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Tu My To Date

Implementation of the GenoType® MTBDR*plus* Assay in a Laboratory in Manila, Philippines:
An Analysis of the Effect on Time to Diagnostic Results and Treatment Initiation

By

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Master of Public Health

Epidemiology

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Tu My To

BA, University of Southern California, 2010

Thesis Committee Chair: Michael Goodman, MD, MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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Abstract

Implementation of the GenoType® MTBDR*plus* Assay in a Laboratory in Manila, Philippines:
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By Tu My To

Background: The steady rise in multidrug resistant tuberculosis (MDR TB) cases challenges global TB control efforts and strains national TB control programs. However, despite the increase, few patients are tested for drug susceptibility and enrolled in treatment regimens. Accurate and timely diagnosis of MDR TB is critical in mitigating its spread, but conventional tests (such as culture or sputum smear microscopy) are slow and do not provide drug susceptibility testing (DST). The WHO recommends that line probe assays (LPAs) be used for rapid TB detection and DST.

Methods: The GenoType® MTBDR*plus* LPA was implemented into routine TB testing in a mycobacteriology laboratory in Manila, Philippines. Patient enrollment was divided into validation (culture-based testing) and demonstration (LPA testing) phases and patients were tested for presence of TB and resistance to RIF and INH. Performance characteristics were calculated for LPA versus the gold-standard culture-based methods. Time from specimen collection to availability of diagnostic results and to treatment initiation were compared between the two phases. The association between patient characteristics and the two outcomes were also examined.

Results: The performance characteristics for detection of RIF-resistance, INH-resistance, and MDR TB status decreased from the validation to the demonstration phase. Although the time from sample collection to availability of diagnostic results was significantly shorter for the demonstration phase ($p<0.0001$), there was no significant difference for time to treatment initiation between the two phases ($p=0.09$).

Conclusion: This study suggests that the MTBDR*plus* assay may serve as a suitable and rapid test for public health practice that may reduce the waiting period for diagnostic results. However, appropriate and timely treatment depends on factors other than diagnostics, and TB control programs should be prepared to manage changes associated with the introduction a rapid diagnostic test.

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BACKGROUND

Tuberculosis (TB) is a highly infectious disease of global concern. The World Health Organization (WHO) estimated 8.8 million incident cases occurred in 2010, with 1.1 million deaths among TB patients who were HIV negative. TB was more commonly found among men than women, and was more likely to affect adults of economically productive ages (i.e. ages 15-59). Fifty-nine percent of all cases originated from Asia, with India and China accounting for 40% of all notified cases (1). In addition, the burden of TB in many countries was greatly exacerbated by the growing HIV epidemic (2, 3). About 1.1 million patients in 2010 were co-infected with both HIV and TB, and the number of deaths from HIV-associated TB was estimated to be 0.35 million (1).

Global efforts to control TB are challenged by the steady rise in multidrug-resistant TB (MDR TB), defined as resistance to at least rifampin (RIF) and isoniazid (INH). In 2008, an estimated 440,000 cases of MDR TB emerged globally, resulting in about 150,000 deaths. From all incident TB cases worldwide, about 3.6% are MDR TB cases, with a much higher frequency among previously treated patients (4, 5). The MDR TB epidemic also places financial strain on TB control programs, as it accounts for almost 70% of the national TB control burden for some countries (6).

Though management of MDR TB requires a large amount of resources, little is known about patients and their access to quality care (5). In fact, despite the increasing numbers of MDR TB cases in the past five years, less than 5% of all patients are tested for drug susceptibility and only 16% of all reported MDR TB patients are enrolled in treatment regimens (1). As a result, the majority of those who have MDR TB are unaware that they are infected and potentially contagious. This dilemma is fueling the MDR TB

epidemic, of which a large part is attributable to the ongoing transmission of resistant strains to susceptible persons, typically from unrecognized or inadequately treated patients infected with resistant strains and in large congregate settings (6, 7).

While accurate and timely diagnosis of MDR TB is critical in controlling the spread of disease, conventional diagnostic tests have important limitations. Culture, the gold standard method, is a slow process that takes weeks or even months for results to be obtained. Sputum smear microscopy (SSM) is valued for its affordability, but often requires multiple patient visits and is unable to test for drug susceptibility. Furthermore, SSM is characterized by low sensitivity, especially among HIV-positive patients (2, 6, 8-9). These deficiencies cause delays in accurate diagnosis and appropriate treatment, adversely affecting TB control programs and further contributing the MDR TB epidemic (6, 10). For these reasons, and in response to the growing problem of MDR TB, the WHO recommends rapid drug susceptibility testing (DST) for both INH and RIF or for RIF alone over conventional testing or no testing at time of TB diagnosis (11). By quickly delivering results needed to prescribe suitable treatment regimens, rapid DST before the start of treatment is a cost effective means to reduce the number of deaths and deter the transmission of MDR TB, as well as prevent the development of additional resistance (5, 11). However, developing a simple, fast, accurate, and effective diagnostic test with DST capabilities proves to be a limiting factor for TB control programs.

