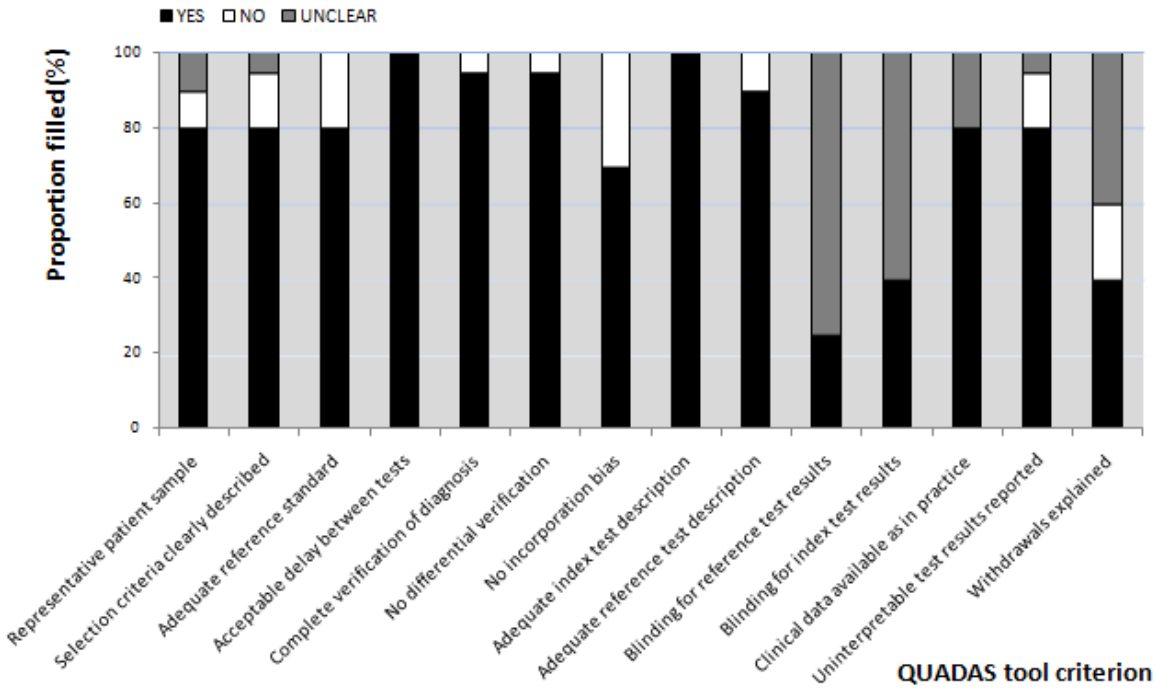
Of the 20 eligible studies, 11 evaluated MODS only, seven evaluated TLA only and two evaluated both. All evaluated detection of active TB and time to detection. Fourteen of the studies used both smear-positive and smear-negative clinical samples, one study included only smear-positive samples, one study included only smear-negative samples of cerebrospinal fluid, and four studies did not report whether samples were smear-positive or negative. Five studies used both solid and liquid culture as the reference test, and nine studies used only solid culture. Six studies incorporated the results of MODS or TLA into the reference standard, meaning that any positive culture was considered a true positive; in these studies specificity could not be estimated. Seven studies (all evaluating MODS) provided information on the HIV status of patients. On average, the study size was 713 specimens per study (ranged from 96 to 3757).

4.2 Quality of Included Studies

By design, in all studies all MODS or TLA results were verified using a reference standard of diagnosis. Twelve studies used a cross-sectional study design, four were case-series of only positive specimens, three used a case-control design and one used a cohort design. Fourteen studies reported using consecutive or random sampling, one used a convenience sample and the remainder did not describe their selection process. Eighteen studies were performed prospectively, one was performed retrospectively, and one did not describe directionality. Eight studies stated that technicians performing the MODS or TLA were blinded with respect to the reference standard results, and the other 12 studies did not report on blinding.

The QUADAS criteria were also used to assess the quality of the studies used. Summaries of their performance are displayed below. Out of 14 quality indicators, the number clearly met by the included studies varied from 5 to 14.

Table 2. Quality of Studies using QUADAS Criteria



4.3 Accuracy Estimates

4.3.1 MODS and TLA for Detection of Active TB

As shown in Table 3, when results from all 20 studies were used, the pooled sensitivity of MODS was 92% (88%, 96%) and for TLA was 87% (79%, 94%). However, there was considerable heterogeneity in these pooled estimates with very high I-squared values. When the studies that incorporated the index test into the reference standard (incorporation bias) were excluded, the pooled sensitivity and specificity for MODS were 92% (87%, 97%) and 97% (95%, 100%), respectively, and for TLA, sensitivity was 86% (73%, 99%), and specificity was 97% (95%, 100%).

Table 3: Pooled Accuracy Estimates of All Included Studies – Overall, By Test, and By Reference Standard

Outcome	Studies (N)	Pos. test/All with disease (N)	Sensitivity		I^2 (95% CI)
			Pooled	(95% CI)	
Overall - and by Type of Test					
Sensitivity- all reference standards combined					
MODS	13	3505/3718	92.3	(88.3, 96.3)	80.8 (68.2, 88.4)
TLA	9	1167/1394	86.5	(79.0, 93.9)	92.8 (88.5, 95.5)
Outcome	**Studies (N)**	**Pos. test/All with disease (N)**	**Specificity**		**I^2 (95% CI)**
			Pooled	**(95% CI)**	
Overall - and by Type of Test					
Specificity- all reference standards combined					
MODS	11	7041/7217	97.1	(94.6, 99.6)	91.4 (87.1, 94.2)
TLA	4	3293/3359	97.9	(95.0, 100)	77.8 (58.0, 88.3)

4.3.2 MODS - According to Reference Standard Used

When the results for MODS were stratified by reference standard (Table 4) sensitivity was 89% (80%, 99%), when MODS was judged against the combination of both liquid and solid cultures, compared to 94% (89%, 100%) if the reference was solid culture only. Specificity was 99% (97%, 100%) if the reference was solid plus liquid cultures, compared to 95% (90%, 100%) with solid cultures only.

Table 4: Pooled Accuracy Estimates of All Included Studies by Diagnostic Gold Standard

Outcome	Studies (N)	Pos. test/All with disease (N)	Sensitivity		I^2
			Pooled	(95% CI)	(95% CI)
MODS Only- By Reference standard					
Sensitivity					
Liquid & solid cultures	5	590/639	89.3	(79.6, 99.1)	88.5 (75.9, 94.6)
Solid cultures only	6	1027/1072	94.2	(88.7, 99.7)	72.5 (36.5, 88.1)
Incorporation† (any positive culture)	2	1888/2005	93.4	(83.6, 100)	--
Outcome	**Studies (N)**	**Pos. test/All with disease (N)**	**Specificity**		**I^2**
			Pooled	**(95% CI)**	**(95% CI)**
MODS Only- By Reference standard					
Specificity					
Liquid & solid cultures	5	3823/3861	98.6	(96.7, 100)	82.9 (61.0, 92.5)
Solid cultures only	6	3218/3356	95.0	(89.6, 100)	93.4 (88.3, 96.3)
Incorporation† (any positive culture)	--	--	--	--	--

† Defined as studies which incorporated the results of MODS or TLA in the reference standard. Hence specificity could not be estimated as all positive cultures considered true positive. One of these studies used both solid and liquid culture and the other used solid culture only.

4.4 Subgroup analysis

All stratified analyses were performed for only the MODS assay because too few studies were identified evaluating TLA, and also excluded the studies that incorporated index test results in the reference standard and the one study that used clinical diagnosis as the reference standard.

4.4.1 Active TB Diagnosis: Stratified By Smear Samples

When results were stratified by smear status, sensitivity was 100% and specificity was 64% in the one study with all smear positive samples (Table 5). There were 11 specimens that were negative by the LJ method and out of these; only seven were negative by MODS. LJ is also a less sensitive culture method, therefore it is possible that in this particular study, the samples that were culture positive with MODS were not false positive but rather the MODS results were true positive, and the LJ results were false negative. In the nine studies which include smear-positive and smear-negative samples, the overall sensitivity estimate was 89% (83, 96) and the overall specificity estimate was 98% (97, 100) (Table 5). The results in the studies were not stratified by smear status and were reported all together. The percentage of samples that were smear-positive ranged from 1.7% to 95.2%.

Table 5: Pooled Accuracy Estimates of All Included Studies by Type of Sample

Outcome	Studies (N)	Pos. test/All with disease (N)	Sensitivity		I^2
			Pooled	(95% CI)	(95% CI)
By results of direct AFB smears†					
All positive (100%)	1	247/247	100	--	--
Some positive (2-95%)††	9	1332/1425	89.4	(83.2, 95.5)	84.4 (73.0, 91.5)
None positive	0	--	--	--	--
Not reported	1	38/41	93.4	(80.1, 100)	--
Outcome	**Studies (N)**	**Neg. test/All without disease (N)**	**Specificity**		**I^2 (95% CI)**
			Pooled	**(95% CI)**	
By Results of Direct AFB smears†					
All positive (100%)	1	7/11	63.6	(7.8, 100)	--
Some positive (2-95%)††	9	6893/7037	98.1	(96.7, 99.5)	92.2 (87.4, 95.2)
None positive	0	--	--	--	--
No information	1	141/169	83.6	(56.0, 100)	--

† The tables have excluded two studies that incorporated the results of MODS or TLA in the reference standard.

††The results in the studies were not stratified by smear status, treatment status or percentage of pulmonary samples; they were reported all together.

4.4.2 Active TB Diagnosis: By Type of Sample

In six studies with only pulmonary samples, the sensitivity was 96% (94, 99) and the specificity was 96% (91, 100). In the two studies that used samples from extra-pulmonary sites, the sensitivity was lower - 87% (72, 100) although specificity remained high - 97% (89.0, 100).

