

# Rapid Molecular Screening for Multidrug-Resistant Tuberculosis in a High-Volume Public Health Laboratory in South Africa

Marinus Barnard<sup>1</sup>, Heidi Albert<sup>2</sup>, Gerrit Coetzee<sup>3</sup>, Richard O'Brien<sup>2</sup>, and Marlein E. Bosman<sup>1</sup>

<sup>1</sup>National Health Laboratory Services (NHLS), Greenpoint, Cape Town, South Africa; <sup>2</sup>Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland; and <sup>3</sup>National TB Reference Laboratory, NHLS, Sandringham, Johannesburg, South Africa

**Rationale:** The dual challenges to tuberculosis (TB) control of HIV infection and multidrug resistance are particularly pressing in South Africa. Conventional methods for detecting *Mycobacterium tuberculosis* drug resistance take weeks to months to produce results. Rapid molecular testing for drug resistance is available but has not been implemented in high-TB-burden settings.

**Objectives:** To assess the performance and feasibility of implementation of a commercially available molecular line-probe assay for rapid detection of rifampicin and isoniazid resistance.

**Methods:** We performed the assay directly on 536 consecutive smear-positive sputum specimens from patients at increased risk of multidrug-resistant (MDR) TB in a busy routine diagnostic laboratory in Cape Town, South Africa. Results were compared with conventional liquid culture and drug susceptibility testing on solid medium.

**Measurements and Main Results:** Overall, 97% of smear-positive specimens gave interpretable results within 1–2 days using the molecular assay. Sensitivity, specificity, and positive and negative predictive values were 98.9, 99.4, 97.9, and 99.7%, respectively, for detection of rifampicin resistance; 94.2, 99.7, 99.1, and 97.9%, respectively, for detection of isoniazid resistance; and 98.8, 100, 100, and 99.7%, respectively, for detection of multidrug resistance compared with conventional results. The assay also performed well on specimens that were contaminated on conventional culture and on smear-negative, culture-positive specimens.

**Conclusions:** This molecular assay is a highly accurate screening tool for MDR TB, which achieves a substantial reduction in diagnostic delay. With overall performance characteristics that are superior to conventional culture and drug susceptibility testing and the possibility for high throughput with substantial cost savings, molecular testing has the potential to revolutionize MDR TB diagnosis.

**Keywords:** tuberculosis; MDR TB; molecular diagnosis.

The dual epidemics of HIV infection and multidrug-resistant (MDR) tuberculosis (TB) threaten global TB control, especially in sub-Saharan Africa. These problems are especially severe in South Africa, which has nearly 20% of the world's reported HIV-associated TB cases (1) and the second largest reported number of MDR TB cases in the world (2, 3). Extensively drug-resistant (XDR) TB, defined as MDR TB (i.e., resistance to rifampicin [RIF] and isoniazid [INH]) with additional resistance to a fluoroquinolone antibiotic and at least one of three injectable drugs used for MDR TB treatment (capreomycin, kanamycin, and amikacin) (4, 5), has heightened the challenge faced in controlling the dual epidemics of TB and HIV. In the much publicized outbreak of XDR TB among HIV-infected patients

## AT A GLANCE COMMENTARY

### Scientific Knowledge on the Subject

Molecular assays for diagnosis of drug-resistant tuberculosis (TB) are available but not widely used. There is no information on their performance in multidrug-resistant (MDR) TB screening in high-burden settings.

### What This Study Adds to the Field

Molecular assays for MDR TB diagnosis from sputum specimens can be implemented in high-burden settings. The high accuracy, large reduction in reporting time, and high-volume capacity suggest the assay may revolutionize MDR TB diagnosis.

in KwaZulu-Natal, South Africa, 52 of 53 patients died, with a median survival time of 16 days from the date of diagnosis (6).