Recently, the search for rapid diagnostic tests with DST has shifted to include molecular methods that identify mutations in the *Mycobacterium tuberculosis* genome leading to drug resistance (12). One particular method, called line probe assay (LPA), is capable of reducing time to TB detection and DST results from months and weeks to

hours. A recent meta-analysis on two versions of a commercially available assay, the GenoType® MTBDR assays (both MTBDR and MTBDR*plus*), provided evidence for its use as suitable test for rapid detection of RIF and INH resistance. The pooled sensitivity for RIF resistance (98.1%; 95% confidence interval [CI]: 95.9-99.1) and specificity (98.7%, 95% CI: 97.3-99.4) were high and consistent in smear positive sputum specimens and culture-positive isolates. Sensitivity for INH resistance was lower (84.3%; 95% CI: 76.6-89.8) and more variable while specificity remained high (99.5%; 95% CI: 97.5-99.9) and more consistent (13). Overall, the GenoType® MTBDR assays proved to be excellent tests for detecting TB and its resistance against first line drugs.

In 2008, the WHO issued a policy statement recommending that line probe assays (LPAs) be used on sputum smear-positive specimens and *M. tuberculosis* culture isolates to quickly detect TB and the presence of RIF and/or INH resistance (14). The policy notes that LPAs do not replace the need for gold standard culture as conventional methods are still required to test smear-negative specimens as well as patients suspected to have extensively drug-resistant TB (XDR TB). Instead, LPAs may be incorporated into specific TB screening algorithms as a supplement to conventional tests that will provide more rapid results and will allow clinicians to make more informed, timely decisions on treatment regimens (15).

In response to the WHO policy, the Foundation for Innovative New Diagnostics (FIND), Centers for Disease Control and Prevention (CDC), and the Tropical Disease Foundation (TDF) collaborated on a demonstration project utilizing the GenoType® MTBDR*plus* assay in a high-burden mycobacteriology laboratory in Manila, Philippines. Sputum specimens collected from TB patients and suspects continued to be assessed

through SSM, culture, and DST per the routine diagnostic algorithm. However, in addition to these routine services, acid-fast bacilli (AFB) smear-positive specimens from MDR TB suspects were also screened for INH and RIF resistance using the MTBDR*plus* assay.

A previous study validating the use of the MTBDR*plus* assay in Vietnam found it to have high specificity and positive predictive value, warranting its use in the national TB control program (16). Likewise, another study set in a high volume laboratory in Southern China also found the assay to be a valuable addition to TB diagnostic algorithms and a suitable method for rapid drug-resistant TB detection (17). Similarly, the objective of this analysis is to use data collected from the demonstration project in Manila to evaluate the GenoType® MTBDR*plus* assay's performance against conventional culture-based methods used to detect MDR TB and to assess the differences in time to availability of diagnostic results for both methods while controlling for patient-related characteristics. Furthermore, because treatment for MDR TB relies on availability of DST results, the effect of using the MTBDR*plus* assay versus conventional culture-based methods on time to treatment initiation will also be evaluated.

METHODS

Data Source

The LPA demonstration project was implemented in a mycobacteriology laboratory in Manila, Philippines as part of the routine testing algorithm for patients suspected of having MDR TB in order to compare the performance of molecular detection against conventional culture-based methods (liquid media and solid media). The project was divided into two phases: the validation phase, which enrolled patients from September 2008 to March 14, 2009, and the demonstration phase, which enrolled patients for one year starting March 15, 2009.

The validation and quality assurance of the MTBDR*plus* assay was carried out by testing each specimen with conventional culture-based methods and with LPA. LPA results were not reported to clinicians during this validation phase. Once the assay was validated, the project then proceeded onto the demonstration phase. During this second phase, all eligible patient specimens were tested with LPA for MDR TB, and those with resistance detected were retested with culture-based DST. Both LPA and culture-based DST results were reported to clinicians during the demonstration phase. For the purposes of this analysis, the validation phase will be equated with results from culture-based methods and the demonstration phase will be equated with results obtained from LPA.

Patients enrolled for both phases included those who: 1) provided at least one sputum smear positive specimen and 2) were at risk for MDR TB due to a failing current treatment, a previous TB treatment, or from contact with individuals infected with drug-resistant TB. In instances where patients were tested in both phases or were tested multiple times, only the first valid result was used for analytic purposes; any duplicates

were removed from the analysis. For example, a patient who submitted specimens during both the validation and the demonstration phases provided data for only the validation phase as this came first chronologically. Additional patient information obtained included age, sex, and previous TB treatment. Age was divided into three categories with relatively equal numbers of suspects in each: less than or equal to 35 years, 36-50 years, and 51 or more years. Prior treatment to TB was dichotomized into ‘yes’ or ‘no’. Information on MDR TB treatment initiation based on diagnostic results from the study was also available for a small portion of MDR positive patients.

Performance Characteristics

Performance characteristics were assessed to determine the sensitivity, specificity, positive and negative predictive values of LPA against gold-standard culture-based methods for the detection of: 1) resistance to RIF, 2) resistance to INH, and 3) MDR TB, defined as resistance to both RIF and INH. Based on reagent availability and the testing algorithms in the laboratory, a combination of liquid media (Becton Dickenson Mycobacteria Growth Indicator Tube, or MGIT) and solid media (Middlebrook 7H10 Agar) was used for phenotypic DST. Both methods are considered to be gold standards, and phenotypic DST performed by either method served as the reference standard in this analysis. These were compared to results provided by LPA testing for detection of RIF and INH resistance along with MDR TB (as determined by LPA). All calculations were based on patients with both valid LPA and culture test readings. As MDR TB was determined using results from both RIF and INH susceptibility tests, observations missing either or both of the two were deleted when calculating performance characteristics for detection of MDR TB.

Survival Analyses

Kaplan-Meier (KM) curves were constructed to examine differences in the specimen collection date (SCD) to result interval for culture-based DST (validation phase) versus LPA (demonstration phase). The SCD-to-result interval was determined as the number of days from date of specimen collection to the date of either culture-based DST or LPA result read. KM curves were also constructed to examine differences in time to treatment initiation, where the treatment regimen included at least one second-line TB drug. The interval was defined as the number of days between date of specimen collection and to the date of treatment initiation for each subject.

Cox regression models were used to examine the association between various patient characteristics and each of the two outcomes – test-to-result interval and time to treatment initiation. Independent variables of interest – study phase, age, sex, and previous TB treatment – were assessed for violations of the proportional hazards (PH) assumption using the log-log survival curves for each variable. The Cox regression model was used only for variables that did not violate the PH assumption (the log-log curves did not cross). For variables violating the assumption, the stratified Cox model was used. Associations between predictor variables and each study outcome were expressed as adjusted hazards ratios (HR) and reported along with the corresponding 95% confidence intervals (CIs).

All analyses were performed using SAS v9.3 for Windows (SAS Institute, Inc., Cary, NC) statistical software package.

RESULTS

As shown in Figure 1, there were 1163 patients enrolled into the study for MDR TB laboratory testing. Of these, 1015 (87.3%) patients had valid test results for their respective phases – 317 had valid culture-based DST results in the validation phase and 698 had valid LPA results in the demonstration phase. From this group of 1015 participants, a total of 597 patients were found to be MDR TB positive – 202 in validation phase and 395 in demonstration phase. However, only 103 (17.3%) of all MDR TB positive patients had treatment data available – 53 in validation phase and 50 in demonstration phase.

According to Table 1, the distributions of patient characteristics in either of the two phases were very similar. Both phases included about 66-68% of male patients. The majority of patients were 50 years of age or younger; those who were older than 50 years accounted for 24.5% and 28.8% of patients in the validation and demonstration phase, respectively. Most patients enrolled in this study had been previously treated for TB: 94.2% of validation phase patients and 90.3% of demonstration phase patients.

Performance Characteristics

Analysis of the LPA test performance against culture-based DST (the gold standard) indicated that LPA was highly sensitive in detecting resistance against RIF, where both phases had a sensitivity of about 97% (95% CI: 92.4-99.2 for validation phase, 93.4-98.4 for demonstration phase). However, as shown in Table 2, the test was less specific – 89.4% (95% CI: 79.4-95.6) for validation phase and 73.1% (95% CI: 64.6-80.5) for demonstration phase. Sensitivity for LPA was lower for detection of INH resistance; around 90% in each study phase (95% CI: 86.0-95.7 for validation phase, 85.8-93.1 for

demonstration phase). The specificity for detection of INH resistance was different for both phases – 92.3% (95% CI: 81.5-97.9) for validation phase but 73.5% (95% CI: 63.6-81.9) for demonstration phase. Lastly, sensitivity for LPA detection of MDR status was similar for both phases: 90.4% (95% CI: 83.8-94.9) and 88.7% (95% CI: 83.9-92.4) for validation and demonstration, respectively. Again, however, LPA specificity was quite different: 88.9% (95% CI: 79.3-95.1) for validation phase and 75.5% (95% CI: 67.7-82.2) for demonstration phase. The positive predictive value in the validation phase for MDR TB detection was 93.4% (95% CI: 87.4-97.1) but lower in the demonstration phase at 85.4% (95% CI: 80.4-89.6). The negative predictive values for MDR TB detection were similar: 84.2% (95% CI: 74.0-91.6) in the validation phase versus 80.4% (95%: 72.8-86.7) in the demonstration phase.

Survival Analyses

Kaplan-Meier curves showing SCD-to-result intervals for validation and demonstration phases are shown in Figure 2. The median time to diagnostic results for the validation phase was 38 days, with an interquartile range (IQR) between 26 and 96 days. The demonstration phase, on the other hand, had much shorter turnaround time: the median was 11 days and the IQR of 7-20 days. The SCD-to-result interval was significantly shorter ($p<0.0001$) in the demonstration phase than in the validation phase (Figure 2).

Figure 3 depicts the Kaplan-Meier curves for time to treatment initiation for MDR TB positive patients in both validation and demonstration phases. The median time to treatment initiation (IQR) estimates were 44.5 (31-124) days and 48.5 (32-79) days for the validation and demonstration phase patients, respectively. The difference between

two phases for time to treatment initiation (Figure 3) was less pronounced than that for test-to-result interval (Figure 2) and was not statistically significant (p=0.09).

Table 3 provides the results of multivariate Cox regression analyses, which evaluated the association between study phase (validation versus demonstration) and SCD-to-result interval while controlling for potential confounders. Examination of the log-log curves for the variables of interest (study phase, age, sex, and previous treatment) demonstrated that the proportional hazards assumption was violated by all except the main exposure (validation versus demonstration phase). While not controlling for any other covariates, the demonstration phase (LPA testing) when compared to validation phase (culture-based DST testing) was associated with a statistically significantly elevated HR of 6.19 (95% CI 5.20-7.37, p-value<0.0001). In order to control for the other variables, the stratified Cox regression models were used. With phase as the main exposure, the effects of covariates were controlled by stratifying on age, sex, and previous treatment separately, in various combinations (age-gender, age-previous treatment, and gender-previous treatment), and all together (age-sex-previous treatment). As shown in Table 3, the HRs for all multivariable stratified models were within 10% of the HR for the unadjusted model and were all statistically significant (p-value<0.0001).

Examination of the log-log curves for variables study phase, age, sex, and previous treatment for time to treatment survival data showed that all variables violated the proportional hazards assumption. Therefore, Cox regression analyses for time to treatment initiation were not performed.

DISCUSSION

Much attention has been directed to developing and evaluating new diagnostics for TB and MDR TB detection, particularly molecular-based tests. Since the WHO recommended the use of LPAs for these purposes, assays such as the GenoType® MTBDR*plus* have been implemented at the population level to assess their performance in public health settings in order to compare them to traditional methods such as culture-based tests. One of the objectives of the demonstration project presented in our study was to introduce the MTBDR*plus* assay into the mycobacteriology laboratory's routine TB testing algorithm in order to determine its performance characteristics in a high-volume setting. As the results indicate, the sensitivity for detecting INH resistance was lower than that for detecting RIF resistance, a pattern also found in the meta-analysis evaluating MTBDR/MTBDR*plus* performance characteristics, although the actual numbers in this particular analysis was higher than those found in our study (13). Our results also show that the assay's specificity for detection of both RIF and INH resistance was much lower than the corresponding estimates reported in the above-mentioned meta-analysis.

The discrepancies between what was found in the literature and what was observed in our study underline a fundamental problem often encountered when a new diagnostic test is implemented for use at the population level: while the test may perform well in research settings, its performance characteristics are likely to change in actual public health practice (18). Although innovative diagnostic tests such as MTBDR*plus* offer promising improvements to existing programs, a key challenge is to adapt these tests for use in high-burden settings (19). In order to fully and correctly utilize these diagnostic tests, constant monitoring is required through routine quality assurance and

proficiency testing, continued training of personnel, and implementation of standard operating procedures (20, 21).

Evaluation of MTBDR*plus* as a suitable diagnostic test extends beyond sensitivity and specificity. Much emphasis has been placed upon developing a method that is not only capable of DST, especially for simultaneous detection of RIF and INH resistance, but will also provide results more quickly compared to slower and more traditional culture-based tests (2, 5, 6, 19). The MTBDR*plus* assay is capable of fulfilling such demands, and our study has indeed found that incorporating the assay into routine TB testing algorithm significantly reduced the wait time for diagnostic results. When the validation phase (culture-based testing) was compared to the demonstration phase (LPA testing), patient samples tested with LPA had a significantly shorter time span between sample collection and availability of results for detection of RIF and INH resistance. Such findings are in agreement with current literature that advocates the use of LPAs due to their rapid turn-around-time.

TB control efforts encompass much more than improved methods of diagnosis. Also of critical importance is providing TB and MDR TB patients with adequate and appropriate treatment. Those who are diagnosed must be treated and, in the case of highly contagious TB and MDR TB, earlier treatment can disrupt the transmission of the disease to susceptible people. This is particularly true for MDR TB, where it has been suggested that acquisition of drug resistant infection may result mostly from transmission of resistant strains (6). Rapid diagnostic tests such as LPAs are promising because they allow clinicians to make timely decisions on appropriate treatment regimens for patients,

rather than allowing treatment initiation to be delayed due to the slow progress of conventional methods that may or may not provide DST.

The literature assessing the impact of LPAs or other rapid diagnostic tests on time to treatment initiation in comparison to conventional culture-based methods is sparse. Our study sought to address this concern by comparing time to treatment initiation for those tested with conventional culture-based methods versus those tested with LPA, and found no significant difference. The lack of a difference between these two groups confirms that many other factors influence treatment initiation in addition to the timeliness of diagnosis. Follow-up with the mycobacteriology laboratory revealed that there was a shortage of medications available and many MDR TB positive patients were unable to initiate treatment. This observation was confirmed in our data as only a small percentage of all MDR TB positive study subjects were included in the survival analysis for treatment initiation.

Our findings highlight an important problem facing TB control programs. As innovative diagnostic tests are introduced into public health settings and as the number of diagnosed patients begins to grow, TB control programs need to be equipped to handle this increase by developing the necessary infrastructure for adequate patient treatment and management (6). Demand for international funding will rise, and there will be greater need for medications, including expensive second and third line drugs, used to treat newly diagnosed MDR TB patients. Therefore, an important matter to consider should be whether or not implementing new tests will result in any significant improvement in patient care and outcome (22). This does not mean that advances in TB diagnostics are not important. Rather, TB and MDR TB elimination should be considered as a multi-fold

approach where diagnostics and patient care and management are viewed as interdependent determinants of the overall success.

Although our study defined time to availability of diagnostic results and time to treatment initiation as the time span between sample collection and event occurrence, we did not consider delays that occur after sample collection and before test initiation. Such delays are unrelated to test performance and are instead attributable to problems of sample shipping or laboratory work flow. Our study also did not address the possibility of untimely reporting of results to clinicians, which would have caused additional delays in treatment initiation and reduced the effect of LPA testing. These problems that affect day-to-day management of the TB control programs need to be considered before drawing conclusions about test-to-result and test-to-treatment intervals (6).

CONCLUSION

The implementation of the MTBDR*plus* line probe assay offers promising results, and the assay may serve as a suitable diagnostic test for public health practice. It provides rapid testing for TB and MDR TB, and significantly reduces the length of time needed before results become available when compared to culture based methods. However, because there was no significant effect on time to treatment initiation, it is important to consider other factors that also affect patient care. TB control programs should be prepared to handle the consequences of increased numbers of diagnosed patients once rapid diagnostic tests are introduced, lest the effects of improved diagnostics become mitigated by lack of treatment availability.

REFERENCES

1. World Health Organization. *WHO Report 2011: Global Tuberculosis Control*. Geneva, Switzerland: World Health Organization; 2011.
2. Weyer K, Carai S, Nunn P. Viewpoint TB Diagnostics: What Does the World Really Need? *J Infect Dis*. 2011; 204:1196-1202.
3. Wells CD, Cegielski JP, Nelson LJ, *et al*. HIV Infection and Multidrug-Resistant Tuberculosis – The perfect storm. *J Infect Dis*. 2007; 196.
4. World Health Organization. *Multidrug and extensively drug-resistant TB (MXDR-TB): 2010 Global Report on Surveillance and Response*. Geneva, Switzerland: World Health Organization; 2010.
5. Falzon D, Jaramillo E, Schunemann HJ, *et al*. WHO guidelines for the programmatic mismanagement of drug- resistant tuberculosis: 2011 update. *Eur Respir J*. 2011; 38:516-528.
6. Hoek KGP, Van Rie A, van Helden PD, Warren RM, Victor TC. Detecting Drug-Resistant Tuberculosis: The Importance of Rapid Testing. *Mol Diagn Ther*. 2011; 15(4):189-194.
7. Nardell E, Dharmadhikari A. Turning off the spigot: reducing drug-resistant tuberculosis transmission in resource-limited settings. *Int J Tuberc Lung Dis*. 2010; 14(10):1233-1243.
8. Lemaire JK, Casenghi M. New diagnostics for tuberculosis: fulfilling patient needs first. *J Int AIDS Soc*. 2010; 13:40.
9. Keeler E, Perkins MD, Small P, *et al*. Reducing the global burden of tuberculosis: the contribution of improved diagnostics. *Nature*. 2006; 444(Suppl 1):49-57.

10. Yimer SA, Hansen CH, Bjune GA. The perspective of private practitioners regarding tuberculosis case detection and treatment delay in Amhara Region, Ethiopia: a cross-sectional study. *BMC Research Notes.* 2011; 4:285.
11. World Health Organization. *Guidelines for the programmatic management of drug resistant tuberculosis: 2011 Update*. Geneva, Switzerland: World Health Organization; 2011.
12. O'Grady J, Maeurer M, Mwaba P, *et al*. New and improved diagnostics for detection of drug-resistant pulmonary tuberculosis. *Curr Opin Pulm Med*. 2011; 17:134-141.
13. Ling DI, Zwerling AA, Pai M. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. *Eur Respir J*. 2008; 32:1165-1174.
14. World Health Organization. *Molecular Line Probe Assays for Rapid Screening of Patients at Risk of Multidrug-Resistant Tuberculosis (MDR-TB): Policy Statement*. Geneva, Switzerland: World Health Organization; 2008.
15. World Health Organization. *A Roadmap for Ensuring Quality Tuberculosis Diagnostics Services Within National Laboratory Strategic Plans*. Geneva, Switzerland: World Health Organization; 2010.
16. Huyen MNT, Tiemersma EW, Nguyen TNL, *et al*. Validation of the GenoType MTBDR*plus* assay for diagnosis of multidrug resistant tuberculosis in South Vietnam. *BMC Infectious Diseases.* 2010; 10:149.
17. Zhang L, Ye Y, Duo L, *et al*. Application of the GenoType MTBDR*plus* in rapid detection of the *Mycobacterium tuberculosis* complex as well as its resistance to isoniazid and rifampin in a high volume laboratory in South China. *Mol Biol Rep*. 2011; 38:2185-2192.

18. Small PM, Perkins MD. More rigour needed in trials of new diagnostic agents for tuberculosis. *Lancet*. 2000; 356:1048-1049.

19. Stop TB Partnership – New Diagnostics Working Group. *Pathways to better diagnostics for tuberculosis*: *a blueprint for the development of TB diagnostics.* Geneva, Switzerland: World Health Organization; 2009.

20. Asencios L, Yale G, Yagui M, *et al*. Programmatic implementation of rapid DST for *Mycobacterium tuberculosis* in Peru. *Int J Tuberc Lung Dis*. 2008; 12(7):743-749.

21. Shulgina MV, Malakhov VN, Hoffner SE, Haile M, Wright A. Results of the external quality assessment of *Mycobacterium tuberculosis* drug susceptibility testing in Russia, 2005-2007. *Int J Tuberc Lung Dis*. 2009;13(10):1294-1300.

22. Pai M, Minion J, Steingart K, Ramsay A. New and improved tuberculosis diagnostics: evidence, policy, practice, and impact. *Curr Opin Pulm Med*. 2010; 16:271-284.

TABLES AND FIGURES

Figure 1. Flow diagram depicting number of patients at various stages of the study, including their separation in validation and demonstration phase.

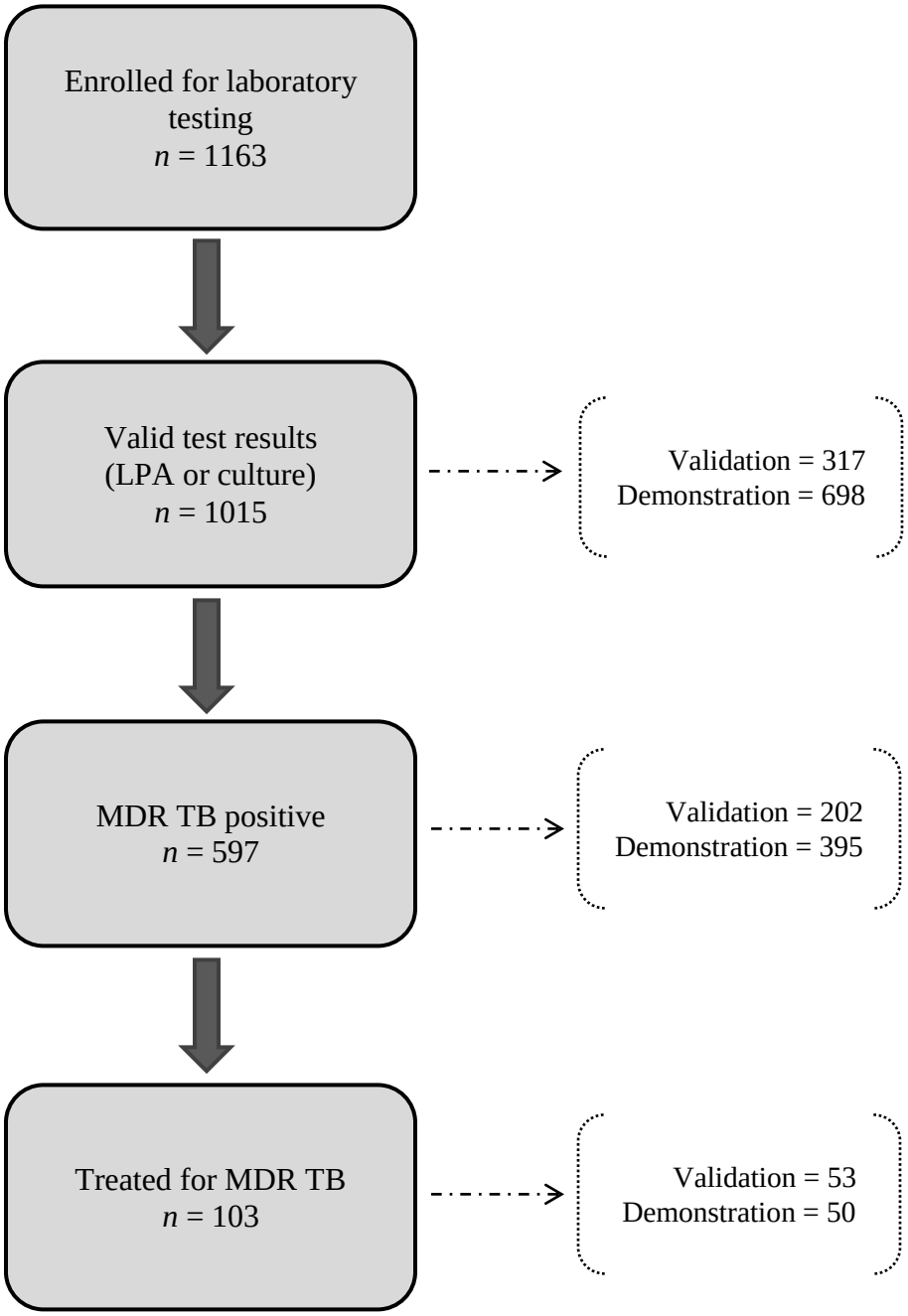

Table 1. Patient characteristics of those who were enrolled into the study and had valid laboratory test results, separated in validation and demonstration phase accordingly.

	Phase	
	Validation	**Demonstration**
	culture-based *n (%)*	*LPA-based* *n (%)*
Total	317	698
Sex[a]		
Male	210 (66.7)	464 (67.9)
Female	105 (33.3)	219 (32.1)
Age *(years)*[b]		
≤ 35	121 (38.6)	222 (32.7)
36-50	116 (36.9)	262 (38.5)
≥ 51	77 (24.5)	196 (28.8)
Previous TB Treatment[c]		
Yes	294 (94.2)	617 (90.3)
No	18 (5.8)	66 (9.7)
MDR TB		
Yes	202 (63.7)	395 (56.6)
No	115 (36.3)	303 (43.4)

[a] missing 2 observations for validation phase and 15 observations for demonstration phase
[b] missing 3 observations for validation phase and 18 observations for demonstration phase
[c] missing 5 observations for validation phase and 15 observations for demonstration phase

Table 2. Performance characteristics for LPA testing versus gold standard culture based methods (either liquid or solid media), separated by validation and demonstration phase.

Validation Phase			
Parameter (95% CI)	**Rifampin**	**Isoniazid**	**MDR TB**
Sensitivity	97.0 (92.4-99.2)	91.7 (86.0-95.7)	90.4 (83.8-94.9)
Specificity	89.4 (79.4-95.6)	92.3 (81.5-97.9)	88.9 (79.3-95.1)
PPV	94.8 (89.5-97.9)	97.1 (92.7-99.2)	93.4 (87.4-97.1)
NPV	93.7 (84.5-98.2)	80.0 (67.7-89.2)	84.2 (74.0-91.6)

Demonstration Phase			
Parameter (95% CI)	**Rifampin**	**Isoniazid**	**MDR TB**
Sensitivity	96.5 (93.4-98.4)	89.9 (85.8-93.1)	88.7 (83.9-92.4)
Specificity	73.1 (64.6-80.5)	73.5 (63.6-81.9)	75.5 (67.7-82.2)
PPV	87.5 (83.1-91.2)	90.9 (86.9-93.9)	85.4 (80.4-89.6)
NPV	91.4 (84.2-96.0)	71.3 (61.4-79.9)	80.4 (72.8-86.7)

Figure 2. Kaplan-Meier curves evaluating the difference in time to availability of diagnostic results for validation (culture-based testing) versus demonstration phase (LPA testing).

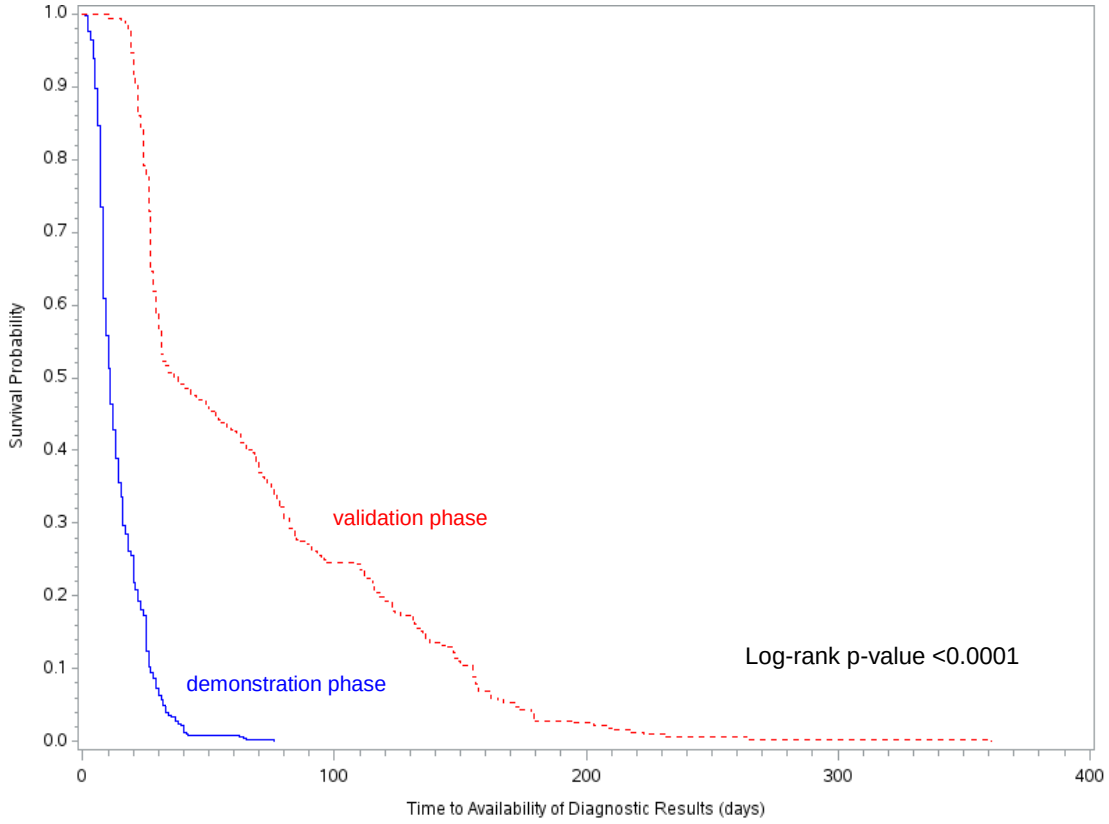

Figure 3. Kaplan-Meier curves evaluating the difference in time to treatment for validation (culture-based testing) versus demonstration phase (LPA testing).

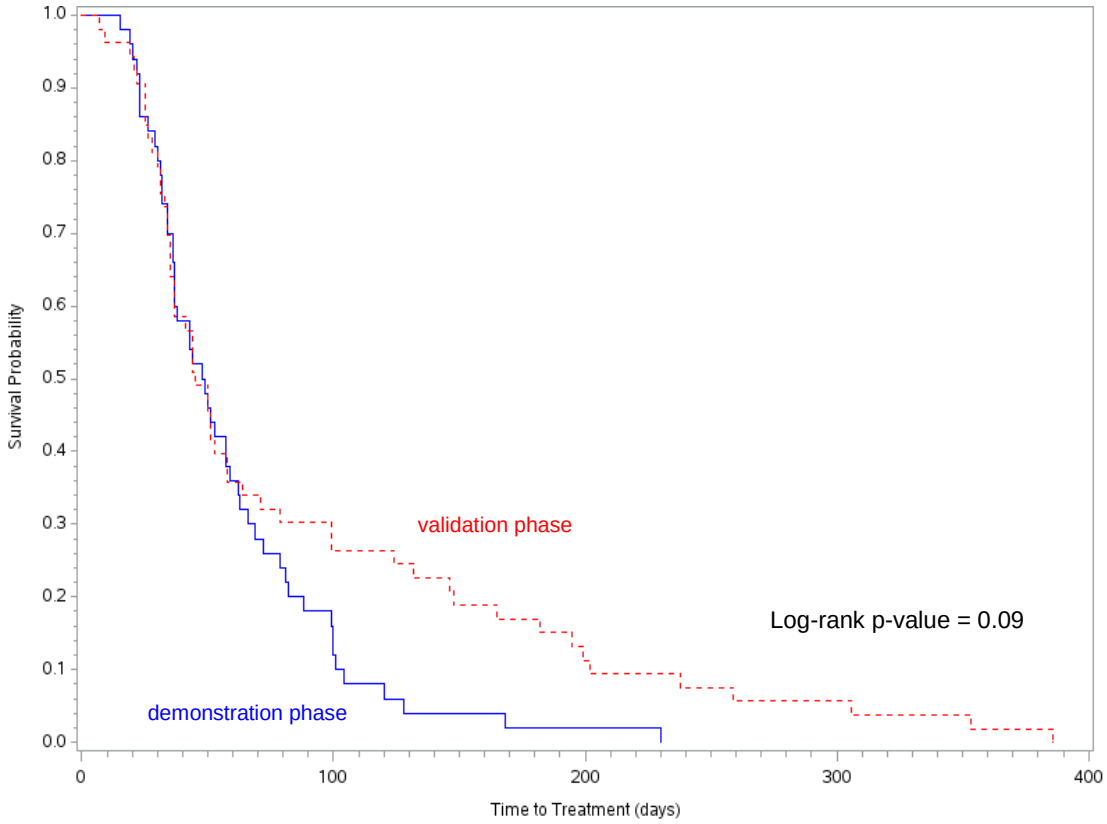

Table 3. Cox regression models evaluating the association between study phase (LPA testing versus culture) and test-to-result interval time to availability of diagnostic results, controlling for various patient characteristics by stratification*.

	Hazards Ratio	**95% Confidence Interval**	**p-value**
Unadjusted	6.19	5.20-7.37	<.0001
Adjusted for			
Age	6.11	5.12-7.29	<.0001
Sex	6.11	5.13-7.28	<.0001
Previous Treatment	6.28	5.26-7.49	<.0001
Age and Sex	6.05	5.07-7.22	<.0001
Sex and Previous Treatment	6.22	5.21-7.42	<.0001
Age and Previous Treatment	6.22	5.21-7.44	<.0001
Age, Sex, and Previous Treatment	6.13	5.12-7.33	<.0001

* Stratification was used because none of the covariates met the proportional hazards assumption

APPENDICES

Appendix A. Emory IRB Letter of Approval.

TO: Tu My To
Principal Investigator
Public Health

DATE: November 9, 2011

RE: **Expedited Approval**
IRB00053226
Analyzing the effectiveness and clinical impact of the GenoType® MTBDRplus assay for presumptive MDR TB diagnosis in smear-positive specimens from patients in the Philippines

Thank you for submitting a new application for this protocol. This research is eligible for expedited review under 45 CFR.46.110 and/or 21 CFR 56.110 because it poses minimal risk and fits the regulatory category F(5) as set forth in the Federal Register. The Emory IRB reviewed it by expedited process on 11/7/2011 and granted approval effective from **11/7/2011** through **11/6/2012**. Thereafter, continuation of human subjects research activities requires the submission of a renewal application, which must be reviewed and approved by the IRB prior to the expiration date noted above. Please note carefully the following items with respect to this approval:

- A waiver of informed consent has been granted for this study

Any reportable events (e.g., unanticipated problems involving risk to subjects or others, noncompliance, breaches of confidentiality, HIPAA violations, protocol deviations) must be reported to the IRB according to our Policies & Procedures at www.irb.emory.edu, immediately, promptly, or periodically. Be sure to check the reporting guidance and contact us if you have questions. Terms and conditions of sponsors, if any, also apply to reporting.

Before implementing any change to this protocol (including but not limited to sample size, informed consent, study design, you must submit an amendment

request and secure IRB approval.

In future correspondence about this matter, please refer to the IRB file ID, name of the Principal Investigator, and study title. Thank you

Sam Roberts, CIP
Research Protocol Analyst
This letter has been digitally signed