Table 6: Pooled Accuracy Estimates of All Included Studies by Type of Sample

Outcome	Studies (N)	Pos. test/All with disease (N)	Sensitivity		I^2
			Pooled	(95% CI)	(95% CI)
By type of samples†					
All sputum (100%)	6	1413/1460	96.3	(93.6, 99.1)	53.0 (0, 80.0)
Some sputum (34-84%)††	3	129/164	78.3	(60.9, 95.7)	24.5 (0, 72.9)
None sputum	2	75/89	86.5	(71.8, 100)	78.1 (4.5, 95.0)
Outcome	**Studies (N)**	**Pos. test/All with disease (N)**	**Specificity**		**I^2**
			Pooled	**(95% CI)**	**(95% CI)**
By type of samples†					
All sputum (100%)	6	5348/5472	95.8	(91.1, 100)	94.7 (91.4, 96.8)
Some sputum (34-84%)††	3	1463/1487	98.8	(96.8, 100)	40.9 (0, 78.2)
None sputum	2	230/258	96.5	(89.0, 100)	98.2 (96.7, 99.0)

† The tables have excluded two studies that incorporated the results of MODS or TLA in the reference standard.. ††The results in the studies were not stratified by smear status, treatment status or percentage of pulmonary samples; they were reported all together.

4.4.3 Active TB Diagnosis: By Blinding Status

Sensitivity did not appear to be affected by blinding status but specificity was 99% (97, 100) for the blinded studies compared to 90% (80, 100) when the information was not provided.

Table 7: Pooled Accuracy Estimates of All Included Studies by Blinding Status

Outcome	Studies (N)	Pos. test/All with disease (N)	Sensitivity		I^2 (95% CI)
			Pooled	(95% CI)	
MODS & TLA - By Blinding of Technicians Performing Tests†					
Double/Single blinding	7	1236/1312	90.0	(81.2, 98.8)	84.7 (70.2, 92.1)
Unclear about blinding	4	381/401	95.4	(89.4, 100)	61.6 (0, 87.1)
Outcome	**Studies (N)**	**Pos. test/All with disease (N)**	**Specificity**		**I^2 (95% CI)**
			Pooled	**(95% CI)**	
MODS & TLA - By Blinding of Technicians Performing Tests†					
Double/Single	7	6323/6443	98.6	(97.3, 100)	92.7 (87.5, 95.8)
Unclear	4	718/774	89.9	(79.5, 100)	92.7 (87.1, 96.9)

† The tables have excluded 2 studies that had an incorporation bias as a reference for MODS.

4.4.4 Head-to-Head Comparisons

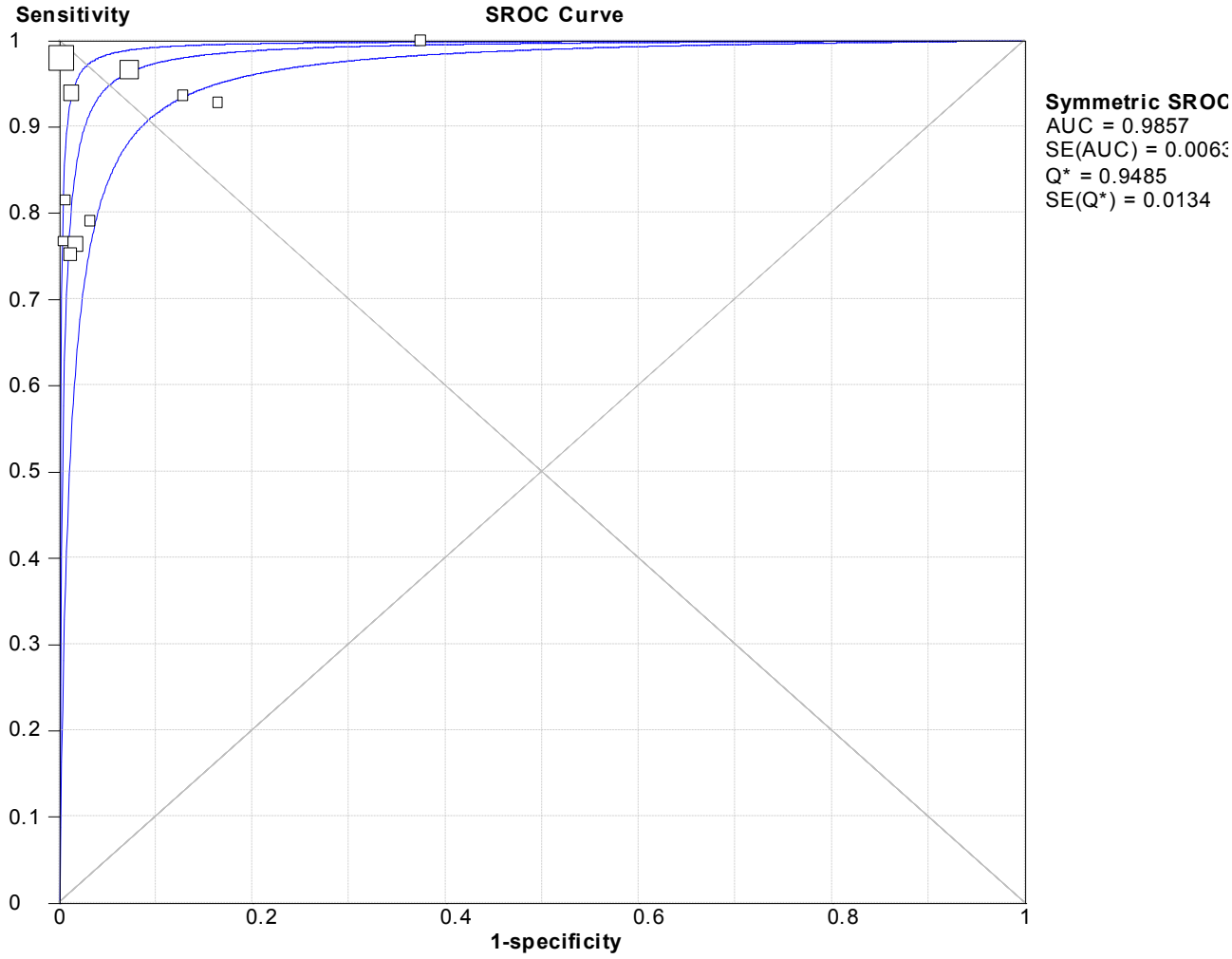
There were two direct comparisons of MODS vs. TLA using the same specimens to detect active TB. In one, the sensitivity of MODS was 76% (95% CI: 66%-84%) compared to 93% for TLA, (95% CI: 85%-97%) [41], but specificity was not reported. In the second, MODS sensitivity was 93% (95% CI: 84%-98%) and TLA sensitivity was 92%, (95% CI: 82%-97%), while specificity for MODS was 87% (95% CI: 80%-92%) and 90% (95% CI: 83%-94%) for TLA[42].

4.5 Hierarchical Summary Receiver Operating Curves

4.5.1 MODS and TLA Combined for Active TB Diagnosis

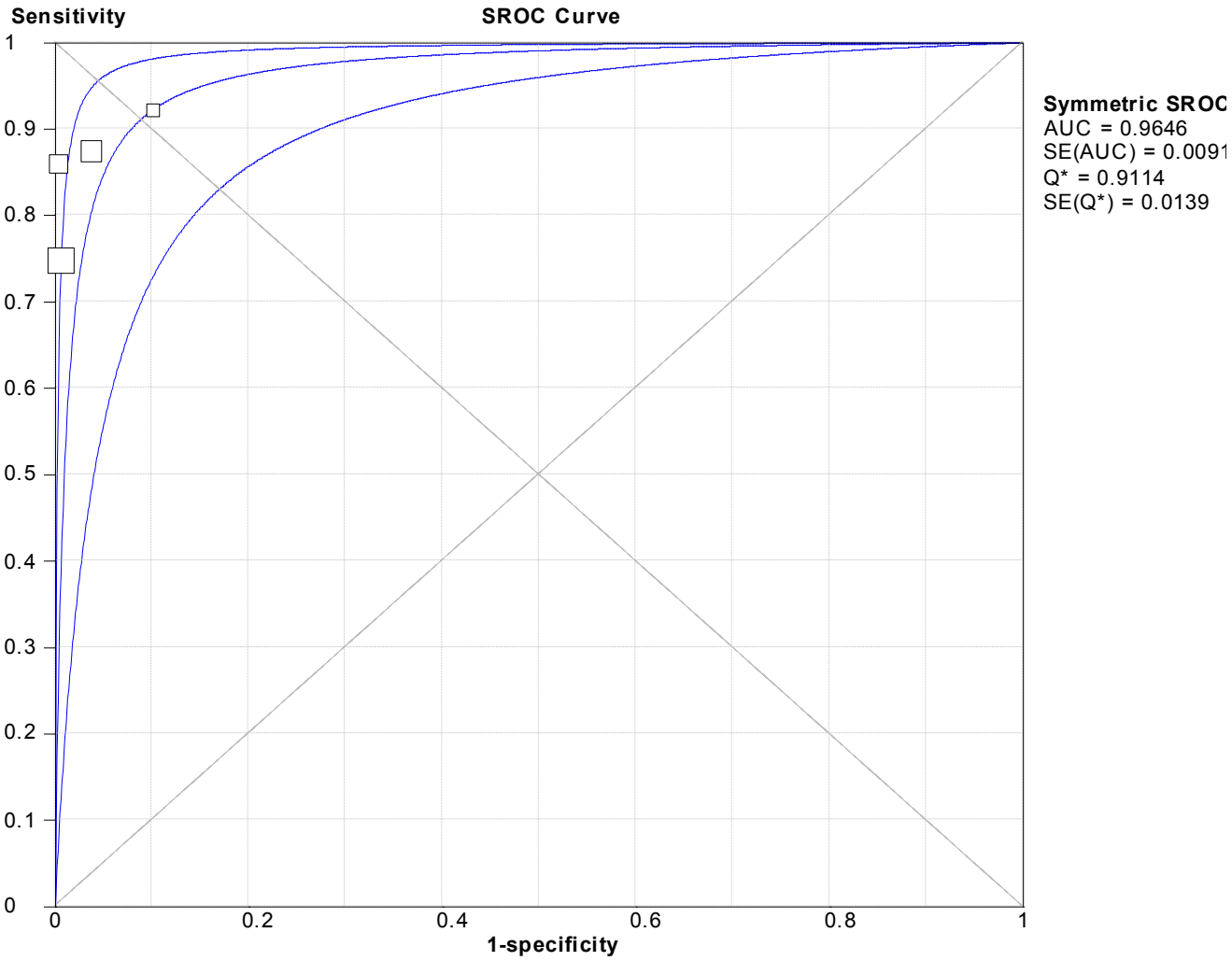
In Figure 4, the sensitivity (or True Positive Rate) and 1-specificity (False Positive Rate) are plotted in a HSROC curve for MODS, using studies which used solid and/or liquid cultures as the reference standard (n=11 studies). The area under the curve was 99% indicating near perfect discriminatory ability, although the sensitivity values were more variable than specificity. As seen in Figure 5, the area under the HSROC was 96% for TLA (n= four studies), also indicating near perfect discriminatory ability.

