Mycobacterial Diseases
ISSN: 2161-1068

Uwimana et al., Mycobact Dis 2017, 7:3
DOI: 10.4172/2161-1068.1000247

Research Article Open Access

Performance Assessment of the GenoType®MTBDR*plus* Assay for Rapid Detection of Multidrug-Resistant Tuberculosis among Clinical Isolates in Low Tuberculosis Burden Setting

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Rec date: July 14, 2017, **Acc date:** July 28, 2017, **Pub date:** August 3, 2017

Abstract

Introduction: New molecular assays for rapid Multidrug-Resistant Tuberculosis (MDR-TB) detection continue to be developed. Rwanda has recently introduced the line probe assay for early TB and MDR-TB diagnosis. We aimed to assess the performance of GenoType®MTBDR*plus* test before its implementation in the routine testing.

Methods: Sputum samples from suspected MDR-TB patients received and processed at Rwanda National Reference Laboratory from 2010-2012 were included in this study. The performance of Genotype®MTBDR*plus* assay was evaluated versus the standard phenotypic conventional Drug Susceptibility Testing (DST) on Lowenstein Jensen. Sensitivity, specificity and predictive values (positive and negative) were calculated. Statistical analyses were performed using Epi Info version 3.5.3. P-values were derived from χ^2 tests applying Fisher Exact where appropriate.

Results: A total of 1548 participants were enrolled in this study, 463 (29.9%) were from new patients and 1085 (70.1%) patients were from retreated patients. The GenoType®MTBDR*plus* assay correctly identified 37 of 39 Isoniazid resistant strains; 33 of 36 Rifampicin resistant; and 30 of 32 MDR-TB strains for both tests. Compared to the reference standard, the sensitivity of the GenoType®MTBDR*plus* assay was 94.8% (95% CI: 79.2-99.2%) to detect Isoniazid resistance, 91.7% (95% CI: 77.5-98.2%) for Rifampicin and 93.8% (95% CI: 79.2-99.2%) for the combination of both, MDR-TB. The specificity was 99.3% for Isoniazid, 98.6% for Rifampicin and 99% for MDR-TB. Positive Predictive Value of GenoType®MTBDR*plus* assay was 96.8% for MDR-TB and its Negative Predictive value 98.6%. The GenoType®MTBDR*plus* performed well in identifying MDR-TB.

Conclusion: GenoType®MTBDR*plus* assay is a rapid and reliable test in detecting MDR-TB cases in Rwanda. Therefore, GenoType®MTBDR*plus* assay can be recommended for detecting MDR-TB in our setting to speed out MDR-TB detection in order to institute early treatment.

Keywords GenoType®MTBDR*plus* assay; Multi-Drug Resistant Tuberculosis; *Mycobacterium Tuberculosis* complex; Drug Susceptibility Testing

Background

Tuberculosis (TB) especially Multi-Drug Resistant Tuberculosis (MDR-TB) is still considered as a major public health concern in developing countries. The World Health Organization (WHO) estimates that one third of world's population is infected with TB whereby 9.2 million and 7.8 million of new TB patients occur annually in Asia and Sub-Saharan Africa. The spread of TB and MDR-TB was enhanced by the occurrence of HIV infection. The risk of developing TB is 5–15% per year in HIV-TB infected individuals (Global Tuberculosis Control [1,2]. About 14% of the global burden of new MDR-TB cases is found in Africa [3,4]. The internationally accepted gold standard for MDR-TB diagnosis is the detection of mycobacteria growth in cultures inoculated either on solid media Lowenstein Jensen (L-J medium) or liquid medium in Mycobacteria Growth Indicator Tube (MGIT): BACTEC MGIT960, Becton Dickinson, containing antibiotics even if it takes several weeks or months to obtain Drugs Susceptibility Tests (DST) results [3]. Due to delays in obtaining DST results to institute early treatment and case management, a rapid technique is needed to diagnose TB and MDR-TB. WHO recommends that countries should expand their capacity for culture-based DST and move to the new molecular based assays for diagnosing drug resistance [5,6].