The diagnosis of MDR and XDR TB is based on mycobacterial culture and drug susceptibility testing (DST) on liquid or solid media, with results available in weeks to months. However, mycobacterial culture and DST capacity is severely limited, especially in resource-poor countries. In response to the growing problem of MDR TB and the threat of an epidemic of virtually incurable XDR TB, the World Health Organization (WHO) and the Stop TB Partnership have issued a call for significant expansion of mycobacterial culture and drug susceptibility testing capacity (7). The Stop TB Partnership's Global Plan to Stop TB 2006–2015 is being revised to include a provision of universal access to diagnose and treat all patients with MDR TB by 2015 (8).

These plans call for accelerated access to rapid testing for rifampin resistance to improve case detection in all patients with suspected MDR and XDR TB. Although rapid molecular methods are available for detecting drug-resistant TB (9), there have been questions surrounding the feasibility of their implementation in high-burden settings in the developing world. To address this concern and to respond to the urgent need for improved MDR and XDR TB diagnosis, the Foundation for Innovative New Diagnostics (FIND) has accelerated large-scale demonstration projects of the Genotype MTBDR<sub>plus</sub> assay, a polymerase chain reaction (PCR) amplification and reverse hybridization assay for detecting RIF and INH resistance (10). The assay detects mutations in the *rpoB* gene for RIF resistance, the *katG* gene for high-level INH resistance, and the *inhA* gene for low-level INH resistance directly from smear-positive sputum. Results are available within 1 day (11).

As a prelude to the demonstration project in South Africa, a validation of the test was undertaken in one of the National Health Laboratory Service (NHLS) laboratories. The aim of the study was to investigate the feasibility of implementation of the

(Received in original form September 27, 2007; accepted in final form January 15, 2008)

Correspondence and requests for reprints should be addressed to Richard O'Brien, M.D., Foundation for Innovative New Diagnostics, 71 av Louis-Casai, 1216 Cointrin, Switzerland. E-mail: rick.obrien@finddiagnostics.org

Am J Respir Crit Care Med Vol 177, pp 787–792, 2008

Originally Published in Press as DOI: 10.1164/rccm.200709-1436OC on January 17, 2008  
Internet address: www.atsjournals.org

assay in a routine diagnostic laboratory in a high-burden setting and to validate its performance compared with conventional culture and DST before the use of assay results for patient management in the demonstration project.

Some of the results of this study have been previously reported in the form of an abstract (12).

## METHODS

### Study Setting

This study took place in the NHLS TB laboratory in Greenpoint, Cape Town, South Africa, a referral laboratory for the Western Cape province, serving a population of approximately 4.25 million people. The estimated TB incidence in the province was 932 per 100,000, with an average TB-HIV coinfection rate of 28.2% in 2001–2002. The estimated MDR rate was 0.9% and 3.9% in new and previously treated cases, respectively (3). The laboratory has an exceedingly high workload for TB diagnostic testing, with approximately 400,000 smears, 150,000 cultures, and 50,000 first-line DSTs performed annually.

Testing was performed on residual portions of routine clinical specimens submitted for culture and DST. Results were delinked from patient identifiers, and no patient information was collected. Therefore, informed consent was not required for the study.

### Sputum Specimens

All manipulations with potentially infectious clinical specimens were performed in a Class II safety cabinet in a BSL2 laboratory. Sputum specimens were decontaminated with N-acetyl-L-cysteine-sodium hydroxide (13). After centrifugation, the pellet was suspended in 1.0 ml of phosphate buffer (pH 6.8). A concentrated auramine smear was prepared and examined under  $\times 500$  magnification using a fluorescent microscope and graded according to International Union Against Tuberculosis and Lung Disease (IUATLD) guidelines (14). A 0.5-ml portion of the sediment was cultured using the BACTEC MGIT 960 system (BD Diagnostics Systems, Sparks, MD), including mycobacterial growth indicator tubes (MGITs) with PANTA and OADC. Culturing on solid media was not done. Positive cultures were confirmed as *Mycobacterium tuberculosis* complex using Ziehl-Neelsen staining and *p*-nitrobenzoic acid testing (15). Indirect DST was performed using the proportion method on Middlebrook 7H11 agar slants with 1.0  $\mu\text{g/ml}$  RIF and 0.2  $\mu\text{g/ml}$  INH (13).