Figure 4: Summary ROC Curve of MODS for Active Tuberculosis Detection
This includes 11 studies using liquid or solid culture media as the reference standard.†



† This curve displays the sensitivity and specificity estimates from each study within the receiver operating characteristic space and a regression curve is fitted through the distribution of pairs of sensitivity and specificity. The area under the curve was 98.6% indicating near perfect discriminatory ability for MODS.

Figure 5: Summary ROC Curve of TLA for Active Tuberculosis Detection This includes four studies using liquid or solid culture media as the reference standard.†



† This curve displays the sensitivity and specificity estimates from each study within the receiver operating characteristic space and a regression curve is fitted through the distribution of pairs of sensitivity and specificity. The area under the curve was 96.5% indicating near perfect discriminatory ability for TLA. The sensitivity is more variable than the specificity.

4.6 Turnaround Time

Median (or mean) turnaround times were provided for eight MODS studies, four TLA studies and one study that compared both media, shown in Table 8. The time from specimen receipt until a diagnostic result was significantly less with MODS than with TLA. The interval from receipt of specimens to results for MODS and TLA were nine days and 11 days respectively, which were significantly shorter for both MODS and TLA than for traditional solid culture, as shown in Table 8. In addition, the interval from receipt of specimens to results was significantly less with MODS than TLA.

Table 8: Average Interval from Receipt of Specimen to Positive Result for MODS or TLA

	Diagnosis Of Active TB with MODS	**Diagnosis Of Active TB with TLA**
MODS		
Studies (N)	9	5
Samples (N)	3865	668
Mean Time to Result in Days (SD)	9.1 (2.3) *	11.5 (1.9) *
Difference from Solid Reference in Days (How many days MODS/TLA faster)	16.2 † (N=7)	11.8 † (N=5)
Difference from Liquid Reference in Days (How many days MODS/TLA faster or slower)	2.6 days faster †† (N=6)	1.5 days slower †† (N=1)

* The difference between MODS and TLA was significant ($p<0.05$).

† The differences between MODS and solid cultures, and between TLA and solid cultures were significant ($p<0.005$)

†† The differences between MODS and liquid cultures, and between TLA and liquid cultures were not significant.

4.7 Contamination Rates

A contaminated culture was defined as a culture whereby there was fungal or bacterial overgrowth in a well[60]. Table 9 shows the pooled contamination rates for MODS and TLA along with contamination rates reported for solid and liquid reference standards in the same studies. The proportion of contaminated specimens using the reference standard method of isolation performed on the same specimens by the same laboratory.

Table 9. Proportion of Contaminated Specimens using MODS or TLA Compared to Reference Method

The proportion of contaminated specimens using the reference or standard culture method performed on the same specimens by the same laboratory.

	Proportion Contaminated using MODS	**Proportion Contaminated using TLA**
Proportion Contaminated†	6.6%	12.3%
(range)	(0.4% – 11.2%)	(1%-26%)
# studies	N=10	N=7
Solid Culture	11.4% ($p<0.05$)	5.4% (p=NS) ‡
(range)	(1%-22%)	(0.9%-17%)
# studies	N=9	N=5
Liquid Culture	3.9% (p=NS) ‡	10.8% (p=NS) ‡
(range)	(0.9%-6.3%)	(4%-21.1%)
# studies	N=5	N=3

† Defined as the proportion of specimens contaminated upon first inoculation

‡ NS= non-significant

In 10 studies, MODS had a pooled proportion of contaminated specimens of 6.6%, compared to contamination proportions of 11.4% with solid culture and 3.9% with liquid culture performed on the same specimens as MODS by the same laboratories (Table 9).

In seven studies, TLA had a contamination proportion of 12.3%, compared to contamination proportion of 5.4% with solid cultures and 10.8% with liquid cultures.

4.8 Cost Estimates

4.8.1 MODS and TLA for Active TB Diagnosis

Table 10 summarizes the estimated costs of performing MODS or TLA, as well as the estimated costs for LJ, BACTEC and automated mycobacterial culture (AMC). The average cost per sample for MODS was $1.48 compared to $2.43 for TLA. The cost for LJ, reported by only three studies, was $2.97 per sample and for BACTEC, it was $5.84. One study provided the cost per sample for automated bacterial culture, which came out to $53.48. Reddy et al reported that it cost approximately $7.31 per sample for MODS and $6.11 per sample for LJ, a value attained by summing the cost of diagnosis and patient cost (which included transport). Usually MODS is more expensive than TLA but these averages are only an artefact of using different studies with different methods of calculating costs. Only Caviedes and colleagues estimated both MODS and TLA costs and he found that MODS cost more than TLA. No studies considered the cost of labour, training, supervision, capital costs, or overhead costs associated with MODS or TLA detection.

Table 10. Average Detection Cost per Sample for MODS or TLA for Reagents and Supplies

	Costs for supplies and reagents
MODS	**$ USD/Sample**
Caviedes et al. (2000)	$0.96
Caws et al. (2007)	$0.53
Michael et al. (2010)	$2.06
Moore et al. (2006)	$2.06
Oberhelman et al. (2006)	$1.30
Tovar et al. (2008)	$1.94
AVERAGE COST PER SAMPLE FOR MODS	**$1.48**
TLA	**$ USD/Sample**
Caviedes et al. (2000)	$0.36
Martin et al. (2009) –Belgium study	$2.53
Mejia et al. (1999)	$3.69
Robledo et al. (2006)	$3.08
AVERAGE COST PER SAMPLE FOR TLA	**$2.42**
LJ	**$ USD/Sample**
Moore et al. (2006)	$6.17
Oberhelman et al. (2006)	$0.83
Tovar et al. (2008)	$1.92
AVERAGE PER SAMPLE FOR LJ	**$2.97**
BACTEC	**$ USD/Sample**
Caviedes et al. (2000)	$3.07
Martin et al. (2009) –Belgium/France Study	$5.21
Oberhelman et al. (2006)	$9.26
AVERAGE PER SAMPLE FOR BACTEC	**$5.84**
AUTOMATED MYCOBACTERIAL CULTURE	**$ USD/Sample**
Moore et al. (2006)	$53.48
AVERAGE PER SAMPLE FOR AMC	**$53.48**

4.9 Other Considerations

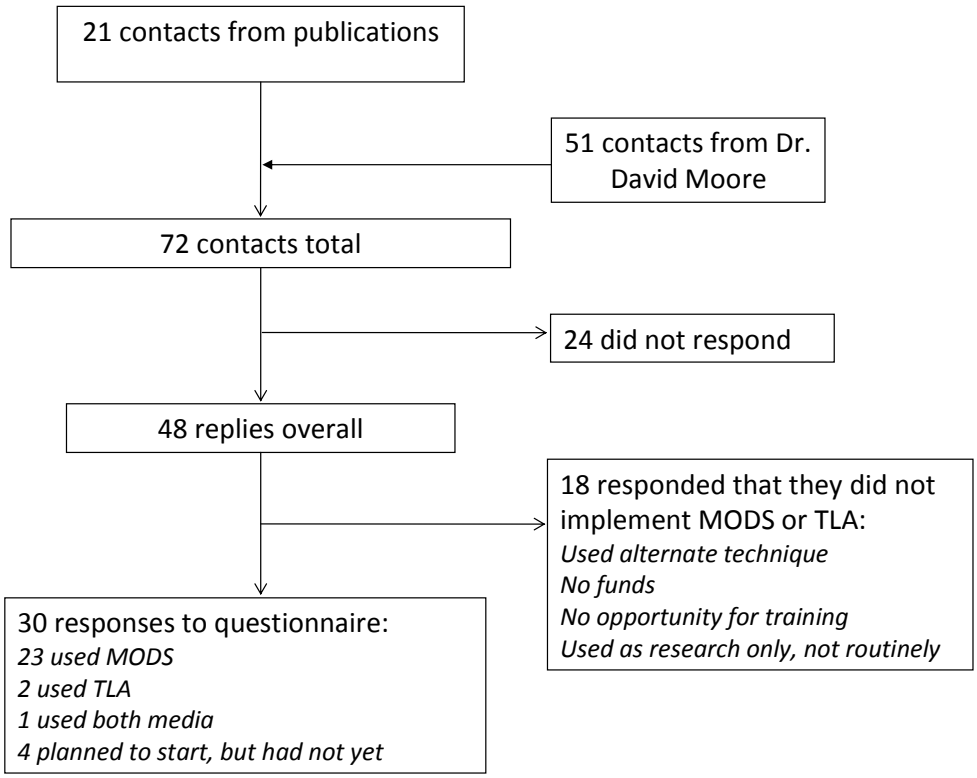
An important advantage of MODS and TLA is that both assays are sealed at the time of specimen inoculation, and remain sealed – considerably reducing the biohazard from handling these cultures. However one concern is whether TB can be differentiated from non-tuberculous mycobacteria (NTM) simply on the basis of micro-colony morphology, as this is subjective, and likely dependent on technician skill. In one study investigators found that they could accurately identify *M. gordonae, M. flavescens* and *M. kubicae* using typical microscopic colony morphology with TLA [77], in a second there was no misidentification of NTM using MODS [67], but in a third investigators reported poor accuracy to distinguish MTB from NTM with MODS [64]. The clinical impact of this problem will depend upon the relative prevalence of NTM and MTB in the population; specificity and positive predictive value of MODS (for MTB) will decrease if the proportion with pulmonary NTM is higher[64]. Further rigorous evaluation of the ability of technologists to differentiate MTB from non-tuberculous mycobacteria (NTM) on the basis of morphology of micro-colonies is needed. A proposed alternative is to include wells in the MODS assay with MTB-specific inhibitor para-nitro benzoic acid (PNB) to distinguish growth of MTB from NTMs. If speciation by micro-colony morphology or use of PNB is accurate, cultures of TLA or MODS could remain sealed, reducing potential biohazard considerably, and allowing these tests to be performed in peripheral settings with minimal environmental controls.

Results for Objective 2

4.10 Characteristics of Included Studies

There were 35 contacts who responded to the first administration. Another five contacts replied to the second administration, sent in September 2009. Finally, another eight contacts responded to the last administration in November 2009, for a total of 48 contacts who replied, representing 67% of all identified potential respondents. Of the 48 respondents, 18 reported they were not using MODS or TLA for various reasons summarized in Figure 6 below. Of the remaining 30 respondents, 23 used MODS, two used TLA, one used both media and four planned on implementing MODS but had yet to start.

Figure 6: Summary of Respondents to Surveys



4.11 General Information for 30 Respondents using MODS/TLA

The majority of the respondents to the questionnaire were from Central America, South America or Asia (77% of respondents). The countries from Central and South America were Bolivia, Brazil, Chile, Colombia, Ecuador, Honduras, and Peru. The countries from Asia were China, Indonesia, Philippines, Vietnam, India, Pakistan and Sri- Lanka. Five countries from Africa responded; these include Egypt, Ethiopia, Nigeria, South Africa, Tunisia and Uganda. There was one respondent from USA. There were four respondents included in the above who had not yet implemented MODS/TLA but planned on doing so.

Table 11. Location of Respondents

Location	**n (%)**
Central / South America	13 (43)
Asia	10 (33)
Africa	6 (20)
North America	1 (3)
Total	30 (100)

As seen in Table 12, 67% of the respondents were from research laboratories, 40% were from a clinical reference laboratory and 17% were primary care laboratories.

Table 12. Type of Facilities of Respondents

Type of Facility	**n (%)**
Research Laboratory	20 (67)
Clinical Reference Laboratory	12 (40)
Primary Care Laboratory	5 (17)
Affiliated with Primary Care Hospital	3 (10)
Affiliated with Tertiary Care Hospital	12 (40)
Affiliated with a University	13 (43)
Other (Includes: Independent Lab, National Reference Lab)	4 (13)

Most of the respondents were funded by the public sector (Table 13). The public sector included funding from the government and Ministry of Health, while the private sector included funding from NGOs and/or private universities.

Table 13. Sources of funding for the Laboratory/Centre

Sector	**n (%)**
Public Sector	20 (67)
Private Sector	8 (27)
Mixed	2 (6)
Total:	30 (100)

All centres and laboratories performed AFB-smear microscopy and mycobacterial culture. Ninety percent of the 30 centres also had the capacity to identify TB and 77% of the centres were capable of performing drug sensitivity testing for TB.

Table 14. Services Provided by Laboratory/Centre

Services	**n (%)**
Perform AFB smear microscopy	30 (100)
Perform TB culture	30 (100)
Have the capacity to identify TB	27 (90)
Perform DST for TB	23 (77)

Of the 26 respondents who had used MODS/TLA, 65% performed MODS/TLA on all clinical specimens received for AFB smear microscopy. Seventy-three percent used MODS/TLA in addition to traditional culture while only one centre used MODS instead of traditional culture.

As an indicator of volume and yield of routine diagnostic services, respondents provided information on the proportion of clinical specimens received in their laboratory that were smear and/or culture positive as well as drug resistant. As shown in Table 15, the proportion positive was high in most of the participating laboratories:

Table 15. Types of Samples

Proportion Positive	**Mean**	**Median**	**Range**
Percentage of samples that were **smear positive** (n=25)	25%	11%	0.53-100
Percentage of samples that were **culture positive** (n=27)	31%	20%	3.76-100
Percentage of samples that were **drug resistant TB** (n=24)	14%	7%	0-60

The average number of samples performed per week was 25, with an inter-quartile range of 5-87. The median number of samples performed per week was 23, with an inter-quartile range of 10 to 33.5.

Table 16. Volume of Activity

Volume of Activity	**Mean/SD**	**Median/IQR**	**Range**
Number of samples performed per week (n=22)	25.1 (+/- 19.4)	23 (10-33.5)	5-87

4.12 Start Up Costs

4.12.1 *Start Up Costs - Lab space Renovation and/or Construction*

When evaluating the start-up costs for renovating a new lab space or constructing a new lab space, there were seven respondents who replied that they constructed a new lab space for MODS/TLA, including one respondent who was not currently implementing MODS but had already constructed a new room for this purpose (Guillermo Pimentel, from Egypt) and there were two that already had the space and only needed to renovate it.

Based on the results, the median number of days to construct lab space was 60 days (IQR: 7-365 days).

Table 17. Time to Construct New Space

Days Needed to Renovate/Construct Lab Space ***Based on 7 respondents who constructed a new lab space***	**n (in days)**
Median (IQR)	60 days (7-365 days)
Mean (Range)	145 days (7-365 days)

The median cost to renovating or constructing new lab space was $60,000 USD (IQR: $6,640-$280,099).

Table 18. Cost to Construct New Space

Costs to Renovate/Construct New Lab Space	$ (USD)
Median (IQR)	$60,000 ($6,640- $280,099)
Mean (Range)	$215,914 ($1,000- $1,000,000)

4.12.2 *Start-up Costs - Capital Costs for Equipment*

The total average cost of newly purchased equipment to start MODS in already established laboratories was $4,630.00 and the cost to start TLA in already established laboratories was $3130.00. However, if a laboratory needed to purchase all the equipment necessary to begin performing MODS for the first time, the capital cost for all the equipment would be $17,300.00. To purchase all the equipment to begin performing TLA for the first time, the cost would be $15,800.00.

For comparison, the cost to initiate Lowenstein Jensen (LJ- traditional egg-based solid media) would be less than MODS because this method does not require an inverted microscope or a biosafety cabinet. Equipment needed to perform LJ includes a refrigerator, a vortex, a centrifuge, an incubator, an autoclave and a balance. The cost for all this equipment needed to initiate LJ would be $ 7,800.00.

On the other hand, the cost to purchase a BACTEC machine for liquid cultures is $40,000.00 USD. This is the preferred price – negotiated between FIND and Becton Dickinson. By contrast, Oberhelman reported that the cost for the BACTEC machine was $70,000[71]. In addition to the machine itself, other equipment would also be needed such as a centrifuge and a freezer, at a cost of another $10,000.00.

Table 19. Information on Purchasing New Equipment

Equipment piece	**Average number per lab**	***n* new**	***n* already had**	**Likelihood needed to purchase**	**Price of equipment $ USD**	**Likelihood X Price**
Refrigerator (n= 24)	1.5	5	19	5/27= 0.185	$500.00	$93.00
Vortex (n=24)	1.2	4	20	4/27= 0.148	$249.00	$37.00
Centrifuge (n=24)	1.1	6	17	6/27= 0.222	$3000.00	$670.00
Incubator (n=24)	1.2	3	21	3/27= 0.111	$1000.00	$111.00
Inverted Microscope for MODS (n=24)	1.1	24	0	24/24= 1	$1500.00	$1,500.00
Microscope for TLA (n=2)	1.0	0	2	0/27= 0	$500.00	$0.00
Autoclave (n=24)	1.3	2	22	2/27= 0.074	$2300.00	$170.00
Balance (n=24)	1.0	2	20	2/27= 0.074	$250.00	$19.00
Biosafety Cabinet	-	7		7/27= 0.259	$8000.00	$2075.00

Several laboratories listed other equipment that they had to purchase in addition to those listed in the questionnaire. This included a sterilizer, a microscope for TLA, micropipettes, a serocoagulator, a pH meter and an agitator.

4.13 Start-Up Costs - Initial Training of Technicians in MODS/TLA Techniques

As seen in Table 20 below, MODS/TLA training sessions lasted on average two weeks and cost $977.00 in salaries for the trainers and $3102.00 in salaries for the technicians being trained. The total training cost was $1245.00 per trainee.

Table 20. Information on Training

Information on training sessions	**n (%)**
Number of labs who provided formal training	4
What is the average duration of each course? (days)	10 (2 weeks)
How many technicians, on average, are trained in each course?	6.6
How many trainers, on average, are trained in each course?	1.75
How long after the initial training until technician is fully proficient? (average)	17.6 (3.5 weeks)
Average cost for trainers per session ($511.00 X 2 X 1.75)	$977.00
Average salary of attendees per session ($279.00 X 2 X 6.6)	$3102.00
Salary of tech while 'learning on the job' ($279.00 X 3.5 X 6.6)	$4135.00
Total training costs – per session	$8214.00
Total Training Cost per Trainee	**$1245.00**

Out of the four laboratories that had provided formal training sessions, all had trained people from their own lab, three had trained technicians from other laboratories in the same country, and two had trained technicians from other countries.

The technicians trained had an average of 5.8 years of previous laboratory experience. Of the 27 technicians, 54% had formal training to learn the MODS/TLA techniques, and the remainder were self-trained using various publications including the MODS website[36].

4.14 Recurrent Costs

4.14.1 Recurrent Costs - Labour

The total average cost of labour per month on MODS/TLA was $1182.00. Assuming 100 samples per month, the recurrent labour cost per sample was $11.82.

Table 21. Cost for Labour

Position	**Average number of people in the position**	**Average number of hrs per month per person spent doing MODS/TLA**	**Monthly Salary**	**Amount of monthly salary spent doing MODS/TLA**
Technician (n=22)	1.3	18	$1115.80	$502.00
Supervision/ Administration (n=18)	1	7.4	$2047.20	$380.00
Clerical/Reporting (n=15)	1	6.8	$1165.60	$200.00
Cleaning/ Housekeeping (n=17)	1	6.8	$597.60	$100.00

For comparison, in previous studies, Kivihya-Ndugga and colleagues estimated the labour cost for smear microscopy to be $0.93 USD per sample[84]. Cornfield and colleagues estimated labour costs for cultures of $3.26 USD per sample when using BACTEC 460, and $1.53 USD per sample when using MGIT[85].

4.14.2 Recurrent Costs - Materials and Supplies

Costs for supplies and reagents estimated in the systematic review were used. The average cost per test for materials and supplies was $1.48 USD for MODS and $2.42 USD for TLA. The average materials cost per test for LJ was $2.97 USD and $5.84 USD for BACTEC from the same studies. One study reported the price per sample for an automated mycobacterial culture test (using BACTEC) was $52.48 USD per sample.

In a separate study, Kivihya-Ndugga and colleagues estimated the cost for materials and supplies for smear microscopy was 0.56$ USD per sample [84].

4.14.3 Total Costs

Table 22. Total Cost

Item	**MODS/TLA $ USD**	**LJ**	**BACTEC**
Start-Up Costs			
Construction Cost To Start Up New Lab (median)	$60,000.00	$60,000.00 (assumed same)	$60,000.00 (assumed same)
Total Average Cost of Equipment to Start Up Lab	$16,800.00	$13,700.00	$50,000.00 to 70,000.
Initial Training (assumes 1.3 technicians/lab)	$1,618.00	No data	No data
Total Start Up Costs (per lab):	**$78,418.00**	**$73,700.00**	**$110,000.00 to $130,000.00**
Recurrent Costs (per sample)			
Labour	$11.82	No data	$3.26
Materials and supplies	$1.36 (MODS) $2.42 (TLA)	$2.97	$5.84
Total Recurrent Costs (per sample):	$13.18 (MODS) $14.24 (TLA)	$2.97	$ 9.10

† Overhead costs were not obtained in the survey due to incomplete responses.

4.15 Quality Assurance

4.15.1 Methods of Quality Assurance

Of the 26 who confirmed MODS/TLA with another culture, only 12 used a method of quality assurance that was judged acceptable.

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Table 23. Quality Control

Evaluation of Quality Assurance Method Used	**n (%)**
Acceptable	12 (46)
Not acceptable	1 (4)
Did not have Quality Assurance of any kind	13 (50)

4.15.2 Supervision/Monitoring of Trainees

Supervision was available after training only for technicians who were trained in the same laboratory. Moore double-checked plate readings for a few weeks until he was assured the trainee was reading positive, negative and contaminated wells precisely and properly, but no formal framework or predetermined performance level had been defined. Robledo stated that all trainees were subject to monitoring by the lab supervisor, who, on a daily basis, checked the work completed by the trainee. This daily supervision lasted for four weeks after which the supervision was performed weekly. Sarojini-Michael stated that their technicians had received no formal training but were essentially self-taught. There was no formal programme of refresher training, and no external quality assurance. However, they were still using MODS as a research tool and had not started using it for routine diagnostics yet. Castillo stated that the observation of MODS was done in pairs for two weeks along with verification by the head of the laboratory.

For quality assurance of trainees outside of the lab, Moore had no formal follow-up. Robledo asked his trainees from outside of the lab to stay to be monitored for at least two weeks with the trainer, who monitored the quality of the work on a daily basis. Sarojini-Michael, based in India, trained one technician from North India whose work was monitored through verifying images via the internet because it was not possible to have in-person supervision. Castillo had not trained any technicians from another laboratory.

4.15.3 *Time to Result*

The average interval between provision of the sample by the patient to receipt in the lab was only 1.8 days (median: one day) compared to 14.3 days (median 10.3 days) from receipt of specimens in the lab until results for MODS/TLA and 11.7 (± 2.3) days for liquid cultures.

Table 24. Time to Result

Average intervals in labs	**Mean/SD**	**Median/IQR**
Days between the sample is provided by the patient and receipt in the lab (n=25)	1.8 (+/-1.7)	1 (1-2)
Days from sample receipt to issue of results for smear-positive samples (n=24)	14.3 (+/-9.8)	10.3 (9-15)
Days from sample receipt to issue of results for smear-negative samples (n=5)	26.5 (+/- 5.0)	21 (21-30)

4.15.4 *Biosafety Issues*

Level Two laboratories are for work involving agents of moderate potential hazard. Personnel have specific training handling pathogenic agents. Access to the laboratory is limited when work is being conducted and extreme precautions are taken with sharp objects. Level Three laboratories are similar to Level Two- but necessary for research in which work is being done with agents that cause serious or potential disease after inhalation or high-risk procedures that require higher-level containment. A Level Three laboratory requires more stringent levels of control than a Level Two laboratory.

For MODS, safe practice requires Biosafety Level Two laboratory facilities. The majority of the respondents had a Level Two laboratory (63%), while the remainder had a Level Three laboratory (37%).

Table 25. Biosafety Levels

Biosafety Level in Lab	**n (%)**
Level Two	19 (63)
Level Three	11 (37)
Total	20 (100)

The Class I cabinets are the lowest level of cabinets possible. This type of cabinet has unrecirculated air flow away from the operator that is discharged to the atmosphere after filtration through a HEPA filter.

The Class II, type A1 cabinets have air that may be recirculated back into the laboratory or exhausted out of the building by means of a "thimble" connection whereby the balance of the cabinet is not disturbed by fluctuations in the building exhaust system. This maintains a minimum average face velocity of 0.38 m/s (75 ft/min).

The Class II, type A2 cabinets are like the Class II, Type A1, cabinets but maintain a minimum average face velocity of 0.5 m/s (100 ft/min).

The Class II, type B1 cabinets have a dedicated exhaust duct connected to the exterior after passage through a HEPA filter. They maintain a minimum average face velocity of 0.5 m/s (100 ft/min) and recirculate 30% of the air within the cabinet.

The Class II, type B2 cabinets are like the Class II, Type B1 cabinets but maintain a minimum average face velocity of 0.38 m/s (75 ft/min).

The Class III cabinets are completely enclosed and gas-tight with HEPA filtered supply and exhaust air. Work is performed with attached long-sleeved gloves. The cabinet is kept under negative pressure of at least 120 Pa and airflow is maintained by a dedicated exterior exhaust system.

For MODS, a well-maintained Class II biosafety cabinet is recommended. Of the laboratories/centres using MODS/TLA, 24 had a Class II cabinet, three had a Class I cabinet and two laboratories had a Class III cabinet

Table 26. Types of Biosafety Cabinets

Biosafety Cabinet	**n (%)**
I	3 (10)
II	1 (3)
II-A	1 (3)
II-A1	3 (10)
II-A2	9 (30)
II-B1	6 (20)
II-B2	4 (13)
III	2 (7)
NA	1 (3)
Total	30 (99)

4.16 Problems with MODS

The survey also addressed problems found with MODS and TLA. Out of the 30 respondents, 26 listed problems they had found with the technique. Some of the common

problems found were: a high contamination rate, difficulty in obtaining the reagents or material in their respective low/middle-income country, difficulty reading the plates, and a lack of personnel trained to do this. A complete list of problems encountered with MODS and TLA can be found in Appendix D.

5 Conclusions

5.1 Summary of Results

The systematic review identified a total of 20 studies evaluating MODS and/or TLA. MODS had a pooled sensitivity of 92% and specificity of 97%, while TLA had a pooled sensitivity of 86% and specificity of 98%. However, there was considerable heterogeneity in these estimates, even in stratified analyses. The sensitivity was slightly lower, but specificity higher when the reference standard was solid plus liquid culture. Costs of reagents and materials for both tests were low, but other costs were not described. The average interval from receipt to results within the lab was 9.1 days for MODS and 11.5 days for TLA, which was much faster than conventional solid and slightly faster than liquid cultures in the same studies [86]. The proportion of contaminated specimens with both assays was low, and comparable to conventional cultures, although higher with TLA than MODS.

The table below summarizes the most important differences between MODS and TLA and the traditional reference standards (liquid culture and solid culture):

	MODS	TLA	LJ	BACTEC
Sensitivity	92.3%	86.5%	~80-85%	~85-95%
Time to Result (average)	9.1 days	11.5 days	25.3 days	10.3 days
Capital Costs (Equipment)	$17,300.00	$15,800.00	$7,800.00	$40,000.00 (machine only)
Recurrent Costs (Material and Supplies)	$1.48	$2.42	$2.97	$5.84

For the survey, there were there were 30 replies in total. Twenty-six were obtained from laboratories that had implemented MODS or TLA and four were obtained from laboratories that were planning to start. The majority of survey respondents were based in countries in Central or South America and Asia, - countries with moderate to high incidence of TB. Initial equipment costs averaged $4,630.00, which is relatively modest, because most of the laboratories initiating these tests already had much of the equipment needed. For MODS all labs needed to purchase an inverted microscope. If a laboratory had no equipment, the cost to purchase all necessary equipment would have been $16,800.00 to perform MODS safely requires only Biosafety Level Two laboratory facilities and a well-maintained Class II Biosafety cabinet. Initial renovation and construction was needed only in a small number of labs; because of minimal biosafety concerns, the space required for these tests is quite modest.

Training costs were also moderate as training required one to two weeks of time, and then several weeks of work under supervision before technicians were considered proficient. Because MODS/TLA was labour-intensive, the labour costs appeared to be higher than published estimates of labour costs with other culture methods. The recurrent labour cost for MODS/TLA was 11.82$ per sample.

Half of the respondents did not have any type of quality assurance procedures in place. Similarly, methods of training and supervision did not appear to be standardised. However, only four respondents had trained and supervised trainees.

5.2 Study Strengths

The study had several strengths. A standard protocol was used when carrying out the systematic review [87] including a comprehensive search of the published literature and efforts to identify unpublished studies as well. Additional data was obtained from four authors (Carlton Evans, Claudio Giacomazzi, David Moore and Jaime Robledo). Two reviewers independently carried out the various stages of the systematic review process including the article selection and data extraction. Moreover, rigorous statistical methods were employed; the binomial random effects models for pooled estimates of accuracy [53] and HSROC curves for estimating diagnostic performance measures [50]. These statistical methods have been recommended by the Cochrane Diagnostic Test Accuracy Working Group as the methods of choice for diagnostic meta-analyses [50].

Another strength was that that this provided novel information regarding the costs and training required for implementing MODS or TLA. This should be useful knowledge for laboratories considering implementing these techniques.

5.3 Study Limitations

Study Limitations for the Systematic Review:

The systematic review had several important limitations. Although all 20 studies presented data on sensitivity, specificity could not be calculated in six of these studies, because the results of MODS or TLA were considered true positive, and therefore were incorporated into the reference standard. Although ten studies reported cost of supplies and reagents, none reported initial start-up costs, nor other important recurrent costs such as labour. None assessed the feasibility of these assays in routine programmatic settings, nor the impact of these tests on patient outcomes.

There were five studies published in languages other than English, French or Spanish that were excluded. Three of the excluded studies were published in Chinese, one in Korean and one in Russian; only the title was available in English for these five papers. Exclusion of these studies could have reduced the power of the systematic review, and could affect the generalisability of the results to these countries.

Publication bias could not be assessed, as available statistical approaches for publication bias (e.g. funnel plots, regression tests) are not recommended for diagnostic meta-analyses [88]. Given this methodological limitation, it would be prudent to assume that publication bias could have occurred, and resulted in an overly optimistic estimate of the accuracy of MODS and TLA.

The presence of incorporation bias in some of the studies reviewed was also a weakness of the systematic review. This is important because this type of bias can lead to an overestimation of diagnostic accuracy. In this systematic review, the studies that had incorporation bias were those that had incorporated the index tests as part of the reference standard- where the diagnosis of active TB was confirmed if any culture, including the index tests, was positive.

Significant heterogeneity of the pooled estimates was found, even with the stratified analysis, which is always a concern for meta-analyses. This heterogeneity could have been due to the different populations studied, the different technicians performing the tests, or variation in the reference standard tests or study quality. Part of the heterogeneity due to different populations could have been due to where the studies were conducted: there were four studies from high-income countries (USA, Belgium, Spain), nine from low-income countries (eg India) and the rest were from middle-income countries (e,g. Peru, Colombia). Heterogeneity due to different reference standard tests could have occurred as well because some studies only used LJ while other studies used both LJ and a liquid culture as well. Therefore the pooled sensitivity and specificity estimates should be interpreted cautiously. Further studies of the sources of heterogeneity may be useful.

Other potential problems in the systematic review could have been caused by some other possible biases. Twelve studies used a cross-sectional study design, but four used a case-series design, using only positive specimens, i.e. no negative specimens were used at all. Fourteen studies reported using consecutive or random sampling, but five did not report their sampling method which could have affected the results if the samples were chosen selectively. Finally, out of the 20 studies used, only eight reported that their technicians were blinded while reading the results. By not blinding the readers, this could also cause bias in reporting the results.

Study Limitations for the Survey:

Despite a total of three different reminders to participate in the survey, only 48 responded out of 72; this response rate of 67% could have led to several problems. Because one third of the targeted population failed to reply to the survey, there was less precision than hoped. It is possible that some respondents only replied if they thought something was to be gained and those who did not see it as important could have decided to forgo responding. However, it seems likely that the non-respondents would be less likely to be using MODS or TLA regularly, so their answers might have added imprecision to the estimates.

It was also difficult to examine complex issues such as quality assurance because there was considerable variation in what each laboratory used, making it difficult to summarize quality assurance. There were also only four laboratories that provided training, making the estimates for training costs less precise.

The language barrier was also a potential study limitation. The questionnaire was written and administered in English. Respondents who were not fluent in English may have not fully understood the meaning of the questions. If a respondent did not respond to the question, this could have led to additional imprecision- there were six respondents who did not respond to all questions initially. If a respondent gave a wrong response, this could have led to non-differential misclassification. To limit this bias, the questions that were not answered were resent to the respondent in their own language (which was Spanish, French or Portuguese). This method of translating the particular problematic questions into the respective language for the respondent was successful as they all subsequently provided the missing responses.

Another limitation of the survey was that there very few labs reported on capital costs, and of those who did, there was substantial variation in the costs reported. Moreover, the labour unit costs, or cost per test for labour, were based on an estimate of the number of tests performed per week. While the labour costs were carefully calculated, the unit cost was less precise as this depended on a rather rough estimate of the number of tests performed regularly.

Moreover, the questionnaire did not address other laboratory methods and costs related to them, which made it harder to compare MODS/TLA with other routine techniques. For other culture methods such as BACTEC or LJ, there was no cost data related to the implementation of the methods such as building new laboratory space and the purchase of material and supplies. Thus, it was more difficult to judge whether or not MODS or TLA was more cost-effective compared to the reference methods. More importantly, the ability to compare labour cost for solid and liquid culture could have been useful but

unfortunately no published data was found on these costs. Because so many of the countries that could potentially use or are currently using MODS/TLA are low- and middle-income countries where salaries vary considerably in different settings, the study would have been stronger if labour costs for these other methods had been ascertained. However, this was not done because to ask the same detailed questions about other lab methods would have increased response burden considerably.

5.4 Implications

In resource limited settings, where the TB burden is also greatest, techniques such as MODS and TLA appear to offer an attractive option for diagnosis of active TB, as they are rapid and accurate yet relatively affordable. The combination of accuracy and speed is important for patient care, allowing therapy initiation sooner in a larger number of patients [72, 89]. These techniques may play a role in expanding access to diagnostic TB services, and enhanced case detection – as they are more sensitive than smear and only slightly less sensitive than automated liquid culture systems – at a fraction of the cost. Although WHO has recommended liquid culture systems [90] and these remain a goal for many TB control programmes, most countries with high TB incidence simply do not have the required infrastructure, expertise or resources required for their implementation [89] Hence, WHO now recommends that selected non-commercial culture methods, including MODS, may be used as an interim solution in specific resource-constrained settings, while the capacity for genotypic and/or automated liquid culture are being developed.

A very important limitation of any non-commercial diagnostic test (ie. any test that does not come as a 'kit') is the lack of standardization. In this regard MODS and TLA are not different from AFB smear microscopy. However, unlike smear microscopy there is not an extensive evidence base, from almost a century of experience, upon which recommendations can be made for training, supervision and external quality control. A few authors have suggested the need for extensive training and standardisation [72], as well as monitoring and evaluation at all stages of implementation of these micro-colony culture techniques[89]. To date groups developing MODS and TLA have published their operating procedures online (MODS is at www.modsperu.org and TLA at: www.tbevidence.org/documents/rescentre/sop/TLA.pdf). These on-line training materials are helpful, but there remains a considerable need for further research to define optimal training, supervision and quality control timing, duration, and procedures.

Although recurrent costs for materials and supplies were very low, the costs for labour were quite high, as these tests are labour intensive. Hence they can be considered

appropriate for low- and middle-income countries, where labour costs are generally low, but may be less useful in high income countries where labour costs are much higher.

Methods of quality assurance were not standardized for MODS and TLA for almost half of the laboratories. This represents an important area for future development, if these tests are to be widely adopted and have a meaningful public health impact. In order to attempt to integrate MODS/TLA into general laboratory diagnostic services for TB in many countries, there is a need to assure that they are performed appropriately. If insufficient attention is given to the quality of the results from MODS/TLA it can lead to serious deficiencies in diagnosis, treatment and monitoring. Ideally, appropriate quality assurance will allow laboratories to assess their capability by comparing their results with those in other laboratories through duplicate testing and rechecking of results. Quality assurance could also include on-site evaluation of the laboratory to review quality of performance and perhaps, on-site rereading of the results[91]. Smear microscopy has previously established very thorough quality assurance mechanisms. Because the quality assurance mechanisms have already been created, it has allowed this method to remain the most cost-effective and widely used test for diagnosing patients with TB and monitoring their progress on treatment. The establishment of QA mechanisms has likely led to fewer preparation and reading errors – two of which are errors that could have resulted in unnecessary treatment or missed diagnoses and continued transmission of infection in the community[91]. Therefore it is vital to have proper quality assurance of MODS/TLA in order to deliver proper diagnostic services.

Cho and Brennan also emphasised the need to apply quality assurance programs in clinical laboratories which employ any new diagnostic approaches where conventional test are applied with new TB diagnostic tests[92].

5.5 Conclusions

The MODS and TLA assays appear to be simple and inexpensive, yet rapid and accurate tests that may be helpful to enhance case detection in resource limited settings with high burden of TB. Because more studies were available that assessed MODS rather than TLA, the findings of high accuracy, rapid time to result and low contamination rates with MODS are more precise and can be viewed with greater confidence.

For the implementation of MODS or TLA, the initial equipment costs and training costs were moderate, costs for materials and supplies were low although labour costs were fairly high.

The systematic review and the survey did not find evidence regarding the performance of these tests under routine field conditions in low- and middle-income countries, nor their impact on patient outcomes, nor their cost effectiveness. Because the incidence of TB is greatest in low- and middle-income countries where resources are limited, cost becomes an important determinant of the implementation of a new diagnostic test. The countries that are most in need of proper TB diagnostic tools are also those with the least resources available. Even though further investigation is necessary, MODS and TLA both appear to be promising diagnostic tools for rapid TB detection.

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Appendix A: Data Extraction Form (for the Systematic Review)

EXTRACTION FORM
Extraction Form: MODS/TLA for the Detection of Active Tuberculosis

Study #: _____ Reviewer: _____

Author: _____ Year: _____ Language: _____

Published: Y / N Country:_____ Sponsor: _____

Index Test:	□ liquid (MODS) media: _____ □ solid (TLA) media: _____ Begin exams day____ Then every ____ days Until ____	□ direct specimen for isolation only □ direct specimen for isolation and resistance □ isolates for resistance only Controls for culture? □ Positive □ Negative □ not specified	Resistance Testing: □ rifampin □ isoniazid □ ethambutol □ pyrazinamide □ others (specify): Controls for resistance? □ Positive □ Negative □ not specified
Reference:	Culture: □ LJ □ MGIT □ BacT □ BACTEC 460 □ other (specify):	Resistance Testing: □ absolute concentration method □ proportion method □ resistance ratio method □ radiometric BACTEC 460 □ non-radiometric (MGIT, BacT) □ other (specify): _____	Validation? □ complete □ partial □ differential Blinding? □ double □ index read blind □ ref read blind □ neither □ not specified
Specimens: □ unit of analysis	□ pulmonary only □ extrapulmonary only □ mixed % pulm: _____ □ not specifed	□ smear + only □ smear – only □ mixed % +: _____ □ not specified	Decontamination? □ yes, NaLC □ yes, other (specify): _____ □ no □ not specified
Patients: □ unit of analysis	□ adults only □ peds only □ mixed □ not specified	□ inpatients only □ outpatients only □ mixed □ not specified	□ HIV included % _____ □ HIV not included □ not specified
Selection:	□ case control design □ cohort design □ unclear □ other: _____	□ consecutive selection □ random selection □ unclear □ other: _____	□ prospective □ retrospective □ not specified

Data on Cost		Index	Ref
☐ no ☐ yes:	Cost/specimen: Cost/positive: [Cost breakdown] Labour: Materials: Capital Costs: Training: Space: Other Measures:		
Data on Time		**Index**	**Ref**
☐ no ☐ yes:	Turnaround from specimen collection Culture Result: Sensitivity Result: Turnaround from specimen received in lab Culture Result: Sensitivity Result: Other measures:		
Data on Biohazards		**Index**	**Ref**
☐ no ☐ yes:	Qualitative Assessment: Incidents reported:		
Data on Implementation		**Index**	**Ref**
☐ no ☐ yes:	Ease of Use Assessment: Length of Training Required: QC/Systems Changes:		

QUADAS Checklist

1. Was the spectrum of patients representative of the patients who will receive the test in practice?	□ YES	□ NO	□ UNCLEAR
2. Were selection criteria clearly described?	□ YES	□ NO	□ UNCLEAR
3. Is the reference standard likely to correctly classify the target condition?	□ YES	□ NO	□ UNCLEAR
4. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	□ YES	□ NO	□ UNCLEAR
5. Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?	□ YES	□ NO	□ UNCLEAR
6. Did patients receive the same reference standard regardless of the index test result?	□ YES	□ NO	□ UNCLEAR
7. Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	□ YES	□ NO	□ UNCLEAR
8. Was the execution of the index test described in sufficient detail to permit replication of the test?	□ YES	□ NO	□ UNCLEAR
9. Was the execution of the reference standard described in sufficient detail to permit its replication?	□ YES	□ NO	□ UNCLEAR
10. Were the index test results interpreted without knowledge of the results of the reference standard?	□ YES	□ NO	□ UNCLEAR
11. Were the reference standard results interpreted without knowledge of the results of the index test?	□ YES	□ NO	□ UNCLEAR
12. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	□ YES	□ NO	□ UNCLEAR
13. Were uninterpretable/intermediate test results reported?	□ YES	□ NO	□ UNCLEAR
14. Were withdrawals from the study explained?	□ YES	□ NO	□ UNCLEAR

Results

Strata	Test	Ref	Specimen	Patient	TP	FP	TN	FN	Total

Appendix B: Survey Questionnaire

This is a short questionnaire about the Microscopic Observation Drug Susceptibility assay (MODS) and the Thin-Layer Agar (TLA) techniques. The questions that will follow will include items regarding local costs (labour time, salaries), space, and infrastructure requirements, expenses and training.

Please provide some information about the person (at the health facility) completing this questionnaire:

NAME:

POSITION:

EMAIL:

PHONE NUMBER:

DATE:

Section 1 – General Facility Information

Name of facility:

City:

State/Province:

1. Is your laboratory a (Please underline all that apply):

Research Laboratory	Clinical Reference Laboratory
Primary Clinical Care Laboratory	Affiliated with a Primary Care Hospital
Affiliated with a Tertiary Care Hospital	Affiliated with a University
Other (Please specify):	

2. Sector (Please underline the appropriate choice):

Public	Please specify (e.g. Government):
Private	Please specify (e.g. NGO, Religious):
Mixed	Please specify:
Other	Please specify:

3. How many specimens does your lab receive for TB diagnostics per _____ YEAR / MONTH (please underline the appropriate choice)?

4. Service Capacity for TB. Do you (Please underline the appropriate choice):

Perform AFB smear microscopy	YES	NO
Perform TB culture	YES	NO
Have capacity to identify TB	YES	NO
Perform drug susceptibility testing for TB	YES	NO

5. What technique do you use: MODS TLA

TB Protocols: (please underline appropriate answer)

6. Do you perform TB cultures on all the specimens you receive for AFB smear? YES NO
7. Is MODS/TLA performed ***in addition*** to TB traditional culture? YES NO
8. Or, is MODS/TLA performed ***instead of*** TB traditional culture (as a replacement)? YES NO

   Comments and Clarifications (If needed):

Rate of Positivity:

9. Of all samples processed, what % are SMEAR positive? _____ %
10. Of all samples processed, what % are CULTURE positive? _____ %
11. Of all samples processed, what % are DRUG RESISTANT TB (any drug)? _____ %

Turn-around Time for Results:

12. On average, how long does it take from the time the sample is submitted to receipt by the lab? _____ days
13. On average, how long does it take from time to sample receipt to issue of results? _____ days
14. What means are used to deliver the results (via telephone, email, post)? _____

Quality Control (Please underline appropriate answer)**:**

15. Do you confirm MODS/TLA results by checking with other culture methods? YES NO
16. If yes, is this done on all MODS/TLA or just a sample? ALL

    SAMPLE
17. Describe any external quality assurance procedures that you use for MODS/TLA:

Section 2 – Recurrent Costs (Labour)

The costs that fall under this category consider how much it costs to perform the MODS/TLA technique in terms of man power.

18. What is the currency used in your lab? Please use this same currency for all questions: _____
19. On average, how many MODS/TLA tests are performed? _____ per week
20. Please indicate in the Table below how many staff are involved in MODS/TLA, and how much time each type of staff worker spends doing MODS/TLA:

Category of Worker	Number in each category	Total hours of work PER PERSON spent doing MODS/TLA per week	Total hours of work IN EACH CATEGORY spent doing MODS/TLA per week	Average salary per category per week
Technician				
Supervisor/Administration				
Clerical/Reporting				
Cleaning/Housekeeping				

Section 3 – Start Up Costs

21. Indicate the biosafety level in the lab in which you perform MODS/ TLA (Please underline appropriate answer):

Level 1 (Suitable for work involving well characterized agents, minimal potential hazard to personnel and environment, precautions against biohazardous materials are minimal, not necessarily separated from general traffic patterns in building, work done on open bench tops using standard microbiological practices, contaminated materials left in open garbage bins)

Level 2 (Similar to Level 1; suitable for work involving agents of moderate potential hazard, personnel have specific training handling pathogenic agents, access to laboratory is limited when work being conducted, extreme precautions are taken with sharp objects and certain procedures may be created in biological safety cabinets or other physical containment equipment)

Level 3 (Similar to Level 2; applicable to clinical/diagnostic/teaching/research in which work is being done with agents that cause serious or potential disease after inhalation, filtered air from laboratory discharged outdoors, recommended "Standard Microbiological Practices, Special Practices and Safety Equipment for Biosafety Level 3" are rigorously followed)

Level 4 (Similar to Level 3; special engineering and design features to prevent microorganisms from being disseminated into the environment, kept at negative air pressure, airlock is used)

22. What type of biosafety cabinet do you use for specimen preparation and performance of MODS/TLA activities? (Please underline appropriate answer):

None

Class I (Unrecirculated air flow away from the operator that is discharged to the atmosphere after filtration through a HEPA filter)

Class II, type A1 (Air may be recirculated back into the laboratory or ducted out of the building by means of a "thimble" connection whereby the balance of the cabinet is not disturbed by fluctuations in the building exhaust system; Maintain a minimum average face velocity of 0.38 m/s (75 ft/min); May have positive pressure contaminated ducts and plenums.)

Class II, type A2 (Air may be recirculated back into the laboratory or ducted out of the building by means of a "thimble" connection whereby the balance of the cabinet is not disturbed by fluctuations in the building exhaust system; Maintain a minimum average face velocity of 0.5 m/s (100 ft/min); Have ducts and plenums under negative pressure.)

Class II, type B1 (Hard-ducted through a dedicated duct exhausted to the atmosphere after passage through a HEPA filter; contain negative pressure plena, Maintain a minimum average face velocity of 0.5 m/s (100 ft/min), Recirculate 30% of the air within the cabinet.)

Class II, type B2 (Hard-ducted through a dedicated duct exhausted to the atmosphere, 100% of cabinet air, after passage through a HEPA filter; negative pressure plena, does not recirculate air within the cabinet, maintain a minimum average face velocity of 0.38 m/s (75 ft/min).)

Class III Class III cabinets are totally enclosed and gas-tight with HEPA filtered supply and exhaust air. Work is performed with attached long-sleeved gloves. The cabinet is kept under negative pressure of at least 120 Pa (0.5 in. w.g.), and airflow is maintained by a dedicated exterior exhaust system.)

For more information, please refer to:
http://www.cdc.gov/OD/ohs/biosfty/bmbl4/bmbl4s3.htm#Biosafety%20Level% 01%20(BSL-1)

The costs that fall under this category include how much it costs to start a lab or open up a lab area that is appropriate for performing MODS/TLA.

23. Did you have to renovate space or construct a new room, in addition to the new equipment purchased (please underline appropriate answer)?

YES NO

If NO, skip to section 4.
If YES, please continue below.

24. How much were the construction costs?

25. How big is the room/space? _____(length) x _____ (width) x _____ (height) (in metres or feet- please underline appropriate answer)

26. How long did it take to construct it? _____ days (1 day = 8 hours)

27. What equipment did you need to purchase to start using MODS/TLA?

Equipment	Number of Units	Newly purchased or Preexisting? (Please underline appropriate answer)	Unit Cost ($) (if had to be newly purchased)
Refrigerator/Freezer		Newly purchased Preexisting	
Vortex		Newly purchased Preexisting	
Centrifuge		Newly purchased Preexisting	
Incubator		Newly purchased Preexisting	
Inverted light microscope		Newly purchased Preexisting	
Autoclave		Newly purchased Preexisting	
Balance		Newly purchased Preexisting	
Other major items (please list):			
		Newly purchased Preexisting	
		Newly purchased Preexisting	
		Newly purchased Preexisting	

Section 4 – Training in MODS/TLA (Please underline answers where appropriate)

Section 4A:

28. When your technician first started using MODS/TLA, how many years of experience did your technician already have?
    _____

29. Have you/your lab provided formal training for MODS/TLA for your own technicians? YES
    NO

30. How did your technician start learning the technique? (Underline all that apply)

    Took a course
    Trained themselves from publications
    From MODS website (www.modsperu.org)
    Other (please specify):

31. If your technician took a course, who provided this course?
    _____

32. How much time was spent in initial training? _____ days (1 day = 8 hours)

33. After that, did your technician take refresher training? YES NO

34. Have you/your lab provided formal training for MODS/TLA for lab technicians from another institution? YES
    NO

    If you answered NO, please skip to SECTION 5.
    If you answered YES, please continue.

35. How many training courses for MODS/TLA have you/your lab given?
    _____

36. What is the duration of each MODS/TLA training course? _____ days (1 day=8hours)

37. How many people are in each course? _____

38. Where do the trainees come from? (Underline all that apply)

People from your own laboratory
People from other laboratory in the same country
People from other laboratory in another country

Section 4B: Training Sessions

39. How long does each course last? _____ days (1 day = 8 hours)

40. How many technicians are involved as trainees per course? _____

41. How many people are involved as trainers per course? _____

42. Are all the trainers present at the same time? (Please underline appropriate answer) YES
NO

43. If NO, please indicate the total amount of trainer hours per course _____ days (1 day = 8 hours)

Section 4C: You have just trained someone. Now what?

Please describe the supervision and monitoring after the initial training, including frequency and duration (hours per week):

44. For trainees from your lab, please describe supervision and monitoring quality control and follow up of newly trained staff including frequency and duration:

45. How long after initial training does a new technician in your lab become fully proficient? _____ weeks (1 week = 5 working days)

46. For external trainees, describe your supervision, monitoring quality control and follow up of newly trained staff in another lab including frequency and duration:

Section 5 - Problems

47. What are some of the problems you have had identified with MODS/TLA testing procedures and results? Also describe any continuing problems.

End of Questionnaire. Thank you for participating.

Appendix C: Reasons for not Implementing MODS and/or TLA

1	AGARWAL, Ashwani	No funds for MODS; did a pilot study last year but no one is actually trained. Has plans to try one day.
2	BEYLIS, Natalie	Uses the MGIT system; implemented uniformly Does not use MODS
3	BWANGA, Freddy	Not using the MODS assay routinely
4	CASE, Karen (With F. Mumbowa)	Does not practice MODS; was stalled by "various factors"
5	CAWS, Maxine (Vietnam)	Not used routinely, only in a research context. They use it routinely for TB meningitis diagnosis at only 200 samples per year. No operational cost evaluations done.
6	FERRO, Beatriz Eugenia	Not using MODS and TLA
7	HAMELMANN, Christoph	Submitted a QST Lawrena Okoro
8	IDIGORAS, Pedro	Doesn't use MODS anymore; uses MGIT
9	INGHAM, Colin	Using a competitor technique to MODS but never actually used MODS or TLA.
10	MATHUR, Murli L	Attempted to use MODS but got fungal contamination in microplate every time. Then gave up. Once tried TLA but MTB did not grow on TLA in the lab. LJ gives best results
11	MUMBOWA, Francis	Sent report Received training but has a new job and works on vaccine studies now.
12	NATHAVITHARANA, Ruvandhi	Does not use MODS; had training with Dr. Moore
13	NOVARISKA, Febriana	Is a respirologist; does not do laboratory work (gave another reference to me)
14	OBERHELMAN, Richard	Results from publications are based from David Moore's research. The results are from research studies funded to carry out work in Peru. TB labs do not use MODS in New Orleans. Not using it in day-to-day work.
15	PITASHNY, Milena	Still has not trained anyone for MODS; has yet to implement
16	RAHIM, Zeaur	Went through MODS training in 2002 but

		did not implement the system in the lab.
17	SALCEDO, Noris	Is not using either MODS or TLA
18	SET, Reena	No opportunity to get trained for MODS so hasn't started it yet

Appendix D: Problems with MODS/TLA

Answers to Question 47 on Survey on Problems Encountered with MODS/TLA

NAME	ANSWERS to Q47
DEVASIA	- In liquid culture like MODS, TB will sometimes form clumps - To observe the usual cording associated with TB, the place must be examined at a higher magnification - Contamination is an issue - Important to inspect the fresh colonies growing on the LJ slant for any sign of contamination prior to preparing the inoculum. - Contamination rate <2% - The cases of contamination were a result of an impure colony population; needed to be decontaminated and restreaked again to a new LH slant prior to inoculum preparation and subsequent DST
SETHI	- Contamination is the major problems - Sometimes cannot make out whether there are actually cords - They see the positives as floccules like in VDRL tests. - They need more training
HU	- Biggest MODS shortcoming: observation by microscopy with the naked eye; can take 10+ mins to finish one plate - Because of the above, some technicians won't do the job - Contamination in wells after PANTA added - With the course of the culture, the water of the liquid culture will vaporize. - In some drug wells, the cord structure is not representative - Difficult to judge results
SHAH	- Initially, the technicians had difficulty with "debris" that clouded the ability to accurately identify positives (cords) and negatives. We started with very high SENS, but low SPEC (i.e., everything was being read as "positive-growth"). Then, after reviewing these results, we moved to a much lower SENS, but better SPEC (i.e., everything was being read as "negative-no

	growth"). - After ~200 samples and trouble-shooting attempts by the lab, we were fortunate to have an on-site training visit by Drs Caviedes and Coronel. The SENS and SPEC improved markedly. - The lesson from this is that training of staff is critical before embarking on MODS but that even a short training (2 weeks) is sufficient for excellent results. - Another major issue has been buy-in due to concerns about bio-safety and the time needed to prepare and read MODS results. We are hopeful that a "MODS kit" will facilitate implementation at the peripheral health center level and that MODS can be viewed as an adjunct (or replacement) for AFB smear, especially in high HIV-prevalence settings where rates of smear-negative TB are high. - Lastly, our setting has extremely high rates of XDR-TB, so there is concern that MODS can only diagnose MDR-TB without further testing for Quinolone or Aminoglycoside resistance. However, we hope to utilize MODS as a method to identify MDR patients in whom an additional sample can be sent for full DST. We hope that this will limit the number of cultures and DSTs, reduce costs and burden on the central lab, and allow patients to start on drug-resistant TB treatment earlier than the current 12-week lag to standard DST results.
HUANG	- Results judgment is some kind of subjective - For second line drugs, no reliable DST results is available for reference - Contamination is always a problem need to deal with - Hard to discriminate NTM from MTC by MODS
GIACOMAZZI	- Problems in ordering materials (24 well plates are impossible to purchase in Ecuador!) -
SENERATNE	None
SHIFERAW	- First used gas impermeable ELISA sealer as a safety measure, which created aerobic conditions in the plate and prevented mycobacterium growth. Finally they identified and fixed the problem (worked without using the sealer)

ALMEIDA DA SILVA	- The main problems with TLA: the number of plates to examine per day and the biosafety - Maybe is not a good idea with high number of samples
CASTILLO ACEVEDO	- Contamination is less than 5% - Lack of sufficient personnel - Occasional indeterminate cultures that must repeat - Equipment repair
PIMENTEL	- Main problem was obtaining all QC strains to conduct MODS
RIVERA LOZADA	- The main problem is that it requires high biosecurity and this limits their use in certain labs where there are no levels of biosecurity. - TLA disadvantage: time to results
NIC FHOGARTAIGH	- Bacterial contamination of media - Time consuming to read the plates in early days of culture where growth may be scanty
CHUNG	- Difficult to obtain data since staff have other functions apart f from MODS - Lack of funds for a new centrifuge is an issue - Storing facility and freezers are continuously getting more difficult
LINEU KRITSKI	- Main problem is the cost of equipment (inverted light microscope and CO2 emissions) and the consumables (medium) - The discordance with the GS and the need for further studies on the standardization of inoculum
DORMAN (Honduras)	- Plates/Liquid media tipping/spilling during the transport from the incubator to the inverted microscope - Challenges in preparing MODS media (learning curve plus need for reagents that were difficult to procure)
DORMAN (Brazil)	- Plates/Liquid media tipping/spilling during the transport from the incubator to the inverted microscope
WERTHEIM	- A problem in Vietnam to obtain reagents (but this is not MODS specific)
LOZANO BELTRAN	- Insufficient personnel in the TB lab of the Escuela Tecnica de Salud but there are young investigators/students that help out in the labs - Recognition of MODS for diagnosis of TB for the National Program of Tuberculosis. - Currently MODS only available for researches

BRIZUELA	- High contamination of the cultures: was controlled after a second training of the technicians who process MODS and prepares the material
BRIGDEN	- Contamination during the decontamination process - Stops to be an issue with contamination
CURTO CHAVEZ	- No money for MODS- everyone wants to do it but no one supports them and gives them the money necessary to do it. - They want to do MODS but need training, something they can't do because they do not have resources to go to Lima
BOUKADIDA	- We are unable to perform drug susceptibility test. Even, we use susceptible strains, but all the time there is growth of mycobacteria
SAROJINI MICHAEL	- No training on MODS prior to testing which affected results initially- both identification of cording as well as time to positivity. - There were several positives for MODS continuously for a period of one week so could not rule out cross contamination because they do not have molecular technique to confirm that these isolates were the same (spoligotyping) - Cross contamination among wells was a problem as they are liquid cultures and can get easily cross-infected from the neighbouring wells during inoculation - MODS positives by cording which does not differentiate MTB from NTM. - Infection control issue especially while training new personnel
DALCOLMO	None
BALAKRISHNAN	- Chance of missing out the ID of other members of the MTB complex (e.g. M bovis, M africanum, M microti…. These do not form cords) -
ROBLEDO	- TLA is suitable for laboratories that do not process high amount of specimens, since it requires labor time from technician to observe under a regular microscope each agar culture - TLA requires a certain amount of experience to distinguish microcolonies compatible with *M.tuberculosis*

	- May be a problem the requirement for a CO2 source although a candle jar is suitable and works perfectible well - It may performs better in laboratories with experience in doing *M. tuberculosis* culture
IRFAN	- I performed Microcolony identification using M7H9 broth and M7H11 agar as a research project- not routinely done During the study I faced following problems: - Fungal contamination rate was very high(8% and 12% for M7H9 broth and M7H11 agar respectively) - Daily microscopy was time consuming in both techniques as well as labour intensive - Using 24 well plates for *Mycobacterium tuberculosis* microscopy, chances of cross contamination are very high. - Microscopy was performed using inverted microscope placed outside the safety cabinet in a BSL3 laboratory. In my opinion this also provides some degree of *Mycobacterium tuberculosis* exposure risk to laboratory worker.
MOORE	None
OKORO	None
PARTAKUSUMA	- Inverted microscope not available in laboratory