In Rwanda, new molecular tools for rapid diagnosis of MDR-TB were recently introduced: as Genotype®MTBDR*plus* in 2010 and Xpert MTB/RIF in 2012. The GenoType®MTBDR*plus* assay has been used as a tool for early TB and MDR-TB diagnosis to support the Programmatic Management of Drug Resistant Tuberculosis in the country to institute early treatment. It has been shown that GenoType®MTBDR*plus* assay is the ideal technique to diagnose TB and MDR-TB and it accelerates the laboratory results for MDR-TB

treatment and control [7,8]. Although the GenoType®MTBDR*plus* assay has been validated in different settings with high burden of tuberculosis like in Thailand, Asia and in Africa like in South Africa [7,9], there is a need to evaluate its performance to ensure the acceptable performance in other settings with low TB burden like Rwanda. We aimed to assess the performance of GenoType®MTBDR*plus* assay to detect Rifampicin and Isoniazid resistance. We compared its performance to the phenotypic drug susceptibility testing DST based method on L-J medium by assessing its sensitivity, specificity and predictive values (positive and negative) in diagnosing MDR-TB.

Material and Methods

Setting, population and design of the study

The present study was conducted at the National Reference Laboratory (NRL) located in Kigali city, Rwanda, in the Microbiology unit. The NRL is the main laboratory in the country which performs specialized tests for TB such as culture, DST, and molecular assay for early MDR-TB diagnosis. NRL acts also as a referral laboratory involved performing quality assurance in the TB network at quarterly basis for all district hospitals in the country. Between 2010 and 2012, GenoType®MTBDR*plus* assay was performed simultaneously on sputum specimens and clinical isolates obtained from 1548 patients from all district hospitals submitted to the NRL for routine culture and drug susceptibility testing to monitor MDR-TB. Sputum samples or *Mycobacterium Tuberculosis* Complex (MTBC) isolates obtained after inoculating L-J medium were used to detect drug susceptibility patterns. This study was conducted after getting ethical approval from School of Public Health.

A written permission was issued for allowing the researchers to conduct this research. Permission was obtained from the National Reference Laboratory and RBC/IHDPC/TB & Other Respiratory Diseases Divisions of Rwanda Biomedical Centre to carry out the laboratory analysis and get access to laboratory records. During the research work, the principle of respect for human dignity was taken into consideration. Testing was performed on residual portions of routine clinical specimens submitted for culture and conventional DST. Results were delinked from patient identifiers, and no patient names were collected. Therefore, informed consent was not being required for this study.

Samples preparation

Sputum samples from new patients or previously treated were digested and decontaminated using N-Acetyl-L-Cystein-Sodium Hydroxide (NALC-NaOH) method by adding an equal amount of NaOH-NALC solution to the sputum followed by washing using prepared Phosphate Buffer Solution (PBS) [10]. Sputum sample concentration was done using a centrifuge for 15-20 minutes at 3000 x g in refrigerated aerosol free centrifuge cups. From the same decontaminated specimen two aliquots were made, one for inoculating LJ medium and the second one for DNA extraction to analyze the presence of mutations conferring resistance to Rifampicin (RIF) and Isoniazid (INH) by GenoType® MTBDR*plus*. The L-J medium was incubated at 370C up to 8 weeks and inspected for growth every week as per NRL's protocol. Positive culture underwent further laboratory testing to detect resistance pattern using both conventional DST by proportional method on L-J medium containing anti-TB drugs and GenoType®MTBDR*plus* Assay (Hain Lifescience GmbH, Nehren, Germany).

GenoType®MTBDRplus assay

The GenoType®MTBDR*plus* assay (Hain Lifescience GmbH, Nehren, Germany) was used according to the manufacturer's instructions [11]. The performance of GenoType®MTBDR*plus* assay was evaluated by calculating its sensitivity, specificity and predictive values (positive and negative) versus the conventional phenotypic DST, proportion method on L-J medium.

Nucleic acid extraction

Deoxyribonucleic Acid (DNA) from decontaminated clinical specimens was extracted by thermal lysis and sonication following the manufacturer's instructions test kits of GenoType®MTBDR*plus* assay. In brief, 500 µL of decontaminated sample was used for DNA extraction by physical method using thermal lysis and sonication and centrifugation at 13000 rpm at 4°C for 15 minutes; pellet were resuspended in 100 µL of sterile distilled water and mycobacteria were lysed by incubation at 95°C for 30 minutes and sonication for 15 minutes.

Master mix preparation

The main composition of Master Mix solution is hot star *Taq* polymerase enzyme in which PNM mix was added within a Polymerase Chain Reaction (PCR) buffer, Magnesium Chloride ($MgCl_2$) and hot grade water. The total reactions were given by the total number of specimens to be processed, plus one positive control (if culture), one negative control, two PCR controls (both negative and positive) and one extra correction volume.

Amplification

A 5-µL of lysate was used for amplification with the provided biotinylated primers. Two units (instead of 1 as reported in manufacturer's instructions) of hot-start Taq DNA polymerase were used in the amplification step. The amplification was performed in a thermal cycler with a protocol consisting of 1 cycle at 95°C for 15 minutes (Taq activation cycle), 10 cycles of denaturation at 95°C for 30 seconds and 40 cycles of denaturation at 95°C for 25 seconds, primer annealing at 53°C for 40 seconds and extension at 70°C for 40 seconds, followed by a final extension at 70°C for 8 minutes.

Hybridization and detection

The GenoType®MTBDR*plus* assay is based on reverse hybridization between amplicons derived from a multiplex PCR and nitrocellulose-bound Wild-Type (WT) and Mutated (MUT) probes targeting the hotspot region of *rpoB* gene, the codon 315 of *katG* gene and the promoter region of *inhA* gene. The assay also includes amplification controls, and a control-probe for MTB complex DNA detection. Hybridization and washing was performed manually using the TWINCUBATOR® Hybridization Tray (Hain Lifesciences, Nehren, Germany) according to the manufacturer's instructions [10,11].

Interpretation of results

To interpret the test results, the sheet provided with the purchased test kits where the test strip was aligned to the bands CC, AC and TUB

then observe for the presence or absence of wild type genes and the presence or absence of *katG, inhA* and *rpoB* genes mutation. Results were interpreted for susceptibility or resistance to RIF and INH according to manufacturer's instructions. When using the evaluation sheet, the developed strips were pasted in the designated fields by aligning the bands CC, AC and TUB with the respective lines on the sheet. Determine the resistance status and write down in the respective column the appropriate results as shown in the example below (Figure 1).