Specimens with a smear grading of 1+ or greater were selected for MTBDR<sub>plus</sub> testing. Five hundred thirty-six acid-fast bacilli smear-positive sputum specimens were collected from 528 patients between January 31 and March 1, 2007, consisting of all smear-positive sputum specimens submitted to the laboratory for smear, culture, and DST during that period. In addition, GenoType MTBDR<sub>plus</sub> assay (Hain Lifescience, Nehren, Germany) (MTBDR<sub>plus</sub>) testing was performed on 100 randomly selected smear-negative specimens submitted for culture and DST.

### Rapid Drug Resistance Testing

The MTBDR<sub>plus</sub> was performed according to the manufacturer's instructions (10). The test is based on DNA strip technology and has three steps: DNA extraction, multiplex polymerase chain reaction (PCR) amplification, and reverse hybridization. A 500- $\mu\text{l}$  portion of the decontaminated sediment was used for DNA extraction, a 1-hour process that included heating, sonification, and centrifugation. The amplification procedure that consisted of preparation of the master mix and addition of the DNA also required 1 hour. These steps were carried out in separate rooms with restricted access and unidirectional workflow. Hybridization was performed with the GT Blot 48 (Hain Lifescience), which is an automated hybridization machine (10). After hybridization and washing, strips were removed, allowed to air dry, and fixed on paper. All tests were performed independent of culture and DST and before the culture and DST results were available.

### Interpretation of Results

Each strip consists of 27 reaction zones (bands), including six controls (conjugate, amplification, *M. tuberculosis* complex, *rpoB*, *katG*, and *inhA* controls), eight *rpoB* wild-type (WT) and four mutant (MUT)

probes, one *katG* wild-type and two mutant probes, and two *inhA* wild-type and four mutant probes (Figure 1). Results were interpreted according to the manufacturer's instructions.

### Turnaround Time of Results

Turnaround time was calculated from the specimen collection date and the date of reporting of the conventional DST result (on the computerized laboratory system) or the date of availability of the MTBDR<sub>plus</sub> result.

### Statistical Methods

Statistical tests were performed using Intercooled STATA 8.0 software (Statacorp LP, College Station, TX). Results were considered significant at  $P < 0.05$ .

## RESULTS

### GenoType MTBDR<sub>plus</sub> Testing from Smear-positive Sputum

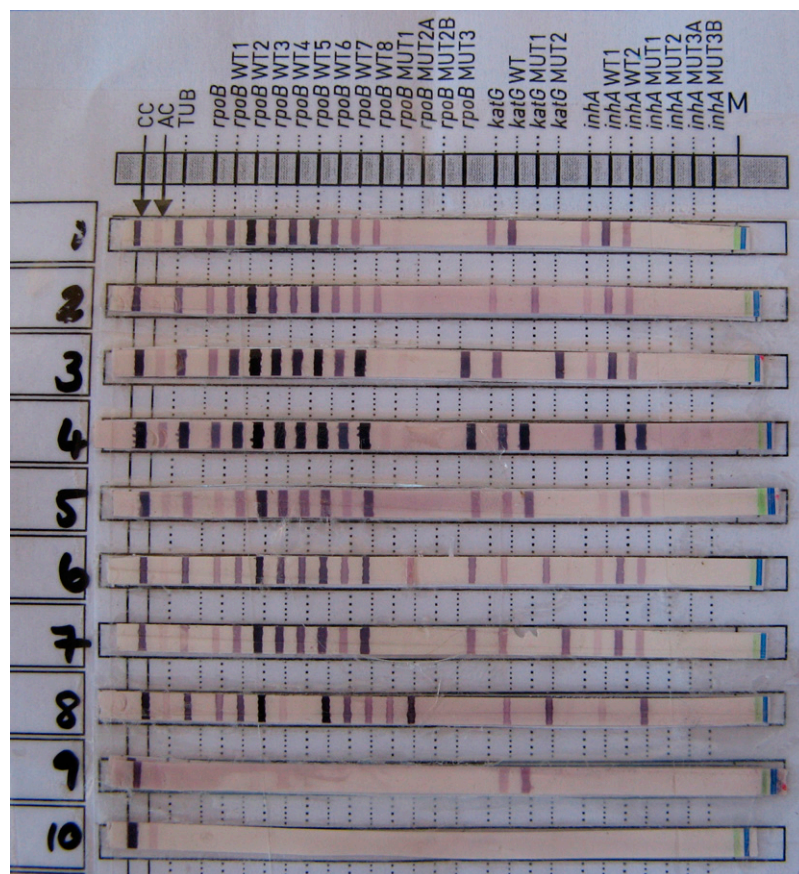
Table 1 is a summary of MTBDR<sub>plus</sub> results of all smear-positive specimens tested ( $n = 536$ ). Eight patients submitted two smear-positive specimens for MTBDR<sub>plus</sub> testing. Identical results were achieved in each case. Fifteen specimens (2.8%) were culture negative, and therefore no conventional DST result was available. Of these 15 culture-negative specimens, 13 (86.7%) gave interpretable results by the MTBDR<sub>plus</sub> method. A further two specimens lost viability during RIF DST, and therefore no results were available (one of these specimens gave an interpretable RIF MTBDR<sub>plus</sub> result). Six specimens lost viability during INH DST, and therefore no results were available (five of these specimens gave an interpretable INH MTBDR<sub>plus</sub> result). Of the specimens with conventional DST results, 88 (19.2%) were MDR, 9 (2.0%) were RIF monoresistant, 34 (7.4%) were INH monoresistant, and 327 (71.4%) were RIF and INH susceptible by conventional DST.

A further 55 (15.4%) conventional DST results were not performed due to contamination of the primary MGIT culture. Of these, 51 (92.7%) gave interpretable RIF MTBDR<sub>plus</sub> results, and 52 (94.5%) had an interpretable INH result. Of the specimens that had a reportable DST result, MTBDR<sub>plus</sub> also had a reportable result in 97.8% (454/464) of specimens for RIF resistance and 98.3% (452/460) for INH resistance. A total of 14 specimens were initially discrepant, with MTBDR<sub>plus</sub> being INH susceptible and INH DST being resistant. Upon reexamination of DST results, an error was detected in the performance of a batch of results due to faulty INH-containing medium. This batch of tests was repeated, and seven results were reassigned as INH susceptible by conventional DST. The data presented are the repeat INH DST results.

Table 2 shows results for detection of multidrug resistance (resistance to RIF and INH). Performance parameters for detection of RIF, INH, and multidrug resistance (Table 3) were calculated from specimens for which rapid and conventional results were available.

Overall, a significantly higher proportion of MTBDR<sub>plus</sub> results (96.8%) was interpretable compared with conventional culture and DST (86.6%) ( $P < 0.001$ ). There was no significant difference in the proportion of interpretable MTBDR<sub>plus</sub> results in specimens with 1+ smear grading (94.6%) compared with 2+ (98.2%) and 3+ (97.1%) smear-positive specimens ( $P = 0.513$  for rifampicin resistance;  $P = 0.350$  for isoniazid resistance).

The MTBDR<sub>plus</sub> test performed well on specimens whose MGIT culture was contaminated, with 92.7% of results from such specimens giving interpretable MTBDR<sub>plus</sub> results compared with 99.6 and 98.2% interpretable results for MTBDR<sub>plus</sub> tests performed on specimens with an uncontaminated MGIT culture and a conventional DST result.



**Figure 1.** Examples of GenoType MTBDRplus strips (Hain Lifescience, Nehren, Germany). (Lane 1) *Mycobacterium tuberculosis*, susceptible to isoniazid (INH) and rifampin (RIF). (Lane 2) *M. tuberculosis*, INH monoresistant (*katG* S315T1 mutation). (Lane 3) Multidrug-resistant tuberculosis (MDR TB), *rpoB* S531L mutation and *katG* S315T2 mutation. (Lane 4) MDR TB *rpoB* S531L mutation and *katG* S315T1 and *inhA* C15T mutations. (Lane 5) *M. tuberculosis*, RIF monoresistant (mutation in *rpoB* 530–533 region). (Lane 6) MDR TB, *rpoB* D516V and *katG* S315T1 mutations. (Lane 7) MDR TB, *rpoB* S531L, and *katG* S315T2 mutations. (Lane 8) MDR TB, *rpoB*, D516V, *katG* S315T1 mutation and *inhA* mutation at –15/–16. (Lane 9) Uninterpretable result, no *M. tuberculosis* complex (TUB) band. (Lane 10) Negative control.

Conventional culture and DST for smear-positive specimens had a total turnaround time of  $42 \pm 9$  days (mean  $\pm$  SD) with a range of 23 to 99 days. For MTBDRplus testing, the test took 1–2 days for smear-positive specimens once specimens had been selected for testing based on positive sputum smear results. Turnaround time for smear-negative specimens was also 1–2 days.

Table 4 shows the distribution of different banding patterns in drug-resistant isolates, including MDR, INH-monoresistant, and RIF-monoresistant strains. Typical banding patterns obtained on the MTBDRplus strips are shown in Figure 1. For detection of RIF resistance, S531L mutation (MUT3 band) occurred the most commonly, with 70.5% of all RIF-resistant strains (76.4% of MDR- and 37.5% of RIF-monoresistant strains) having the mutation. This difference in prevalence of the S531L mutation

between MDR- and RIF-monoresistant strains was significant ( $P = 0.0115$ ). One MDR strain had a S531L and a D516V mutation, whereas one RIF-monoresistant strain had S531L and H26Y mutations. Other mutations in the 530–533 region were common, as detected by the lack of binding to the WT8 probe in the absence of S531L mutation. A significantly higher proportion of RIF-monoresistant strains (18.8%) had a H526Y mutation (MUT2A band) compared with MDR strains (2.2%) ( $P = 0.0015$ ). Other mutations occurred at *rpoB*516 (9.5% overall), and one MDR strain had complete deletion of the *rpoB* gene. There was no significant difference in the presence of other bands between MDR- and RIF-monoresistant strains. Two “false” RIF-resistant results were obtained compared with the conventional DST result. One strain had the WT2 band missing (Q513L

**TABLE 1. SUMMARY OF RESULTS OF RIFAMPICIN AND ISONIAZID RESISTANCE BY GENOTYPE MTBDRplus COMPARED WITH MYCOBACTERIAL GROWTH INDICATOR TUBE CULTURE AND DRUG SUSCEPTIBILITY TESTING**

Genotype MTBDRplus	Conventional MGIT Culture and DST				
	Resistant	Susceptible	Not Done (MGIT contaminated)	Not Done (MGIT culture negative)	No Growth on DST Control
Rifampicin					
Resistant	94	2	4	0	0
Susceptible	1	357	47	13	1
Uninterpretable	2	8	4	2	1
Isoniazid					
Resistant	114	1	4	1	0
Susceptible	7	330	48	12	5
Uninterpretable	1	7	3	2	1

Definition of abbreviations: DST = drug susceptibility testing; MGIT = mycobacterial growth indicator tube.

**TABLE 2. SUMMARY OF RESULTS OF MULTIDRUG RESISTANCE BY GENOTYPE MTBDR<sub>plus</sub> COMPARED WITH CONVENTIONAL CULTURE AND DRUG SUSCEPTIBILITY TESTING**

Genotype MTBDR <sub>plus</sub>	Conventional MGIT Culture and DST						
	MDR	RIF Monoresistant	INH Monoresistant	RIF and INH Susceptible	Not Done (MGIT contam)	Not Done (MGIT culture negative)	No Growth on DST Control
MDR	85	0	0	0	3	0	0
RIF monoresistant	1	8	0	2	1	0	0
INH monoresistant	0	0	28	1	1	1	0
RIF and INH susceptible	0	1	6	318	46	12	5
Uninterpretable	2	0	0	8	4	2	1

Definition of abbreviations: DST = drug susceptibility test; INH = isoniazid; MDR = multidrug resistant; MGIT = mycobacterial growth indicator tube; MGIT contam = MGIT culture contaminated; RIF = rifampicin.

mutation), a rare mutation that had previously only been detected theoretically (*in silico*). The second strain had a S531L mutation, which is likely to be indicative of true resistance.

Of all INH-resistant strains, 64.1% (70.8% of MDR strains and 42.9% of INH-monoresistant strains) had a mutation in the *katG* gene, and 41.9% (38.2% of MDR strains and 53.6% of INH-monoresistant strains) had a mutation in the *inhA* gene. This difference in prevalence of mutations in MDR strains compared with INH-monoresistant strains was significant for *katG* ( $P = 0.0073$ ) but not for *inhA* ( $P = 0.1497$ ). Twelve strains had mutations in both the *katG* and *inhA* genes. There was one "false" INH-resistant MTBDR<sub>plus</sub> result compared with conventional DST. This isolate had an *inhA* C15T mutation.

Twenty-seven percent (24/89) of MDR strains did not have a mutation in the *katG* gene and were detected as INH resistant by a mutation in the *inhA* gene. Fifteen of 28 (53.6%) INH-monoresistant strains were detected by the presence of a mutation in *inhA* only.

#### Genotype MTBDR<sub>plus</sub> Testing from Smear-negative Sputum

A secondary part of the study included testing of 100 smear-negative sputum specimens. Of 100 smear-negative specimens tested, 25% ( $n = 25$ ) were culture positive. Twenty culture-positive specimens were put up for conventional DST (three specimens were contaminated, and two specimens did not have DST requested). Of these specimens, 16 out of 20 (80%) gave interpretable MTBDR<sub>plus</sub> results for RIF, and 14 out of 19 (74%) gave interpretable MTBDR<sub>plus</sub> results for INH resistance. Furthermore, MTBDR<sub>plus</sub> results were available for two specimens in which no conventional DST result was available due to contamination. No MTBDR<sub>plus</sub> results were interpretable for any of the culture-negative specimens ( $n = 77$ ).

There was 100% correlation in the results for detection of RIF (16/16 sensitive strains) and INH resistance (4 resistant and

10 susceptible strains) in the specimens for which both MTBDR<sub>plus</sub> and conventional DST results were available.

## DISCUSSION

The performance of the GenoType MTBDR<sub>plus</sub> test directly from smear-positive sputum correlated very highly with conventional culture and DST. The sensitivity for detection of rifampicin resistance, isoniazid resistance, and multidrug resistance was 99, 94, and 99%, respectively. The specificity for detection of rifampicin, isoniazid, and multidrug resistance was 99, 100, and 100%, respectively. These performance characteristics suggest that the assay is equivalent to conventional Lowenstein-Jensen medium-based DST performed in quality-assured reference laboratories (16). Considering that the test performs well on specimens that subsequently are contaminated on culture, its overall performance for detection of MDR TB is superior to conventional methods.

Detection of mutations in the 81-bp region of the *rpoB* gene correlated very highly with RIF resistance (17, 18), with 99% of RIF-resistant strains being identified in this study. However,

**TABLE 4. PATTERN OF GENE MUTATIONS IN RESISTANT *Mycobacterium tuberculosis* STRAINS USING GENOTYPE MTBDR<sub>plus</sub> ASSAY**

Gene	Band	Gene Region or Mutation	MDR	INH Monoresistant	RIF Monoresistant
			( $n = 89^*$ )	( $n = 28$ )	( $n = 16$ )
<i>rpoB</i>	WT1	506–509	88 (99)	28 (100)	16 (100)
	WT2	510–513	88 (99)	28 (100)	14 (88)
	WT3	513–517	78 (88)	28 (100)	16 (100)
	WT4	516–519	78 (88)	28 (100)	16 (100)
	WT5	518–522	88 (99)	28 (100)	16 (100)
	WT6	521–525	88 (99)	28 (100)	16 (100)
	WT7	526–529	83 (93)	28 (100)	14 (88)
	WT8	530–533	19 (21)	28 (100)	9 (56)
	MUT1	D516V	8 (9)	0 (0)	2 (13)
	MUT2A	H526Y	2 (2)	0 (0)	3 (19)
	MUT2B	H526D	3 (3)	0 (0)	1 (6)
	MUT3	S531L	68 (76)	0 (0)	6 (38)
	WT	315	26 (29)	16 (57)	16 (100)
<i>katG</i>	MUT1	S315T1	34 (38)	10 (36)	0 (0)
	MUT2	S315T2	31 (35)	3 (11)	0 (0)
	WT	–15/–16	55 (62)	13 (46)	16 (100)
<i>inhA</i>	WT2	–8	89 (100)	28 (100)	16 (100)
	MUT1	C15T	32 (36)	15 (54)	0 (0)
	MUT2	A16G	1 (1)	0 (0)	0 (0)
	MUT3A	T8C	0 (0)	0 (0)	0 (0)
	MUT3B	T8A	0 (0)	0 (0)	0 (0)

Definition of abbreviations: INH = isoniazid; MDR = multidrug resistant; RIF = rifampicin.

Values are numbers, with percentages in parentheses.

**TABLE 3. PERFORMANCE OF GENOTYPE MTBDR<sub>plus</sub> IN DETECTING RIFAMPICIN, ISONIAZID, AND MULTIDRUG RESISTANCE FROM SMEAR-POSITIVE SPUTUM SPECIMENS**

	Rifampicin	Isoniazid	Multidrug Resistance
Sensitivity	98.9 (94.3–100.0)	94.2 (88.4–97.6)	98.8 (93.7–100.0)
Specificity	99.4 (98.0–99.9)	99.7 (98.3–100.0)	100 (99.0–100.0)*
Overall accuracy	99.3 (98.1–99.9)	98.2 (96.5–99.2)	99.8 (98.8–100.0)
PPV	97.9 (92.7–99.7)	99.1 (95.3–100.0)	100 (95.8–100.0)*
NPV	99.7 (98.5–100.0)	97.9 (95.8–99.2)	99.7 (98.5–100.0)

Definition of abbreviations: NPV = negative predictive value; PPV = positive predictive value.

Values are percentages with 95% confidence interval in parentheses.

\* One-sided, 97.5% confidence interval.

test sensitivity for RIF resistance may be lower in other settings where mutations outside the 81-base-pair region of the *rpoB* gene, which are not detected by the assay, are responsible for RIF resistance (19). In agreement with most other studies, we found the most common mutations at codons 531, 526, and 516. We found a higher proportion of RIF resistance due to S531L mutations than that reported in other geographical locations (70.5% in all rifampin-resistant strains in this study compared with between 36.1 and 56.7%). Mutations at codon 526 were less common than reported in many other countries (8.6% in all rifampin-resistant strains compared with between 6.8 and 39.3%, with six of nine countries having >20% mutations at codon 526). The rate of mutations at 516 (9.6% in all rifampin-resistant strains) was within the range reported elsewhere (20). