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Discordance of Drug Susceptibility Test Data between the CDC Mycobacteriology Laboratory and Local Public Health Laboratories Participating in Tuberculosis Clinical Trials, TBTC, CDC

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DISCORDANCE OF DRUG SUSCEPTIBILIY TEST DATA BETWEEN THE CDC MYCOBACTERIOLOGY LABORATORY AND LOCAL PUBLIC HEALTH LABORATORIES PARTICIPATING IN TUBERCULOSIS CLINICAL TRIALS, TBTC, CDC

By

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DEDICATION PAGE

The following thesis document is dedicated to God and my family for their unconditional love and constant support.

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I am thankful to God who has guided me through each step of my life. I want to thank my parents and the rest of my family for all their support and love. I would like to extend my deepest gratitude to Lorna Bozeman MS, Dr. Stefan Goldberg, and Dr. Ruth Moro for their guidance, support and enthusiasm. I would also like to thank Dr. Richard Rothenberg MD, MPH for his invaluable guidance and support throughout this thesis writing process. The skills and knowledge gained by working closely with all of you are beyond measure.

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DISCORDANCE OF DRUG SUSCEPTIBILIY TEST DATA BETWEEN THE CDC MYCOBACTERIOLOGY LABORATORY AND THE LOCAL PUBLIC HEALTH LABORATORIES IN DRUG EFFICACY TESTING CLINICAL TRIALS, TBTC, CDC

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ABSTRACT

BACKGROUND: Multi drug resistant Tuberculosis (MDR-TB) is a serious public health concern in many parts of the world. As per the WHO- 2010 global report on Surveillance and response 3.6% of all incident TB cases globally are multidrug resistant. In this regard, there is an increasing demand for timely, reliable and comprehensive drug susceptibility testing (DST) as MDR-TB surveillance is being geared up. The intent of this analysis is to determine whether there is a need to continue routine confirmatory DST testing at CDC in addition to just sending the isolates for genotyping. Analysis is done by measuring the discordance between the results of laboratory DST at CDC and the local labs drug type, drug testing concentrations, and study sites.

METHODS: The data for this analysis was provided by the Tuberculosis Trials Consortium (TBTC), CDC. Data for this analysis was collected over nearly two decades (1993-2011), gathered from 7 clinical trials. Discordance between the local and CDC lab DST results was measured using Kappa statistic. Sensitivity and specificity analysis was done by taking the CDC DST lab results as the gold standard. Discordance levels were calculated by local sites and baseline drug resistance for each antibiotic in each study was measured.

RESULTS: Average Kappa values for inter rater agreement for all the studies was 0.6444 whereas the overall level of discordance across all studies is 7.786%. Drug resistance at baseline was highest for Isoniazid and Streptomycin (except Study 23 and 22).

CONCLUSION: Though the current results show few DST result discordances between local and CDC labs, it is better to continue to send isolates to the centralized lab (CDC) in view of the worldwide threat of drug resistant TB epidemic, the recommendations of the current literature and the benefits of reliable confirmatory testing services and availability of other molecular diagnostic methods.

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CHAPTER I

INTRODUCTION

Tuberculosis (TB), one of the most deadly diseases throughout history, is the second leading cause of death among infectious diseases. While effective treatment regimens are continuously being improvised, emergence of multidrug resistance to anti TB drugs is currently a huge hindrance in combating this lethal infection. TB Drug susceptibility testing (DST), documented to be one of the most complex procedures to standardize in the Mycobacteriology laboratory requires technical expertise to produce valid and reliable results and requires up to 8 weeks to get results by commonly used methodology [51]. DST on initial isolates from all patients enrolled in Tuberculosis Trials Consortium (TBTC) studies is done to identify an effective anti-TB regimen at Local Public Health Laboratories to assure an effective treatment regimen is prescribed. Subcultures of initial isolates from local labs are subsequently sent to the Mycobacteriology laboratory (CDC) for confirmatory DST.

The goal of this study is to measure the discordance between the results of laboratory DST at CDC and the local labs. The intent of the analysis is to determine whether there is a need to continue routine confirmatory DST testing at CDC in addition to just sending the isolates for genotyping. Also measuring discordance by various factors such as the drug type, drug testing concentrations, and study sites is critical part of this analysis. Since the clinical decisions are based on the local lab results, the reliability of lab test results and the study site's potential for DST lab testing is imperative. Another aspect of this analysis is to measure how much drug resistance exists among patients

entering TBTC studies (22, 23, 24, 25, 27, 28 and 29) as most studies assume a pan sensitive population. This will help understand if the current lab techniques are enabling the timely detection of drug resistance in new patients. At sites with considerable background rates of drug resistant TB, suggestions to use new molecular DST (Rapid MDR TB identification tests such as Gene xpert) which give results in hours might be evaluated.

CHAPTER II

REVIEW OF LITERATURE

2.1 Global burden of Tuberculosis

Tuberculosis (TB), a worldwide pandemic, is the seventh leading cause of mortality globally and ranks second only to human immunodeficiency virus (HIV) as a cause of death from an infectious agent [1]. TB bacilli infect around one third of the world's population, approximately 2 billion people [3]. According to the 2009 WHO report, the estimated global incidence of TB was 9.4 million, the estimated global prevalence 11 million with the largest proportion of estimated cases occurring in South-East Asia region (34%), the Western Pacific region (21%) and African Region (30%) [2]. Among the 22 High Burden Countries (HBCs) which account for 80% of new cases every year, India, China, Indonesia, South Africa and Nigeria are among the highest TB incidence countries [4].

2.2 TB history and overview:

TB is an airborne, infectious disease caused by *Mycobacterium tuberculosis* (MTB) that primarily attacks the lungs and sometimes other organs such as kidney, spine and brain. It can cause two reactions in the human body: either the latent TB infection (LTBI) or active TB disease. Latent infection occurs when the person is infected with MTB (shows no symptoms) but the immune system fights progression to TB disease. People with latent TB cannot spread the bacteria and can be identified by tuberculin skin

Test (TST) or special TB blood test. Active TB disease occurs when the bacteria are rapidly multiplying in the body and the immune system is incapable of stopping the proliferation. Most often (around 75%) active TB is pulmonary (affecting the lungs). Extra–pulmonary TB is less frequent at higher rates in immune compromised individuals. (In this paper we follow common usage and take TB to mean pulmonary tuberculosis. Others forms will be specified.). Clinical manifestations of pulmonary TB include chronic cough, weight loss, fever, fatigue, sweating, and blood tinged sputum [18, 19, 20].

Evidence exists in the form of skeletal remains with TB (4000 BC) and tubercular decay in the spines of Egyptian mummies (3000-2400 BC), thus proving that TB is one of the oldest infectious diseases [21]. With the industrial revolution in 1600 AD, TB became widespread with the growth and expansion of urban areas. The same epidemiological trend is reflected in current urban areas wherein overcrowding, lack of sanitation and malnutrition are the breeding grounds for TB [22].

2.3 Close association of TB with poverty:

Along with malaria and HIV, TB is a preventable and curable disease most closely linked to poverty. Ninety-eight percent of TB deaths and 95% of TB incidence occur in low and middle income countries [5]. According to WHO estimates, average incidence in low income countries is twenty times higher than in high income countries. Several studies done in different locations showed that over -crowding, poor living and working settings, HIV, malnutrition, homelessness, smoking, alcohol abuse, indoor air pollution are environmental risk factors for TB [6,7,8,10] . TB not only thrives on poverty but also worsens it. Estimates show that TB might lead to loss of 20-30% of annual wages

among the poor [13]. Even in the developed world, similar epidemiology is documented - higher rates of TB are found in poorer, underprivileged sections of the society and thus underlining the close interaction between social determinants of health and existence of TB [8,9,10,11,12,13,17]. As the world's population is rising, the number of people living in poverty is also rising- posing a real threat to TB eradication programs.

The importance of tuberculosis among other infectious diseases is chiefly attributed to the high case fatality rate among untreated and improperly treated patients. According to Styblo & Enarson, two thirds of untreated smear positive patients will die within five to eight years and most of them in the first 18 months [24]. Even in smear positive patients receiving anti TB drug treatment, the case fatality rate can be more than 10 percent in areas with low adherence rates or high HIV co infection and drug resistance rates [25].

2.4 Current trends in TB:

Currently the incidence of TB is gradually declining in most countries (since the peak in 2004) and also the death rate is declining (since 2000) due to the diagnosis and treatment of TB. However, treatment programs have not had a major, detectable impact on incidence on the whole [65]. In the United States, there has been a steady decline and in 2010 there was lowest recorded incidence [66, 67].

2.5 Evolution of public health approaches for tackling TB:

The approaches to control TB changed over the years (1948-present). The DOTS strategy formulated by the WHO in 1993 emphasized the five elements needed for

controlling this world wide public health emergency which included political commitment, increasing case detection rate using sputum smear microscope, standardizing short course therapies including (Directly Observed Therapy) DOT, regular supply of drugs and this DOTS strategy is estimated to be one of the most cost effective interventions currently available [33, 34].

2.6 TB drugs:

Before the introduction of the anti TB drugs in the 1950s and the development of drug regimens during 1980s, mortality due to pulmonary TB was estimated to be 50%. The discovery of streptomycin and its clinical use as the first specific anti tuberculosis drug is a significant milestone in efforts to fight TB [23]. Anti TB drugs today are classified into first line, second line and third line drugs. First line drugs are highly effective and essential components of a short course regimen while second line drugs frequently produce adverse events. Ethambutol (E or EMB), Isoniazid (H or INH), Pyrazinamide (Z or PZA), Rifampicin (R or RMP) (equivalent to Rifampin (RIF) in US), streptomycin (S or STM) are classified as first line drugs. Second line drugs include aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine, p-aminosalicyclic acid [26]. Examples of newly discovered drugs are Fluoroquinolones- levofloxacin, gatofloxacin and moxifloxacin [35]. The current recommended drug regimen for most patients with pulmonary TB is a 6 month multi drug regimen with two phases – Intensive phase (four first line drugs, isoniazid, rifampin, pyrazinamide, and ethambutol) for 2 months and continuation phase (Isoniazid and Rifampin alone) for 4 months. Likewise various drug

regimens are explored based on the drug susceptibility results, toxicity patterns and administration strategies (Example: DOTS) [27, 28]

2.6.1 Isoniazid:

Isoniazid (INH), one of the primary drugs in TB treatment today has been in use since 1952 due to its efficacy, low toxicity and reasonable cost. When Isoniazid was used in a clinical trial for the first time, it was so successful that patients were reported to be 'dancing in their wards'. Oxygen is important for the action of INH and it is active against growing tubercle bacilli and not the resting organisms. Isoniazid is a "pro" drug which needs to be activated by the catalase-peroxidase enzyme which is coded by *katG* gene and once the drug is activated it shows highly specific activity against mycobacteria by inhibiting the biosynthesis of mycolic acids which are an essential component of the mycobacterial cell wall (36). According to Mitchinson, Isoniazid kills 95% of mycobacteria in the first 2 days of treatment while Rifampicin is more effective in the continuation phase (28, 29).

2.6.2 Rifamycins:

Rifamycins are among the most potent antibiotics against tubercle bacilli both in log and stationary phases. They possess unique characteristics of not only acting rapidly after exposure to bacilli but also being bactericidal months after the start of the treatment regimen (29). Mechanism of action is by inhibiting an enzyme DNA dependent RNA polymerase synthesis through binding to the growing DNA chain (37). 95% of rifamycin resistance is due to mutations in a sub unit of this RNA polymerase enzyme (38).

2.6.3 Ethambutol:

Ethambutol, first introduced in 1961, is still an enigma with respect to its mechanism of action and molecular basis for drug resistance. It is believed that it is effective against mycobacteria by specifically inhibiting various steps in the bacterial cell wall synthesis (39).

2.6.4 Pyrazinamide:

Pyrazinamide, one of the first line drugs has an incredible sterilizing activity thus killing the persisting bacilli and enabling the shortening of treatment regimen from 9 months to 6 months. It is also a prodrug and converted into Pyrazinoic acid by the enzyme Pzase produced by *M.tuberculosis*. Resistance is mostly attributed to mutations in this Pzase enzyme (40).

2.6.5 Streptomycin:

Streptomycin, first discovered by Waksman and colleagues in 1941 was effective as an anti TB drug previously but not currently used as a mono therapy drug due to increase in drug resistance and treatment failure [60]. The mode of action of streptomycin is inhibition of translation of m RNA and aberrant proofreading [41].

2.6.6 Fluoroquinolones:

Fluoroquinolones are currently used in drug resistance and for those who do not tolerate therapy. In view of changing resistance patterns, they are now considered for inclusion as first line agents. The key factor in their mode of action is DNA gyrase and

mutations in this enzyme are the most common reason for resistance. Mouse models confirm these findings [42].

2.7 Fighting TB- challenges:

2.7.1 TB and HIV:

Globally around nine percent of TB incidence is attributed to HIV but in some regions it is higher- WHO African region (31%) and also in industrialized nation- United States (8.6%) [67]. Also 12 % of TB deaths are attributed to HIV [14]. There are more than 1 million TB cases in people with HIV. In a HIV infected person, TB is harder to diagnose and progresses more rapidly [15]. In addition, HIV infection weakens the immune system and increases the probability of getting infected and progressing to active TB [15, 16]. Studies have shown that co-infection with HIV increases the risk of TB infection developing into active TB by 10 fold [68]. Out of the 1.8 million persons who died with TB in 2007, estimates show that around 456,000 were HIV positive [3]. These numbers prove that the HIV pandemic poses a massive threat to global TB control programs.

Though some adverse drug-drug interactions between HAART (Highly active anti retroviral therapy) and TB drugs (rifamycins), DOTS and anti retroviral therapies are synergistic and without undergoing both of these therapies in combination, the life expectancy of a HIV infected TB patient will be typically less than five years. Emphasis on early detection and cure will help in minimizing TB related cases and deaths in a most cost effective way according to results shown by mathematical models [45].

2.7.2 Multidrug resistance:

Multidrug resistant TB (MDR-TB), the condition where Mycobacteria are resistant at least to Isoniazid (INH) and Rifampicin (RIF), the two most powerful drugs against TB, is one of the huge challenges impeding public health efforts to control tuberculosis. Drug resistant TB can occur in two ways- acquired or secondary drug resistance and initial (primary) drug resistance. Acquired drug resistance which occurs owing to discontinuous and ineffective therapy that selects a small number of resistant mutants, is normally seen from 1 to 4 months after initiation of therapy. Serial exposure to inadequate regimens enables the emergence of multi drug resistance. Initial resistance occurs when a person is infected by drug resistant TB strains and can only be distinguished from acquired resistance by comparing a patient's baseline and follow up drug susceptibility patterns. In the absence of microbiology data, it can be inferred by knowing the past treatment regimens followed by the patient [31, 32].

Though the true levels of multidrug resistance are unknown, according to the WHO 2008 estimates, around 440 000 cases of multidrug resistant TB emerged globally, implying that around 3.6% of TB incident cases are multi drug resistant (MDR). Moreover, national and regional anecdotal evidence points towards an increase in the number of drug resistance cases throughout the world. Half of these cases occur in China and India, and MDR-TB accounts for approximately 150 000 deaths. The most common form of drug resistance in the US is Isoniazid resistance which has been documented in 10% of TB patients. The standard treatment regimen is not effective against MDR-TB, and the alternatives are far more expensive, less potent, more toxic and takes longer for effective treatment (at least two years) [30].

According to the Stop TB initiative 2006-2015, there is a huge need for resources to address this issue since estimates show that 1.3 million MDR-TB cases will need treatment in 27 high MDR-TB burden countries (2010-2015) and this would cost USD 16.2 billion. 29,423 MDR-TB cases reported throughout the world in 2008 represent only 7% of the estimated number of cases that year. The limited surveillance for MDR-TB is attributed to deficiency of local laboratory resources and drug susceptibility testing to identify incident MDR-TB cases. Only 1% of newly incident cases underwent drug susceptibility tests (DST) in 2008. The distressing fact is that only 1% of the MDR-TB cases identified are enrolled in treatment [30]. Increase in Multidrug resistance throughout the world underlines the importance of need for accurate DST and availability of alternate regimens to these patients. Molecular level understanding of medicinal chemistry of anti TB drugs is necessary to understand multidrug resistance. Analyzing drug susceptibility results is imperative to address the need for surveillance, to propose improved treatment regimen and guidelines, to understand the risk factors in proven cases of resistance and several other potential benefits.

2.8 Current methods for MTB detection and drug susceptibility testing:

Valid and reliable DST is important to design appropriate drug regimens. As per the recommendations by the American Thoracic Society, initial isolates from all patients must be tested for first line drug resistance (INH, RIF, ETH, and PZA). Subsequently, isolates resistant to first line drugs must be tested for resistance to second line drugs (fluoroquinolones, amikacin, kanamycin and capreomycin) [50, 69]. DST results define clinical resistance using terms ‘susceptible’ and ‘resistant’ based on quantitative

analysis. 'Resistance' is defined as growth of greater than 1% of bacteria when 'critical' drug concentration is present. Critical concentration implies the minimum concentration of the drug that inhibits 95% of wild strains of MTB. These concentrations have been determined empirically and adopted worldwide [51].

There is an increasing need for development of rapid tests due to the high rates of multidrug resistant TB (MDR-TB) and emergence of extensively drug resistant TB (XDR-TB). It is highly important to identify cases of MTB and treat them in a timely and efficient way. It normally takes 7 to 14 days to detect TB using methods such as MGIT (Mycobacterium Growth Indicator) or BACTEC (Becton Dickinson) in laboratories that are well established and funded [46]. If traditional methods for culturing MTB are followed (using Lowenstein-Jensen (LJ) and the less expensive Ogawa media for example), it may take an average of 3 weeks for detection alone and DST may take an additional 3 to 4 weeks [48]. Lengthy periods for DST tests might lead to adverse consequences such as assignment of inappropriate treatment, spread of drug resistance in the community and augmentation of resistance in the patient [47]. In the developing world most TB control programs use stained sputum smears for case finding and mostly the treatment regimen is given in the absence of Drug Susceptibility Testing [49]. Development of rapid tests will have individual and also public health benefits such as enhanced diagnosis, improved survival chances, prevention of acquisition of further drug resistance and reduced spread of resistance in the population. Nevertheless, as of now, there is no single test which is good, quick, cheap and easy [47]. Also most TB cases occur in resource-limited countries where costly and sophisticated equipment requiring conventional rapid detection methods (BACTEC and MGIT) are not available.

The National Centre for Clinical Laboratory services recommends the agar proportion method which is an inexpensive and comparatively simple method for DST testing. The procedure for agar proportion method includes steps such as plating bacteria on media with either no drug or critical concentration of the drug and then incubating for 3 weeks followed by counting of colonies. An isolate is defined as resistant if the number of colonies on drug- containing media is greater than 1% of number of colonies on drug-free media. This method has been a "gold standard" in the US. In general, for first line DST testing in the US, commercial broth systems is used rather than the time-consuming agar proportion method. The minimum time for agar proportion method is 21 days [50, 52, 53]. Nevertheless, second line DST is mostly done using the agar proportion method. FDA-cleared rapid broth DST tests exist for first line drugs but not for second line drugs so far [50]. Due to the emergence of multiple drug resistant TB, the CDC recommended DST on all baseline isolates from each patient and repeated testing if the patient was not culture converted after 3 months of therapy or failed to respond clinically to the treatment. In addition, it is mandatory to report susceptibility results within 4 weeks after receiving the specimen.

In the summary report on the TB drug susceptibility testing by the APHA and CDC, several issues and concerns about current practices have been raised. Some of the problems are due to the differential capabilities of Mycobacteriology labs and the discrepancies that arise due to piece-meal services offered at each lab. Inefficient communication between labs has been an issue of concern. Lack of confidence in drug resistance results leads to continued retesting, further delaying the reporting of results. High costs and limited trained laboratory expertise are additional subjects of concern[51].

2.9 Description of drug treatment testing clinical trials by TB Trials Consortium, CDC (Studies- 22, 23, 24, 25, 27, 28 and 29):

CDC Tuberculosis Trials Consortium (TBTC) conducts clinical, microbiological and epidemiological research in areas such as diagnosis, clinical management and prevention of TB infection and disease. It has several sites in various parts of the globe- United States, Canada, Brazil, Peru, Spain, South Africa, Uganda, Vietnam and China. For laboratory services, the sites rely on local Mycobacteriology labs and also the CDC Mycobacteriology lab which acts as the central lab for confirmatory drug susceptibility testing (where second line drug resistance testing is also performed, unlike the local labs where only first line drug resistance is tested). Below is a synopsis of some of the clinical trials done by TBTC:

- *Study 29* is a prospective, multicentre, open label Phase II clinical trial designed to evaluate the efficacy and safety of an experimental intensive phase (first 8 weeks of treatment) tuberculosis treatment regimen in which daily Rifapentine is substituted for Rifampin in combination with INH, ETH and PZA. Patients with suspected pulmonary tuberculosis and other inclusion criteria were enrolled in the study [43].
- *Study 28* is a multicenter, placebo-controlled, Phase II- double-blind trial designed to evaluate the effect of using Moxifloxacin (M) in place of Isoniazid (H), in combination with Rifampin (R), Pyrazinamide (Z) and Ethambutol (E) on 2-month culture conversion rates among patients with sputum smear-positive pulmonary tuberculosis [44, 70].
- *Study 27* is a double-blind, Phase II, randomized, multicenter study by the Tuberculosis Trials Consortium (TBTC) undertaken in United States, Uganda,

Canada and Brazil to assess the effect of using Moxifloxacin (Moxi) in place of Ethambutol (E), in combination with Isoniazid (H), Rifampin (R), and Pyrazinamide (Z) on 2-months culture conversion among patients with sputum smear-positive pulmonary tuberculosis[28, 72].

- *Study 25* is a prospective, randomized, double-blind study to test the tolerability of three different doses (900 mg, 1200mg, 600mg) of Rifapentine in the treatment of tuberculosis. HIV seronegative patients with culture positive drug susceptible tuberculosis were enrolled into this study[59, 73].
- *Study 24* aimed at determining the efficacy of a largely intermittent regimen for INH-resistant or INH intolerant Tuberculosis. It is a prospective, open-label, nonrandomized trial where in the patients enrolled must be sensitive to Rifampin, Ethambutol and Pyrazinamide, INH resistant or intolerant[58].
- *Study 23* is a non randomized, open label, single arm, prospective study to treat HIV-Related Tuberculosis and to determine the rate of confirmed treatment failure and relapse with an intermittent rifabutin-based regimen for the treatment of Isoniazid and Rifamycin-susceptible HIV-related tuberculosis[54, 71].
- *Study 22* is an open label, randomized controlled trial to compare, at completion of the follow-up phase, the clinical and bacteriologic relapse rates associated with the two study regimens- Once-Weekly Rifapentine and Isoniazid compared to twice-Weekly Rifampin and Isoniazid for treatment of Pulmonary Tuberculosis[57, 74].

Studies 23, 27, 28, and 29 enrolled patients at the beginning of intensive phase treatment. Studies 22 and 25 enrolled patients at the beginning of continuation phase treatment, after approximately 2 months of TB therapy. Study 24 enrolled patients after a period of up to approximately 2 months on pre-study TB treatment.

CHAPTER III

METHODOLOGY

3.1 Data Sources and Study Population

The data for this analysis were provided by the Tuberculosis Trials Consortium (TBTC), CDC. TBTC conducts programmatically relevant clinical trials, partnering with US and international clinical sites to expand the current clinical and epidemiologic knowledge of TB and enhance the scope for diagnosis, clinical management, and prevention of tuberculosis infection and disease. Data for this analysis were collected over nearly two decades (1993-2011), gathered from 7 clinical trials. Many variables, methods of data collection differ among these studies.

3.2 Objectives:

The objectives of this analysis are four-fold for each study (22, 23, 24, 25, 27, 28 and 29)

1. Measure the amount of discordance between the DST results done at CDC Mycobacteriology laboratory and local site laboratories.
2. Compute discordance by drug type, drug testing concentration and study sites.
3. Calculate the Kappa values, the statistic to measure inter rater agreement for each antibiotic in each study.

4. Evaluate the sensitivity and specificity of the local laboratory DST results considering CDC results as the 'gold' standard.
5. Determine how much drug resistant MTB exists among the patients entering TBTC studies (22, 23, 24, 25, 27, 28 and 29) as most studies assume a pan sensitive population. (The notable exception is Study 24, for which resistance to INH was one possible entry criterion.)

3.3 Hypothesis:

For each study, DST results of the CDC Mycobacteriology lab do not differ from DST results of public health labs at local TBTC sites.

3.4 Study Design:

All clinical trials conducted by the TBTC have been approved by the institutional review boards of CDC and each clinical site. Patients give written informed consent before being enrolled into these studies. TBTC, CDC provided this previously collected data for secondary analysis free of identifiers. The IRB at Georgia State University approved this analysis (Appendix A).

3.4.1 Common Methodology for all studies for Secondary data analysis:

All analysis was done using SAS 9.2 version (The SAS Institute, Cary, NC). Each study ID corresponds to a single participant in that study. All study ids without comparable DST results in either CDC dataset or the local lab results dataset were

excluded from the analysis. DST results for other antibiotics such as Amikacin which were only tested at the CDC Mycobacteriology lab were not considered for analysis.

After merging the CDC and the local DST results dataset, the records meeting the above criteria were considered for further cleaning. Nevertheless there were a few study IDs which were present more than once. This might be due to the reasons such as the initial culture being non viable, culture contamination (after which the site resubmits the isolate) or absence of Mtb growth on susceptibility testing medium. In such a scenario, the condition used to pick the right record was matching the variables 'date_collected' (from CDC) and 'specdate'(from local labs). These two fields 'date_collected' and 'specdate' as discussed above come from two different datasets- CDC and local respectively and both the dates indicate when the specimen was collected and hence are comparable. Multiple DST results for the same patient exist due to various reasons such as culture being contaminated or failing in grow, repetition of lab tests to confirm drug resistance, isolates that are not collected at baseline etc. Since one of the objectives of the analysis is to capture how much drug resistance exists in the general TB population by measuring drug resistance when patients first enroll in the study (Baseline isolates- isolate collected at the time of enrollment), it is imperative to pick the baseline DST results for the analysis and not include any acquired drug resistance results which might sometimes lead to additional discordance. If repetition of lab tests to confirm drug resistance was identified ('date_collected' -period is within few months or looking at comments section: 'resend specimen') the record that have earliest concordant DST results was picked.

Further when the above criteria were met when duplicates and triplicates persisted, checking the 'comments' field in CDC dataset for information regarding culture contamination, isolate resubmission and other factors helped in picking the right record. Thus the final cleaned dataset was created for each study based on all the above criteria and these datasets was used for not only determining the levels of discordance between the two DST results but also to answer all the questions listed in the objectives of the analysis.

3.4.2 Kappa Statistic Analysis:

3.4.2.1 Background

Kappa statistic, the most commonly used statistic to measure the agreement between two or more observers takes into account that observers may agree or disagree just by chance. If a kappa value is 1, it indicates perfect agreement while a kappa value of 0 indicates agreement equal to chance. One of the limitations of Kappa statistic is that it is dependent on the prevalence of the condition being tested. Precision (agreement between observers) is reported using kappa statistic [61]. This statistic is used in situations where two or more observers are calculating the same thing- CDC lab DST results and Local lab DST results as in this case. The formula for calculation is the based on observed agreement and expected agreement (expected due to chance alone). Observed agreement (P_0) being the ratio of results where both the labs agreed (a+d) to the total number of results (N) where (a) and (d) represent the number of times the raters agree, (b) and (c) represent the number of times the raters disagree. If N_1 is the sum of (a) and (c), N_0 is the sum of (b) and (d), M_1 is the sum of (a) and (b) and M_0 is the sum of (c) and

(d), then expected agreement (P_E) is given by the formula- [((N_1/ N)*(M_1/ N)) + ((N_0/ N)*(M_0/ N))]. Finally, kappa is calculated as the ratio of (P_0 - P_E) and (1- P_E) [62].

3.4.2.1 Kappa analysis specifically for DST data analysis:

Kappa statistic was calculated for each antibiotic (Isoniazid (0.2µg/ml), Isoniazid (1 µg/ml), Rifampin (1µg/ml), Ethambutol (5µg/ml), Pyrazinamide (100µg/ml), Streptomycin (2µg/ml) in each study (22, 23, 24, 25, 27, 28, and 29) using proc freq-kappa procedure in SAS 9.2.

3.4.3 Sensitivity, Specificity and PPV (Positive Predictive Value) analysis:

For each antibiotic, sensitivity and specificity of the local lab DST results were measured by first counting the number of true positives, false positives, true negatives and false negatives. True positives are those results that tested positive (resistant) and were truly positive (resistant) in CDC lab test results. False Positives are those that tested positive (resistant) in local lab but tested negative (susceptible) in CDC lab. True negatives are those that tested negative (susceptible) in local lab test and were truly negative (susceptible) in CDC lab results. False Negatives are those that tested negative (susceptible) in local lab test but were positive (resistant) in CDC lab results. Sensitivity, the statistical measure signifying the proportion of true positives (antibiotic resistant specimens) correctly identified so, was calculated by using the following formula: TP/(TP+FN) where TP represents the number of true positives and FN represents the number of false negatives. Likewise, specificity represents the proportion of true negatives (antibiotic sensitive specimens) correctly identified so is calculated as TN/(TN+FP). Positive predictive value which is the ratio of True Positives (TP) and the sum of True Positives and false positives (TP+FP) (Denominator: number of patients

testing positive for resistant MTB) is the probability that the patient has drug resistant TB when restricted to those patients who test positive (resistant) in local lab results. PPV was calculated for each antibiotic in each study.

3.4.3 Discordance between the two lab test results is the sum of false positives and false negatives.

After measuring the total discordance, discordance by local lab site for each study was measured as the sum of false positive and false negatives. Baseline drug resistant MTB among the patients entering these clinical trial studies (22, 23, 24, 25, 27, 28 and 29) was measured as the ratio of number of resistant isolates for each study (per antibiotic) measured by both labs-CDC and local and the total number of specimens tested for that antibiotic. Discordance is calculated by considering individual DST results independently. In other words, there could be multiple discordances for a single isolate but each of the discordances is counted independently by drug tested. Similar procedure was followed for all other studies with a few differences in variable names and this will be discussed in sections below.

3.5 Methodology for each study specifically and variables analyzed:

3.5.1 Study 29: (Rifapentine substituted for Rifampin)

Variables analyzed:

CDC lab results-Variable Name	Comparable-Local lab results-Variable name	Label
PATIENT_ID1	STUDY_ID	Study ID number which

		is unique for each patient
DATE_COLLECTED	SPEC_DATE	Date when the specimen was collected at site
DATE_RECEIVED		Date when the specimen was received at CDC
DATE_SENT		Date when the specimen was sent to CDC
RIFAMPIN_1	RIF	Rifampin-1µg/ml
STREPTOMYCIN_2	SM	Streptomycin-2µg/ml
ISONIAZID_0_2	INH_02	Isoniazid-0.2µg/ml
ISONIAZID_1	INH_1	Isoniazid-1µg/ml
PYRAZINAMIDE_100	PZA	Pyrazinamide-100µg/ml
ETHAMBUTOL_5	ETH	Ethambutol-5µg/ml

The CDC and local datasets had 402 and 473 records respectively. After merging the CDC and the local DST results dataset by study id and taking only the ones that have DST results in both there were 359 records. After duplicate records were further cleaned by using criteria mentioned and information in the comments section there were 333 records in the final data set (as there were several duplicates and triplicates) and this dataset was used to address the objectives. Sensitivity and specificity and discordance were calculated as per the procedure mentioned in 3.d.2 and 3.d.3 respectively

3.5.2 Study 28 (Moxifloxacin substituted for INH):

The variables analyzed were similar to that of Study 29. The CDC and local datasets had 522 and 421 records respectively. After merging the CDC and the local DST

results dataset the 435 records meeting the criteria mentioned in 3.4.1. After duplicate records were further cleaned by using criteria mentioned and information in the comments section there were 342 records in the final data set and this dataset was used to address the objectives. Sensitivity and specificity and discordance were calculated as per the procedure mentioned in 3.4.2 and 3.4.3 respectively.

3.5.3 Study 27 (Moxifloxacin substituted for Ethambutol):

Variables analyzed:

CDC lab results-Variable Name	Comparable-Local lab results-Variable name	Label
LAST_NAME	STUDY_ID	Study ID number which is unique for each patient
DATE_RECEI		Date when the specimen was received at CDC
SPECIMEN_T		Date when the specimen was sent to CDC
RIF_1	RIF	Rifampin-1µg/ml
SM_2	SM	Streptomycin-2µg/ml
INH_02	INH_02	Isoniazid-0.2µg/ml
INH_1	INH_1	Isoniazid-1µg/ml
BACTEC_PZA	PZA	Pyrazinamide-100µg/ml
ETHAMBUTOL_5	ETH	Ethambutol-5µg/ml

The CDC and local datasets had 374 and 323 records respectively. Merging the CDC and the local DST results dataset records after crosschecking whether they meet the criteria mentioned in 3.4.1 was done (351 records). There were 28 distinct patient ids

with duplicates. After removing the duplicates, the final cleaned dataset had 321 records and this was used for Sensitivity and specificity and discordance analysis as per the procedure mentioned in 3.4.2 and 3.4.3.

3.5.4 Study 25 (tolerability- 600, 900, 1200 mg Rifapentine):

The variables analyzed for this study were exactly similar to that of study 27 (Except- variable for PZA in CDC dataset was PZA_25). The CDC and local datasets had 158 and 150 records respectively. The merged dataset had 149 records. The final cleaned datasets after merging had 135 records meeting the criteria mentioned in 3.4.1. The records with comments regarding culture contamination and other culture non-viability were removed. Also the records which seemingly showed discordance but where the comments section had additional information about the results being pending were removed from the analysis. Also 2 records that seemingly showed Pyrazinamide (PZA) discordance but the comment had information that though the results show resistance the cultures were sensitive on another medium were removed as this cannot be considered as discordance as there is information about the true drug susceptibility of the culture. There was one duplicate and one record with culture contamination –both excluded from the final analysis. So the cleaned dataset had 135 records and this was used for Sensitivity and specificity and discordance analysis as per the procedure mentioned in 3.4.2 and 3.4.3.

3.5.5 Study 24 (Alternate regimen for INH intolerant or resistant patients):

The variables analyzed for this study are exactly similar to that of Study 27 (Except- variable for PZA in CDC dataset was PZA_25). There were 50 and 92 records

in the CDC and local datasets respectively. The merged dataset had 50 records. There were 4 duplicates. Then the records were cleaned as per the criteria mentioned in 3.4.1 and the final dataset had 43 records after removing the duplicates. This dataset was used for Sensitivity and specificity and discordance analysis as per the procedure mentioned in 3.4.2 and 3.4.3.

3.5.6 Study 23 (treatment of Isoniazid and Rifamycin-susceptible HIV-related tuberculosis):

The variables analyzed for this study are exactly similar to that of Study 27 (Except- variable for PZA in CDC dataset was PZA_25). There were 191 and 168 records in the CDC and local datasets respectively. The merged dataset had 172 records. There were 15 patient IDs with duplicates. When there were multiple records for the same ID, the one that had 'Final Report' in comments section were picked. Then the records were cleaned as per the criteria mentioned in 3.4.1 and the final dataset had 142 records after making sure there is no redundancy. . This dataset was used for Sensitivity and specificity and discordance analysis as per the procedure mentioned in 3.4.2 and 3.4.3.

3.5.7 Study 22 (once weekly Rifapentine INH in continuation phase):

The variables analyzed for this study are exactly similar to that of Study 27 (Except- variable for PZA in CDC dataset was PZA_25). There were 1416 and 311 records in the CDC and local datasets respectively. The merged dataset had 487 records. There were 111 distinct patient ids with multiple duplicate records. They were picked based on the

field ‘date recei’ such that DST results of the valid isolate were picked for the analysis. Then the records were cleaned as per the criteria mentioned in 3.4.1 and the final dataset had 303 records after making sure there is no redundancy. For Pyrazinamide, a new dataset was created from the final dataset as there were a few records with a null value in CDC- DST results for PZA. This new dataset used for PZA alone (271 records) served a dual purpose - the records with null values in CDC lab-DST results are not picked up as discordance and also other antibiotic related DST results are not lost due to exclusion from the analysis. These datasets were used for Sensitivity and specificity and discordance analysis as per the procedure mentioned in 3.4.2 and 3.4.3.

CHAPTER IV

RESULTS

This section describes the answers to the research questions in detail.

4.1 Discordance analysis:

4.1.1 STUDY 29:

The total number of records included in the analysis was 333 (Figure 1). Very good inter rater agreement (high kappa value) was found for INH and RIF (Table 14). Due to the high number of false negatives for Pyrazinamide (PZA), Ethambutol (EMB) and Streptomycin (SM), the sensitivity was very low. No discordance was observed for Rifampin (RIF) leading to 100% sensitivity, specificity and Positive predictive value. Drug resistant population at baseline (%) was calculated as the ratio of number of true positives and total number of records analyzed multiplied by hundred to understand the drug resistance pattern in patients enrolling into these studies. The drug resistance at baseline in Isoniazid (both concentrations-especially (1µg/ml)) was found to be high (8.25% and 11.34% respectively) (Table 1).

Discordance between the two labs for each antibiotic was also categorized by site to identify frequent discordances so that appropriate recommendations for local lab resource strengthening can be made (Table 8).

4.1.2 STUDY 28: The total number of records included in the analysis was 342 (Figure 2). High Kappa values were found for INH and RIF (Table 15). Low sensitivity was observed for PZA whereas high specificity was documented for INH (both concentrations) and Rifampin. Drug resistant population at baseline (%) was calculated as per above discussion in Study 29 results and highest was found in INH (1µg/ml). Discordance between the two labs for each antibiotic was also categorized by site (Table 9).

4.1.3 STUDY 27: The total number of records included in the analysis was 321 (Figure 3). ETH and SM had very high kappa values (Table 16). Ethambutol (ETH) had 100% sensitivity, specificity and positive predictive value (PPV) whereas INH (1µg/ml) and SM had high specificity and PPV. INH (0.2µg/ml) had low sensitivity (Table 3). There were no PZA DST results in CDC dataset. Drug resistant population at baseline (%) was calculated as per above discussion in Study 29 results and it was highest in INH (0.2µg/ml) - 10.31% when compared to all other antibiotics. Discordance between the two labs for each antibiotic was also categorized by site. Site 30 had 10 discordances (INH (0.2 µg/ml) alone) given the higher N (total patients tested) value after comparing the results with the gold standard- CDC (Table 9).

4.1.4 STUDY 25: The total number of records included in the analysis was 135 (Figure 4). The fewer number of records in the final dataset compared to other studies might explain the low drug resistance found in this study-25 (Table 3). Discordance between the two labs for each antibiotic was also categorized by site (Table 10).

4.1.5 STUDY 24: The total number of records included in the analysis was 43 (Figure 5). High Kappa value was found for INH (1µg/ml) (Table 18). Drug resistant population at baseline (%) was found to be very high for INH (75.76% and 40.74% for both concentrations) which is reasonable as the requirement to be a part of this study is INH resistance or intolerance (Table 4). Discordance between the two labs for each antibiotic was also categorized by site (Table 10).

4.1.6 STUDY 23: The total number of records included in the analysis was 142 (Figure 5). High kappa statistic was found for INH (0.2µg/ml), INH (1µg/ml) and PZA (Table 19). Sensitivity was particularly low for Streptomycin (SM) whereas specificity was almost 100% for all antibiotics (Table 5). Higher PZA resistance at baseline (around 13%) was found in this study. Discordance between the two labs for each antibiotic was also categorized by site (Table 11).

4.1.7 STUDY 22: The total number of records included in the analysis for PZA were 271 where as for all the other antibiotics - 303 (Figure 7). There were no local lab results for INH (1µg/ml). The two lab DST results agreed less than would be expected just by chance alone for PZA and ETH and overall the Kappa values were lower when compared to other studies (Table 20). Sensitivity was low for INH (0.2µg/ml), SM and RIF. PPV and specificity were 100% for INH (0.2µg/ml) (Table 7). SM had the highest drug resistance at baseline (around 6%). Discordance between the two labs for each antibiotic was also categorized by site (Table 14).

Overall statistics (Table 22) indicate a total discordance percentage of 7.786% (N=1708) and an average kappa value of 0.6444 which indicates good agreement overall. A closer

look indicates lowest kappa value for study 22 (0.2216) and highest agreement for study 23 (0.8497). Though it might not be appropriate to calculate average values (across all studies) for kappa, sensitivity, specificity, PPV and discordance due to several reasons mentioned in Discussion section, the calculations were done only to indicate overall values. High specificity (97.75) and low sensitivity (70.24) were found overall. Average positive predictive value across all studies was 77.96%.

Box Plots for Kappa statistic and Positive predictive values across studies (Figures 3 and 4):

All kappa values that are zero or undefined were excluded from the box plots for Positive predictive values and kappa statistics. Especially in the case of Kappa statistic- a calculated value of zero resulted as no drug resistance was observed for that antibiotic in that study. So it actually means perfect agreement even though the kappa value is zero.

The median for kappa statistic were mostly closer across different studies even though the range was large. For study 22- there were no local lab results for Isoniazid (INH) at 1 µg/ml concentration. Also there were negative kappa values for 2 antibiotics. In all the studies clumping of lower values is seen (especially study 24 and 27).

A Box plot for positive predictive values (for each antibiotic) across studies was drawn. In case of no drug resistance for a particular antibiotic, the positive predictive value is not defined. These cases were excluded for drawing the box plot. Most often the lower values are clumped but the range is high. For study 25 there was no drug resistance

found except in streptomycin, so the box plot is entirely based on the values from Streptomycin.

CHAPTER V

DISCUSSION AND CONCLUSION

5.1. Discussion

There are numerous striking variations in the objective of each clinical trial, the clinical disease status of participants (though all have active TB – some have pulmonary, extra pulmonary, HIV, cavitation, etc), participating sites (some changed over time), number of patients enrolled at each site, number of patients enrolled in each study, number, concentration of antibiotics tested for drug susceptibility, and test method at each site and in each study.

Also the long time span between the earliest and the later studies (1997-2010) creates variability in the emphasis placed on DST, pursuing local sites to resend specimens in case cultures are contaminated or failed to grow, the resources available for DST and sophistication of methods used for testing.

Merely combining the results of all studies and calculating pooled values without paying attention to the variations will be inappropriate. However, among all studies DST results are compared between a local lab and CDC lab for paired isolates from each patient. Therefore, merging the results just to get an overall picture of trends over time by site was helpful to make appropriate recommendations. Nevertheless, all these variations need to be considered while examining the results.

The isolates sent to CDC laboratory are subcultures of the original culture used for local lab tests. This might influence the capacity to identify low level resistance at CDC lab. Another factor that might influence discordance is the lack of indication of partial drug resistance at the local lab. Local lab DST results only indicate whether the isolate is resistant or susceptible unlike the CDC lab where drug resistance is expressed as percentage. For this analysis, any percentage drug resistance above 0 at CDC lab was considered as 'resistant'.

The eligibility criteria for each study vary and this has significant impact on the various measures computed. Patients in Study 29, 28 and 27 are enrolled prior to being tested for drug resistance during intensive phase therapy. Patients in study 24 are known to be infected with *M. tuberculosis* resistant to Isoniazid (primary) or are intolerant to INH at the time of enrollment. Patients in study 23 are HIV seropositive adults with positive cultures for TB, known to be susceptible to INH and Rifampin at enrollment. Patients in study 22 were tested in the continuation phase of therapy, having been eligible to enroll with a baseline isolate susceptible to INH and Rifamycin. Thus, Drug-resistance rates between studies are partly attributable to differences in study design. Studies that enrolled later in the course of TB therapy would be expected to find lower rates of drug resistance, since patients with drug resistance would more likely have been discovered and excluded from the study, except for Study 24, which had higher rates of INH-resistance by design. DST can take a month or two in the laboratory, due to the slow growth of MTB in culture.

The current global drug resistance percentages for Isoniazid are 6·7% (IQR 4·2–11·6)[63]. According to the Global surveillance for anti tuberculosis drug

resistance, 1994-1997, primary drug resistance to Isoniazid (7.3 percent) or streptomycin (6.5 percent) was more common than resistance to Rifampin (1.8 percent) or Ethambutol (1.0 percent) [64]. Similar trend was observed in all studies (except study 23). For Study 24, the baseline drug resistance for INH and SM was very high compared to other TBTC studies. This is expected as being INH intolerant or INH resistant is one of the inclusion criteria.

Discordance rates for Study 27, 28 and 29 (tested after enrollment-resistance at enrollment is not known) were not unusually different from the other studies (Studies 22, 23 and 25 – DST results known at enrollment) (Table 22). However, employing rapid tests for determining drug resistance prior to initiating treatment at sites where rates of drug resistance is high would be beneficial. Such screening would avoid starting patients on sub optimal treatment regimens, who are later found to have baseline drug resistant TB.

Though overall statistics for kappa, sensitivity, specificity, Positive predictive value and discordance % have been calculated for all studies combined, results should be interpreted with caution due to all the mentioned variations. Box plot for kappa statistic across studies was done. There was skewed distribution in all studies and it might be concluded that with time the concordance between the two lab results improved for the most part based on the distribution of kappa values.

5.2 Limitations of the Study:

- Considering each antibiotic DST results for single patient independently might be a problem especially if discordance exists in several antibiotic DST results for the same isolate.

- Due to the variable number of patients at each site and in each study, it is difficult to give equal value for their results.
- The time span between the earliest and latest studies is around 11 years which might have impact in the sophistication of methods used, resources available, the emphasis on the importance of DST results
- Some local sites did not test certain antibiotics (or certain antibiotics at different concentrations) which resulted in an irregular distribution of sites testing the antibiotics and these were excluded from the analysis. Thus the total number of records will not reflect the number of valid unique DST results. These variations need to be considered while interpreting the results.
- In this analysis discordance was not measured by DST method, which might play a role in outcome of DST.
- Kappa statistic is influenced by base rates of diagnosis and might not be appropriate to compare across studies with different base rates.

5.3 Recommendations

Timely detection of drug resistance in patients is most important to prevent a worldwide epidemic of incurable multidrug resistant tuberculosis even though rarity of resistance is the rule, for now. Most often, underestimation of the problem rather than affordability is currently paralyzing laboratory services to detect resistance. Since the currently available methods are all laboratory based, it is understandable that at least for the next few years, avoiding lab based methods is not possible. Currently traditional approaches using phenotype (culture media detecting drug

resistance based methods) are in use. The minimum inhibitory concentration ranges (MIC s) or critical concentration ranges (between resistant and susceptible strains) vary for each drug and the gap (range) indicates whether the lab based test is reliable for that antibiotic. Drugs such as Isoniazid (INH) and rifampin (RIF) have wide gap between the highest MIC s for susceptible strains and lowest MIC s for resistant strains, thus improving the reliability of the DST results[55]. Literature shows that for drugs such as Ethambutol (ETH) the difference is narrow and this might give rise to a number of false positives and false negatives[56].

There are several rapid and sensitive genotypic methods also but their affordability is the issue of concern. Though the current results show few DST results discordances between local and CDC labs, it is better to continue to send isolates to the centralized lab (CDC) (even though it means more investment) in view of the worldwide threat of drug resistant TB epidemic, the recommendations of the current literature [51, 56] and the benefits of reliable confirmatory testing services and availability of other molecular diagnostic methods. The key role that local laboratories play by providing timely DST reporting to clinicians, which is critical for tailoring effective drug regimens to treat patients is also recognized.

5.4 Conclusion

Though the current results show few DST result discordances between local and CDC labs, it is better to continue to send isolates to the centralized lab (CDC) in view of the worldwide threat of drug resistant TB epidemic, the recommendations

of the current literature and the benefits of reliable confirmatory testing services and availability of other molecular diagnostic methods.

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TABLES

Table 1: Comparison of discordance results for antibiotics in Study 29

Antibiotic (µg/ml)	True Positives (TP)	False Positives (FP)	True Negatives (TN)	False Negatives (FN)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (PPV)	Discordance (FP+FN)	Drug resistant population at baseline (%)((TP+FN)/N *100)) N=TP+FP+TN+FN
Isoniazid (0.2)	22	1	277	3	88	99.64	95.65	4	8.25
Isoniazid (1)	9	1	85	2	81.81	98.83	90	3	11.34
Rifampin (1)	9	0	322	0	100	100	100	0	2.72
Pyrazinamide (25)	2	1	231	5	28.57	99.56	90	6	2.93
Ethambutol (5)	1	0	324	6	14.28	100	100	6	2.11

Streptomycin (2)	9	3	270	9	50	98.90	75	12	6.19

Table 2: Comparison of discordance results for antibiotics in Study 28

Antibiotic (µg/ml)	True Positives (TP)	False Positives (FP)	True Negatives (TN)	False Negatives (FN)	Sensitivity (%)	Specificity (%)	PPV	Discordance (FP+FN)	Drug resistant population at baseline (%)(TP/N*100)
Isoniazid (0.2)	20	0	289	2	90.90	100	100	2	7.07
Isoniazid (1)	8	0	111	1	88.88	100	100	1	7.50
Rifampin (1)	4	0	337	1	80	100	100	1	1.46
Pyrazinamide (25)	3	2	257	2	60	99.22	60	4	1.89
Ethambutol (5)	0	2	337	0	-	99.41	-	2	0.00
Streptomycin (2)	9	5	284	2	81.81	98.26	64.28	7	3.67

Table 3: Comparison of discordance results for antibiotics in Study 27

Antibiotic (µg/ml)	True Positives (TP)	False Positives (FP)	True Negatives (TN)	False Negatives (FN)	Sensitivity (%)	Specificity (%)	PPV	Discordance (FP+FN)	Drug resistant population at baseline (%)((TP+FN)/N)*100)
Isoniazid (0.2)	15	2	233	12	55.55	99.14	88.23	14	10.31
Isoniazid (1)	2	0	108	1	66.66	100	100	1	2.70
Rifampin (1)	3	1	309	2	60	99.67	75	3	1.59
Pyrazinamide (25)	2	1	243	2	50	99.59	66.66	3	1.61
Ethambutol (5)	4	0	311	0	100	100	100	0	1.27
Streptomycin (2)	10	0	262	1	90.90	100	100	1	4.03

Table 4: Comparison of discordance results for antibiotics in Study 25

Antibiotic (µg/ml)	True Positives (TP)	False Positives (FP)	True Negatives (TN)	False Negatives (FN)	Sensitivity (%)	Specificity (%)	PPV (%)	Discordance (FP+FN)	Drug resistant population at baseline (%)((TP+F)/N *100)
Isoniazid (0.2)	0	0	87	0	-	100	-	0	0.00
Isoniazid (1)	0	1	71	0	-	98.61	-	1	0.00
Rifampin (1)	0	1	133	0	-	99.25	-	1	0.00
Pyrazinamide (25)	0	0	59	2	-	100	-	2	3.28
Ethambutol (5)	0	2	125	0	-	98.42	-	2	0.00

Streptomycin (2)	2	3	94	0	100	96.90	40	3	2.02

Table 5: Comparison of discordance results for antibiotics in Study 24

Antibiotic (µg/ml)	True Positives (TP)	False Positives (FP)	True Negatives (TN)	False Negatives (FN)	Sensitivity (%)	Specificity (%)	PPV (%)	Discordance (FP+FN)	Drug resistant population at baseline (%)(TP+FN/N *100)
Isoniazid (0.2)	23	4	4	2	92	50	85.18	6	75.76
Isoniazid (1)	11	2	14	0	100	87.5	84.61	2	40.74
Rifampin (1)	0	0	43	0	-	100	-	0	0.00
Pyrazinamide (25)	0	0	26	1	-	100	-	1	3.70
Ethambutol	0	0	43	0	-	100	-	0	0.00

(5)									
Streptomycin (2)	6	2	20	3	66.66	90.90	75	5	29.03

Table 6: Comparison of discordance results for antibiotics in Study 23

Antibiotic (µg/ml)	True Positives (TP)	False Positives (FP)	True Negatives (TN)	False Negatives (FN)	Sensitivity (%)	Specificity (%)	PPV (%)	Discordance (FP+FN)	Drug resistant population at baseline
Isoniazid (0.2)	3	0	89	1	75	100	100	1	4.30
Isoniazid (1)	2	0	87	0	100	100	100	0	2.25
Rifampin (1)	0	0	134	3	-	100	-	3	2.19
Pyrazinamide (25)	2	0	68	8	20	100	100	8	12.82
Ethambutol (5)	0	0	139	0	-	100	-	0	0.00

Streptomycin (2)	4	1	110	5	44.44	99.09	80	6	7.50

Table 7: Comparison of discordance results for antibiotics in Study 22

Antibiotic (µg/ml)	True Positives (TP)	False Positives (FP)	True Negatives (TN)	False Negatives (FN)	Sensitivity (%)	Specificity (%)	PPV (%)	Discordance (FP+FN)	Drug resistant population at baseline (%)(TP/N*100)
Isoniazid (0.2)	1	0	*236*	5	16.66	100	100	5	2.48
Isoniazid (1)	No Local Lab results for Isoniazid (1)								
Rifampin (1)	1	4	234	3	25	98.31	20	7	1.65
Pyrazinamide (25)	0	1	73	4	0	98.64	-	5	5.13
Ethambutol (5)	0	3	235	1	0	98.73	-	4	0.42
Streptomycin (2)	8	3	198	5	61.53	98.50	72.72	8	6.07

Table 8: Discordance by local site –Study 29

Antibiotic (µg/ml)	Sites and Frequency of discordance		No. of patients at each site	Discordance %
Isoniazid (0.2)	20	2	22	9.09
	22	1	8	12.50
	40	1	30	3.33
Isoniazid (1)	20	2	22	9.09
	28	1	10	10.00
Streptomycin (2)	24	2	16	12.50
	30	4	144	2.78
	31	2	15	13.33
	40	2	30	6.67
	62	2	25	8.00
Pyrazinamide (25)	13	1	9	11.11
	20	2	22	9.09
	30	2	144	1.39
	70	1	3	33.33

Ethambutol (5)	20	1	22	4.55
	30	2	144	1.39
	31	1	15	6.67
	40	2	30	6.67
Rifampin (1)	-			

Table 9: Discordance by local site –Study 28

Antibiotic (µg/ml)	Sites and Frequency of discordance		No. of patients at each site	Discordance %= (freq of discordance/No. of patients)*100
Isoniazid (0.2)	17	1	10	10.00
	32	1	27	3.70
Isoniazid (1)	32	1	27	3.70
Streptomycin (2)	16	1	10	10.00
	29	1	18	5.56
	30	3	190	1.58
	40	1	19	5.26
	66	1	3	33.33
Pyrazinamide (25)	16	1	10	10.00
	30	1	190	0.53
	32	2	27	7.41
Ethambutol (5)	16	1	10	10.00
	30	1	190	0.53

Rifampin (1)	14	1	3	33.33

Table 10: Discordance by local site –Study 27

Antibiotic (µg/ml)	Sites and Frequency of discordance		No. of patients at each site	Discordance %
Isoniazid (0.2)	24	1	3	33.33
	30	10	168	5.95
	32	3	36	8.33
Isoniazid (1)	25	1	3	33.33
Streptomycin (2)	30	1	168	0.60
Pyrazinamide (25)	22	1	13	7.69
	32	2	36	5.55
Ethambutol (5)	-	-	-	-
Rifampin (1)	13	1	15	6.67
	15	1	10	10.00
	30	1	168	0.60

Table 11: Discordance by local site –Study 25

Antibiotic (µg/ml)	Sites and Frequency of discordance		No. of patients at each site	Discordance %
Isoniazid (0.2)	-	-	-	-
Isoniazid (1)	20	1	32	3.13
Rifampin (1)	20	1	32	3.13
Pyrazinamide (25)	25	2	11	18.18
Ethambutol (5)	54	1	7	14.29
	70	1	8	12.50
Streptomycin (2)	20	1	32	3.13
	61	1	6	16.67
	62	1	4	25.00

Table 12: Discordance by local site –Study 24

Antibiotic (µg/ml)	Sites and Frequency of discordance		No. of patients at each site	Discordance %
Isoniazid (0.2)	13	1	1	100.00
	14	1	1	100.00
	21	1	5	20.00
	22	1	4	25.00
	40	1	6	16.67
	59	1	3	33.33
Isoniazid (1)	40	1	6	16.67
	59	1	3	33.33
Streptomycin (2)	21	1	5	20.00
	22	1	4	25.00
	26	1	2	50.00
	40	2	6	33.33
Pyrazinamide (25)	22	1	4	25.00

Ethambutol (5)	-	-	-	-
Rifampin (1)	-	-	-	-

Table 13: Discordance by local site –Study 23

Antibiotic (µg/ml)	Sites and Frequency of discordance		No. of patients at each site	Discordance %
Isoniazid (0.2)	22	1	3	33.33
Isoniazid (1)	-	-	-	-
Streptomycin (2)	17	1	24	4.17
	20	1	15	6.67
	62	3	14	21.43
	68	1	8	12.50
Pyrazinamide (25)	17	4	24	16.67
	28	3	7	42.86
	53	1	2	50.00
Ethambutol (5)	-	-	-	-
Rifampin (1)	17	1	24	4.17
	22	1	3	33.33
	70	1	10	10.00

Table 14: Discordance by local site –Study 22

Antibiotic (µg/ml)	Sites and Frequency of discordance		No. of patients at each site	Discordance %
Isoniazid (0.2)	18	1	46	2.17
	20	1	40	2.50
	51	1	6	16.67
	53	1	5	20.00
	61	1	17	5.88
Isoniazid (1)	No Local lab results for this antibiotic			
Streptomycin (2)	17	1	8	12.50
	20	3	40	7.50
	51	1	6	16.67
	55	1	4	25.00
	59	2	9	22.22
Pyrazinamide (25)	13	1	9	11.11
	18	1	46	2.17

	51	1	6	16.67
	64	1	17	5.88
	65	1	11	9.09
Ethambutol (5)	18	3	46	6.52
	51	1	6	16.67
Rifampin (1)	13	1	9	11.11
	16	1	5	20.00
	17	1	8	12.50
	51	1	6	16.67
	59	1	9	11.11
	61	1	17	5.88
	64	1	17	5.88

Table 15: Study 29- Kappa statistic and interpretation

Antibiotic (µg/ml)	N	Simple Kappa Coefficient	Interpretation
INH (0.2)	303	0.9095	Very good agreement
INH (1)	97	0.8398	Very good agreement
RIF (1)	331	1	Perfect agreement
PZA (25)	239	0.3893	Fair Agreement
ETH (5)	331	0.2460	Fair Agreement
SM (2)	291	0.5792	Moderate agreement

Table 16: Study 28- Kappa statistic and interpretation

Antibiotic (µg/ml)	N	Simple Kappa Coefficient	Interpretation
INH (0.2)	311	0.9490	Very good agreement
INH (1)	120	0.9368	Very good agreement
RIF (1)	342	0.8875	Very good agreement
PZA (25)	264	0.5924	Moderate agreement
ETH (5)	339	**TP=0;FN=0;TN=337;FP=2;**No 2*2 table Calculated value=0	Not feasible to calculate Kappa values for non-square tables (using SAS);
SM (2)	300	0.7082	Good agreement

Table 17: Study 27- Kappa statistic and interpretation

Antibiotic (µg/ml)	N	Simple Kappa Coefficient	Interpretation
INH (0.2)	262	0.6543	Good agreement
INH (1)	111	0.7956	Good agreement
RIF (1)	315	0.6619	Good agreement
PZA (25)	248	0.5654	Moderate agreement
ETH (5)	315	1	Perfect Agreement
SM (2)	273	0.9505	Very good agreement

Table 18: Study 25- Kappa statistic and interpretation

Antibiotic (µg/ml)	N	Simple Kappa Coefficient	Interpretation
INH (0.2)	87	**TP=FP=FN=0;TN=87;** No 2*2 table; Calculated value=undefined	Not feasible to calculate Kappa values for non-square tables (using SAS); Since there is no discordance, kappa can be interpreted as 1
INH (1)	72	0.9861	Very good agreement
RIF (1)	134	**TP=FN=0; TN=133;FP=1;** No 2*2 table; Calculated value=0	Not feasible to calculate Kappa values for non-square tables (using SAS); There is discordance (due to FP), nevertheless a kappa of 0 is calculated.
PZA (25)	61	**TP=FP=0; TN=59;FN=2;** No 2*2 table; Calculated value=0	Not feasible to calculate Kappa values for non-square tables; There is discordance (due to FN), nevertheless a kappa of 0 is calculated.
ETH (5)	127	**TP=FN=0;FP=2;TN=125;** No 2*2 table; Calculated value=0	Not feasible to calculate Kappa values for non-square tables; There is discordance (due to FP), nevertheless a

			kappa of 0 is calculated
SM (2)	99	0.5587	Moderate agreement

Table 19: Study 24- Kappa statistic and interpretation

Antibiotic (µg/ml)	N	Simple Kappa Coefficient	Interpretation
INH (0.2)	33	0.4590	Moderate agreement
INH (1)	27	0.8508	Very good agreement
RIF (1)	43	**TP=FP=FN=0;TN=43;** No 2*2 table; Calculated=undefined	Not feasible to calculate Kappa values for non-square tables; Not feasible to calculate Kappa values for non-square tables (using SAS); Since there is no discordance, kappa can be interpreted as 1
PZA (25)	27	**TP=FP=0;TN=26;FN=1;** No 2*2 table Calculated=0	Not feasible to calculate Kappa values for non-square tables; There is discordance (due to FN), nevertheless a kappa of 0 is calculated
ETH (5)	43	**TP=FP=FN=0;TN=43;** No 2*2 table; Calculate=not defined	Not feasible to calculate Kappa values for non-square tables; Not feasible to calculate Kappa values for non-square tables (using SAS); Since there is no discordance, kappa can be interpreted as 1

SM (2)	31	0.5953	Moderate agreement

Table 20: Study 23- Kappa statistic and interpretation

Antibiotic (µg/ml)	N	Simple Kappa Coefficient	Interpretation
INH (0.2)	93	0.8517	Very good agreement
INH (1)	89	1	Perfect agreement
RIF (1)	137	**FP=TP=0;TN=134;FN=3;** No 2*2 table	Not feasible to calculate Kappa values for non-square tables; Not feasible to calculate Kappa values for non-square tables; There is discordance (due to FN), nevertheless a kappa of 0 is calculated
PZA (25)	78	1	Perfect agreement
ETH (5)	139	**TP=FN=FP=0;TN=139;** No 2*2 table; Calculated= not defined	Not feasible to calculate Kappa values for non-square tables; Not feasible to calculate Kappa values for non-square tables (using SAS); Since there is no discordance, kappa can be interpreted as 1
SM (2)	120	0.5472	Moderate Agreement

Table 21: Study 22- Kappa statistic and interpretation

Antibiotic (µg/ml)	N	Simple Kappa Coefficient	Interpretation
INH (0.2)	242	0.2806	Fair
INH (1)		No Local Lab DST results	-
RIF (1)	242	0.2077	Fair
PZA (25)	78	-0.0209	The two lab results agreed less than would be expected just by chance
ETH (5)	239	-0.0063	The two lab results agreed less than would be expected just by chance
SM (2)	214	0.6470	Good

- Poor agreement = Less than 0.20
- Fair agreement = 0.20 to 0.40
- Moderate agreement = 0.40 to 0.60
- Good agreement = 0.60 to 0.80
- Very good agreement = 0.80 to 1.00

Table 22: Overall Statistics for all studies

Study Number	% Discordance	Kappa Statistic	Kappa Interpretation	Sensitivity	Specificity	PPV
22	(29/303)*100= 9.57	0.2216	Fair Agreement	34.396 (3)	98.836 (6)	64.24 (3)
23	(18/142)*100= 12.67	0.8497	Very good agreement	59.86 (4)	99.848 (6)	95 (4)
24	(14/58)*100= 24.13	0.6350	Good agreement	86.22 (3)	88.06 (6)	81.59 (6)
25	(9/135)*100= 6.66	0.5587	Moderate agreement	100 (1)	98.86 (6)	40 (1)
27	(22/351)*100= 6.26	0.7712	Good agreement	70.51 (6)	99.73 (6)	88.315(6)
28	(17/382)*100= 4.45	0.8147	Very good agreement	80.318 (5)	99.48 (6)	84.856 (5)
29	(31/337)*100=9.19	0.6603	Good agreement	60.44 (6)	99.48(6)	91.775(6)
	Total Discordance % = (133/1708)*100= **7.786**%	**0.6444**(Average Values across all studies)	Good Agreement	Avg:70.24	Avg: 97.75	Avg:77.968

Table 23: Data cleaning methodology (record particulars) for all studies

Study number	No. of records in CDC dataset	No. of records in local dataset	No. of records is merged dataset (after exclusion of records with no match in either datasets)	No. of records in final dataset (after removal of duplicates and further cleaning using information in comments section)
29	402	473	359	333
28	522	421	435	342
27	374	323	351	321
25	158	150	149	135
24	50	92	50	43
23	191	168	172	142
22	1416	311	487	303 (PZA dataset:271)

Figure 1: Model diagram of methodology for data cleaning (All studies)

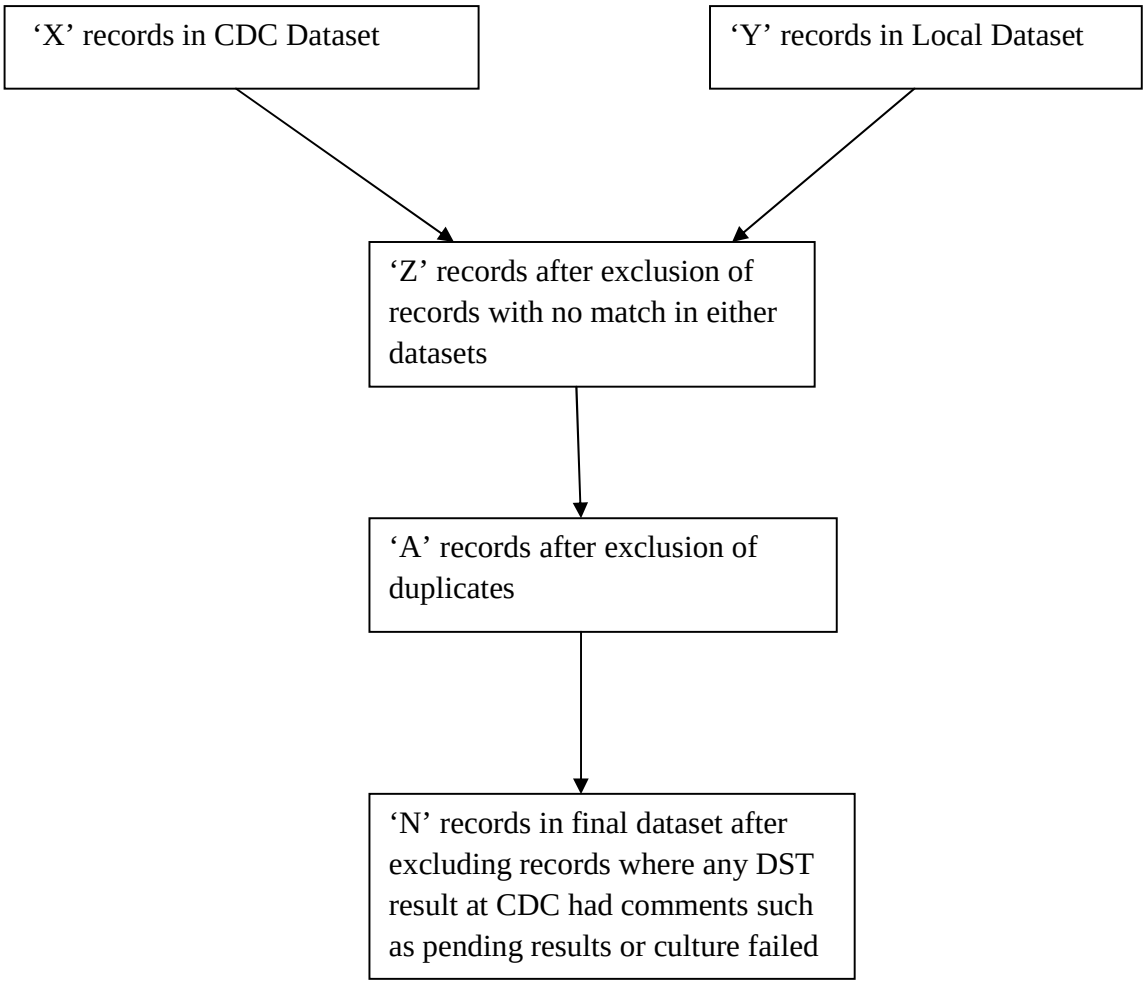


Figure 2: Box Plot- Positive predictive values (PPV) across studies

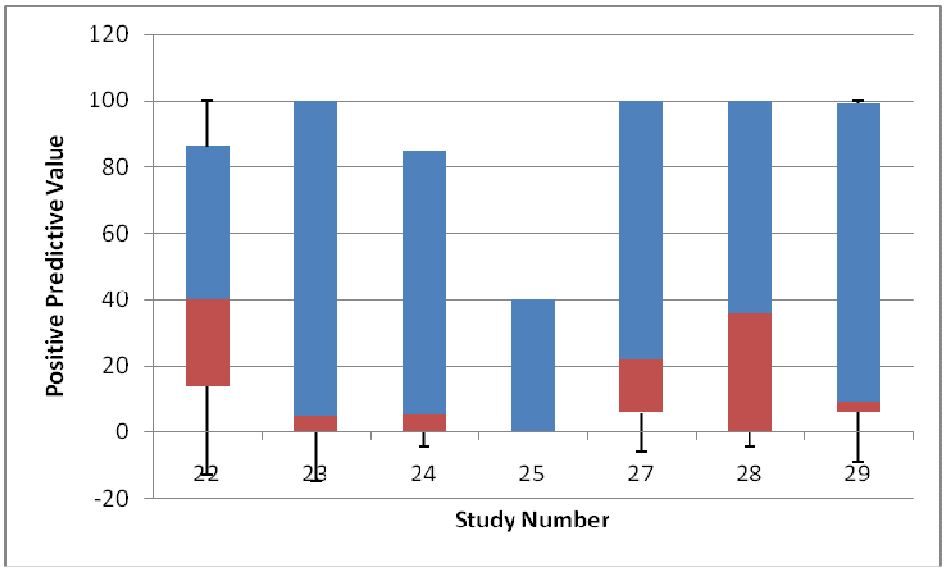
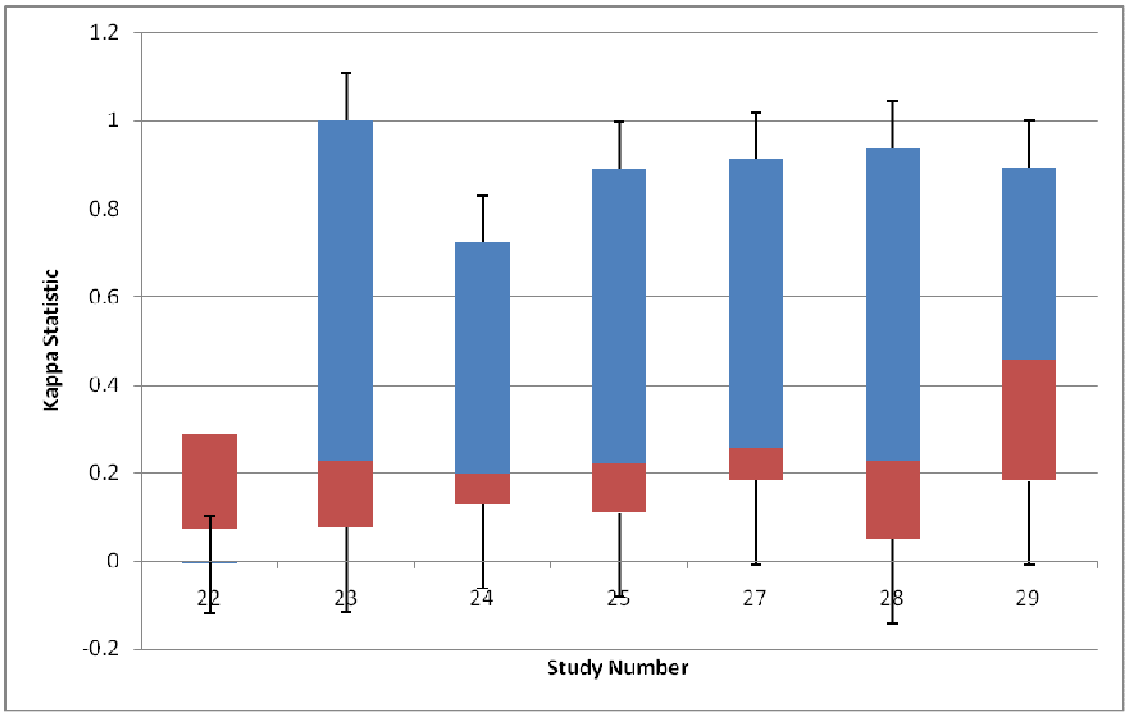


Figure 3: Box Plot- Kappa Statistics across studies



APPENDIX A

INSTITUTIONAL REVIEW BOARD

Mail: P.O. Box 3999
Atlanta, Georgia 30302-3999

In Person: Alumni Hall
30 Courtland St, Suite 217

Phone: 404/413-3500

Fax: 404/413-3504

January 25, 2011

Principal Investigator: Rothenberg, Richard B
Protocol Department: Institute of Public Health
Protocol Title: Concordance of Drug Susceptibility test data between CDC Mycobacteriology Laboratory and Local Public Health Laboratories in drug efficacy testing trials by TB trials Consortium, CDC.

Submission Type: Protocol H11285

Review Type: Exempt Review

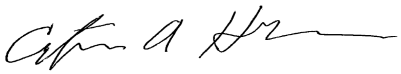
Approval Date: January 25, 2011

The Georgia State University Institutional Review Board (IRB) reviewed and approved your IRB protocol entitled Concordance of Drug Susceptibility test data between CDC Mycobacteriology Laboratory and Local Public Health Laboratories in drug efficacy testing trials by TB trials Consortium, CDC.. The approval date is listed above.

Exempt protocols do not require yearly renewal. However, if any changes occur in the protocol that would change the category of review, you must re-submit the protocol for IRB review. When the protocol is complete, a Study Closure Form must be submitted to the IRB.

Any adverse reactions or problems resulting from this investigation must be reported immediately to the University Institutional Review Board. For more information, please visit our website at www.gsu.edu/irb.

Sincerely,



Cynthia A. Hoffner, IRB Vice-Chair

Federal Wide Assurance Number: 00000129