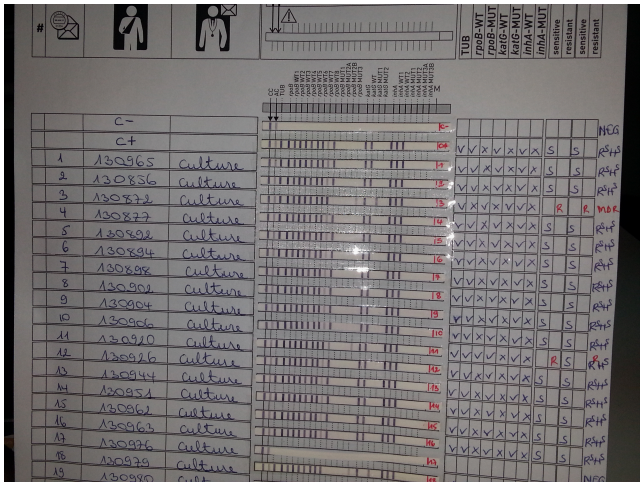

Figure 1: Interpretation of GenoType®MTBDR*plus* test results

Conventional drug susceptibility testing method

Conventional DST using proportional method known as Petroff method was used by inoculating L-J medium containing 20.0 µg and 40.0 µg of Rifampicin, 0.1 µg and 2.0 µg of Isoniazid. Both Rifampicin and Isoniazid were tested at high and low antibiotic concentration. A patient may be resistant at low antibiotic concentration or at high antibiotic concentration. To determine if an isolate is resistant, we first observe drug containing tube at each drug concentration and there should be no growth in the tube containing drug for an isolate to be susceptible. If there was growth, to determine whether an isolate is susceptible or resistant we compared with the control tube that contains the most diluted antibiotic of interest as described by Canetti et al,. as follow: In case, the growth on the drug-containing tube is equal or more than the growth observed on the most diluted control tube 10^{-4}, representing 1% of possible growth of a valid test, the isolate is considered as resistant. In case, growth on the drug-containing tube is less than the growth observed on the most diluted control tube 10^{-4}, represents 1% of possible growth of a valid test, the isolate is considered to be susceptible for the specific drug as described elsewhere [12].

Turnaround time of results

Turnaround time was obtained by the difference between the time of the inoculation date of the test (conventional DST and/or GenoType®MTBDR*plus* test) and the date of the availability conventional DST results or the date of GenoType®MTBDR*plus* results from the computerized National Reference Laboratory database.

Statistical methods

Statistical analyses were performed using Epi Info version 3.5.3. P-values were derived from $\chi 2$ tests applying Fisher Exact where appropriate.

Results

The GenoType®MTBDR*plus* assay was evaluated on 1548 specimens obtained from patients in which TB and MDR-TB was suspected. Out of 1548 samples tested by GenoType®MTBDR*plus* assay, 463 (29.9%) were from new patients and 1085 (70.1%) patients were from retreated patients. Moreover, out of 1548 samples included in this evaluation, 1106 (71.4%) were fresh sputum specimens and 442 (28.6%) were isolates from colonies harvested from L-J solid media (Table 1). Among sputum samples, the detection rate was 15.8% (175/1106) while 84.2% (931/1106) were negative for *Mycobacterium tuberculosis* Complex. In all isolates, culture positive from L-J medium, the detection rate of MTBC was found in 99.3% (439/442). In sputum samples, susceptibility patterns by GenoType®MTBDR*plus* assay show that INH was susceptible at the rate of 13.1% (145/1106) while MTB susceptible to RIF rate was 12.9% (143/1106). From isolates, patterns show that INH was susceptible at the rate of 79.8% (353/442) while Rifampicin sensitive in 78.7% (348/439).

Resistance characteristics	Type of specimens analyzed			p-value
	AFB+ Sputum n(%)	Isolates n(%)	Total n(%)	
All samples analyzed	1106 (71.4)	442 (28.6)	1548 (100.0)	
Treatment history				
New	406(36,7)	57(13.0)	463(29.9)	0.55
Previously treated	700(63.3	385(87.0)	1085(70.1)	
Detection of MTBC	175 (15.8)	439 (99.3)	614 (39.7)	0.0001
No detection, negative reaction	931 (84.2)	3 (0.7)	934 (60.3)	
Susceptible				
INH	145 (13.1)	353 (79.8)	498 (32.1)	0.73
RIF	143 (12.9)	348 (78.7)	491 (31.7)	
Monoresistance				
INH	30 (2.7)	86 (19.5)	116 (7.5)	0.52
RIF	32 (2.9)	91 (20.5)	123 (8.0)	
Multi Drug Resistance (MDR)				
INH & RIF	29 (2.6)	86 (19.5)	115 (7.4)	

Key: AFB+: Acid Fast Bacilli smear positive, INH: Isoniazid, RIF: Rifampicin, MDR: Multidrug resistance

Table 1: Resistance characteristics according to type of specimen analyzed by GenoType®MTBDR*plus*

The monoresistance as tested by GenoType®MTBDR*plus* assay was 2.7%, 2.9% in sputum samples from INH and RIF respectively. The monoresistance from culture positive was 19.5% and 20.5% for INH

and RIF respectively. The results of the present study revealed that 7.4% (115/1548) are MDR-TB (Table 1). Out of 614 conventional DST processed, 5 (0.8%) were contaminated and the DST could not be ascertained hence therefore there was no DST result. Although DST results were not available, these samples gave an interpretable Isoniazid and Rifampicin results by GenoType®MTBDR*plus* assay. Of which, 2 (0.3%) and 1 (0.2%) were resistant to Isoniazid and Rifampicin respectively. While, 3 (0.5%) and 4 (0.6%) were susceptible to Isoniazid and Rifampicin respectively (Table 2).

GenoType®MTBDR*plus*	Conventional Drug Susceptibility Testing		
	Positive growth n(%)	DST contaminated n(%)	Total n
Growth outcome	609 (99.2)	5 (0.8)	614
Isoniazid			
Susceptible	495 (80.6)	3 (0.5)	498
Resistant	114 (18.5)	2 (0.3)	116
Rifampicin			
Susceptible	487 (79.3)	4 (0.6)	491
Resistant	122 (19.8)	1 (0.2)	123
Key: DST: Drug Susceptibility Testing			

Table 2: GenoType®MTBDR*plus* assay compared with conventional Drug Susceptibility Testing

Performance of GenoType®MTBDRplus assay to detect drug resistance

The performance of GenoType®MTBDR*plus* assay (Hain Lifescience GmbH, Nehren, Germany) were assessed by comparing GenoType®MTBDR*plus* results with those of the conventional DST on L-J solid media considered as gold standard. The performance of GenoType®MTBDR*plus* assay was assessed in terms of its sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) calculated considering the results of the conventional DST performed on LJ as gold standard (Table 3).

	Isoniazid	Rifampicin	Multidrug Resistance
Sensitivity	94.8% (95% CI: 79.8-99.3)	91.7% (95% CI: 77.5-98.2)	93.8% (95% CI: 79.2-99.2)
Specificity	99.3% (95%CI: 96.2-100.0)	98.6% (95% CI: 94.9-99.8)	99.0% (95%CI: 96.2-100.0)
PPV	96.7% (95% CI: 93.8-99.6)	94.3% (95% CI: 91.8-98.5)	96.8% (95% CI: 93.9-99.8)
Key: PPV: positive predictive value, NPV: negative predictive value			

Table 3: Performance of GenoType®MTBDR*plus* Assay to detect susceptibility patterns of Isoniazid, Rifampicin and Multidrug resistance

Compared to the reference standard the GenoType®MTBDR*plus* assay had a sensitivity of 94.8% (95% CI: 79.8-99.3), 91.7% (95% CI: 77.5-98.2), 93.8% (95% CI: 79.2-99.2) for Isoniazid, Rifampicin and multidrug resistance (MDR) respectively. The specificities were 99.3% (95%CI: 96.2-100.0), 98.6% (95% CI: 94.9-99.8) and 99.0% (95%CI: 96.2-100.0) for Isoniazid, Rifampicin and multidrug resistance respectively. PPVs were 96.7% (95% CI: 93.8-99.6), 94.3% (95% CI: 91.8-98.5) and 96.8% (95% CI: 93.9-99.8) for Isoniazid, Rifampicin and MDR-TB respectively. NPVs were 98.6% (95% CI: 94.9-99.8), 99.3% (95%CI: 96.2-100.0) and 98.6% (95% CI: 94.9-99.8) for Isoniazid, Rifampicin and for MDR respectively (Table 3).

Turnaround time

DST results from sputum (including primary culture and DST) were 4 weeks plus 42 days in average 28+42 (70 days) with a range of 26 to 82 days. When applying GenoType®MTBDR*plus* testing directly on sputum samples with smear positive, the turnaround time was 1 to 2 days and it was 28 days+2 days (30 days) when the GenoType®MTBDR*plus* assay was applied to isolates from positive culture.

Discussion

The Mycobacteriology Section Laboratory within the Rwanda National Reference Laboratory is the referral laboratory for culture of Mycobacteria and DST for TB diagnosis and patients follow-up in Rwanda. The laboratory is receiving approximately 20 to 30 sputum samples for primary culture and DST daily. Even though GenoType®MTBDR*plus* assay is considered as the most effective, reliable and fast line probe assay used for rapid detection of pulmonary TB and MDR-TB from AFB smear positive and MTB isolates [7], evaluating the performance of the new laboratory tool is a prerequisite before its implementation in the routine testing in our setting.

The present study assessed the performance characteristics including sensitivity, specificity, Positive Predictive Values (PVP) and Negative Predictive Values (NPV) of the GenoType®MTBDR*plus* assay for the detection of resistance to INH, RIF and MDR-TB from sputum specimens or isolates from primary culture. The GenoType®MTBDR*plus* assay was performed in parallel with conventional isolation and indirect susceptibility testing procedures routinely used in mycobacteriology laboratories on egg-based L-J medium [13].

The current study presents the results of 1548 suspected MDR-TB patients from all health facilities in Rwanda enrolled in our study. At the same time, DST using conventional method "the proportional method", taken as gold standard, was applied. GenoType®MTBDR*plus* assay was performed directly on sputum samples whose smear was positive and culture positive isolates. In total, 1548 patients were enrolled in the present study, from which 1106 (71.4%) were sputum samples and 442 (28.6%) were isolates from positive cultures. The detection rate in isolates (99.3%) compared to the detection performed from sputum (15.8%) was statistical significant ($p<0.0001$) if the GenoType®MTBDR*plus* assay was applied directly on isolates. We observed at the same time a high number of MDR-TB patients (19.5%) detected from isolates while there was a relative low number of MDR-TB (2.6%) detected from smear positive sputum (Table 1). However, the results of the present study show a slight low number of MDR-TB (7.4%) patients compared to the previous study conducted in Rwanda (9.4%) from previous treated patients [14].

Resistance patterns studied were monoresistance to isoniazid and rifampicin as well as multi-drug resistance profiles from either fresh sputum samples or culture positive isolates. The

GenoType®MTBDR*plus* assay showed a good performance in detection of both INH and RIF monoresistance. However, there was no statistical significant difference between detection of INH monoresistance (2.7%) and RIF monoresistance (2.9%) from sputum samples smear positive, or between detection of INH monoresistance (19.5%) and RIF monoresistance (20.5%) from isolates (p=0.52). Moreover, the difference in detection of monoresistance for INH and RIF monoresistance was statistically significant from isolates culture positive compared to that from fresh sputum specimens smear positive ($p < 0.05$). When the test was performed directly on sputum, the yield was not as good as when it was performed on isolates. One observation was that the GenoType®MTBDR*plus* assay performed well on specimens whose primary or DST culture was contaminated what constitutes an advantage of the molecular test over the phenotypic DST. One RIF and two INH resistant results could be missed out by conventional DST but were revealed by the GenoType®MTBDR*plus* test (Table 2). These results sound the same with those found elsewhere in which contaminated culture GenoType®MTBDR*plus* assay gave an interpretable results [9]. Misdiagnosing TB and MDR-TB can lead to late treatment, thus spreading the infection in the entire population.

Performance of GenoType®MTBDR*plus*: sensitivity, specificity, positive predictive values and negative predictive values

To assess the performance characteristics of GenoType®MTBDR*plus* test, the sensitivity, specificity, PPV and NPV were calculated from samples for which both GenoType®MTBDR*plus* and conventional DST tests were performed as well. Our findings show that GenoType®MTBDR*plus* assay has a good sensitivity and specificity as performance characteristics. Its sensitivity to detect Isoniazid resistance is 94.8% (95% CI 79.8-99.3), and Rifampicin 91.7% (95% CI 77.5-98.2). The sensitivity for both INH and RIF was 93.8% (95% CI 79.2-99.2%) for MDR TB. The specificity to detect resistance patterns was 99.3% (95% CI 96.2-100.0) for Isoniazid, 98.6% (95% CI 94.9-99.8) for Rifampicin and for the detection of MDR-TB it was 99.0% (95% CI 96.2-100.0) for both INH and RIF resistance. The test has a quite high specificity compared to its sensitivity in detecting INH, RIF resistance and MDR-TB cases (Table 3). The low sensitivity to detect INH and RIF resistance patterns could be explained by the lack of some probes to detect all alleles conferring any given mutation and if these all probes are included, it could improve the detection of all RIF and INH resistance patterns. Results from several studies conducted elsewhere in the world to evaluate the performance of GenoType®MTBDR*plus* assay, have reported similar results to our findings. A meta-analysis to validate GenoType®MTBDR*plus* assays for the diagnosis of Multidrug-Resistant Tuberculosis showed 98%, 89% of sensitivity and specificity respectively [8]. Others studies presented similar results with a slight high specificity (100%) for RIF resistance in South Africa. Moreover, similar sensitivity was documented in these studies respectively as follow: 94.2% and 98.9% for INH respectively in Thailand and South Africa. In Thailand the sensitivity to detect INH, RIF resistance and MDR-TB was 95.3%, 100%, and 94.4% respectively [7,9]. However, when compared to what has been found in other studies, our assessment showed that the GenoType®MTBDR*plus* assay in our setting did not achieve 100% specificity to detect MDR-TB cases.

Compared to our findings, a study conducted in South Vietnam by Huyen M.N. et al, has found that by conventional DST, 55 strains were classified as MDR-TB, four strains were rifampicin mono-resistant and 52 strains were susceptible to all first-line drugs. The sensitivity of the GenoType®MTBDR*plus* assay was 93.1% for rifampicin, 92.6% for isoniazid and 88.9% for the combination of both; its specificity was 100% [15]. However, the specificity of GenoType®MTBDR*plus* assay to detect MDR-TB, in our study, was lower than that found in other studies conducted elsewhere in the world [7,9,15].

The positive predictive value (PPV) of GenoType®MTBDR*plus* assay is 96.7% (95% CI: 93.8-99.6) while its negative predictive value (NPV) is 98.6% (95% CI: 94.9-99.8) for Isoniazid. The pooled PPV and NPV for multidrug resistance (MDR) are 96.8% (95% CI: 93.9-99.8) and 98.6% (95% CI: 94.9-99.8) respectively. The lower NPV and PPV could be explained by low TB prevalence in our setting where the estimated TB incidence rates in Rwanda which is lower than the Global and AFRO Regional average, 86 incident TB cases -new and relapse- per 100,000 habitants in Rwanda in 2012 vs. 122 and 255 respectively at global and AFRO Region level [16].

Turnaround time

The turnaround time (TAT) evaluated included only the time between inoculation of the test sample and the availability of conventional DST results or the date of GenoType®MTBDR*plus* results from the computerized National Reference Laboratory database. The additional time including the date of sample collection, the period of storage and transport were not included in the calculation of TAT. We considered the date between the inoculation of the test and the availability of the results. In the routine testing for laboratory TB diagnosis, using solid culture and indirect susceptibility testing, the total turnaround time takes weeks and even months in many laboratories [17]. In the present evaluation, the difference in TAT is statistical significant when applying GenoType®MTBDR*plus* assay on fresh sputum as well as on clinical isolates ($p<0.05$). The reduction of TAT confers to the GenoType®MTBDR*plus* assay the ability of rapid TB and MDR-TB diagnosis, thus initiation early TB treatment and MDR-TB case management is made possible with the new test method. Thus, GenoType®MTBDR*plus* assay can serve as an early guidance of MDR-TB treatment and case management. The GenoType®MTBDR*plus* assay is considered as alternative method to diagnose TB and MDR-TB in few days rather than DST performed on L-J solid medium which takes several weeks to months. The present performance assessment ensured that GenoType®MTBDR*plus* assay can be used for early TB and MDR-TB diagnosis so early appropriate treatment can be instituted.

Conclusions

GenoType®MTBDR*plus* assay is a rapid and reliable test to be implemented in Rwanda. Considering the short turnaround time, ease of use, its accuracy, specificity and low infrastructure requirements, the GenoType®MTBDR*plus* assay is an alternative tool for routine testing in Rwanda of MDR-TB detection in order to institute early treatment. Furthermore, monoresistance of multidrug resistance were neither associated with drug resistance nor type of specimen analyzed.

Acknowledgements

We acknowledge the Mycobacteriology staff from Rwanda National Reference Laboratory who assisted in sample processing, culture and drug susceptibility testing. We express our gratitude to the Rwanda Biomedical Centre, National Reference Laboratory Division and University of Rwanda, College of Medicine and Allied Health Sciences-

School of Public (UR-CMHS/SPH), CDC-Rwanda Program for administrative and financial support.

References

1. (2008) WHO report, Global Tuberculosis Control. Geneva, Switzerland.
2. (2010) WHO report, TB Control. Geneva, Switzerland.
3. Srisuwanvilai LO, Monkongde P, Podewils LJ, Ngamlert K, Pobkeeree V, et al. (2008) Performance of the BACTEC MGIT 960 compared with solid media for detection of Mycobacterium in Bangkok, Thailand. Diagn Microbiol Infect Dis 61: 402–407.
4. Schaaf HS, Moll AP, Dheda K (2009) Multidrug- and extensively drug-resistant tuberculosis in Africa and South America: epidemiology, diagnosis and management in adults and children. Clin Chest Med 30: 667-683.
5. (2007) Stop TB Department WHO, Seventh meeting. Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) report on conclusions and recommendations. Geneva, Switzerland.
6. (2008) World Health Organization, Policy Statement: Molecular line probe assays for rapid screening of patients at risk of Multi-Drug Resistant Tuberculosis (MDR-TB). Geneva, Switzerland.
7. Vorapong AR, Sinthuwattanawibool C, Podewils LJ, McCarthy K, Ngamlert K, et al. (2010) Validation of the GenoType® MTBDRplus assay for detection of MDR-TB in a public health laboratory in Thailand. BMC Infect Dis 10: 123.
8. Ling DI, Zwerling AA, Pai M (2008) GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: A meta-analysis. Eur Respir J 32: 1165–1174.
9. Barnard M, Albert H, Coetzee G, O'Brien R, Bosman ME (2008) Rapid molecular screening for Multidrug-Resistant Tuberculosis in a high-volume public health laboratory in South Africa. Am J Respir Crit Care Med 177: 787-792.
10. Deutsches Institut für Normung, (1986) Medical microbiology. Diagnosis of tuberculosis. Parts 3.
11. Hain Lifescience, Genotype MTBDRplus, version 1.0 Nehren, Germany: Hain Lifescience.
12. Canetti G, Fox W, Khomenko A, Mahler HT, Menon MK, Mitchison DA, et al. (1969) Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bull World Health Organ 41: 21-43.
13. Oles'Jolanta P, Montewka MK, Magrys A, (2009) New Microbiologica 32: 147-152.
14. Umubyeyi AN, G Vandebriel, M Gasana, P Basinga, JP Zawadi, et al. (2007) Results of a national survey on drug resistance among pulmonary tuberculosis patients in Rwanda. Int J Tuberc Lung Dis. 11: 189–194.
15. Huyen MN, Tiemersma EW, Lan NT, Cobelens FG, Dung NH, et al. (2010) Validation of the GenoType MTBDRplus assay for diagnosis of multidrug resistant tuberculosis in South Vietnam. BMC Infect Dis 10: 149.
16. (2013) Ministry of Health, Rwanda National Strategic Plan for tuberculosis. Annual report, July 2011- June 2012.
17. Hart CA, Beeching NJ, Duerden BI (1996) Tuberculosis into the next century. J Med Microbiol 44: 1- 34.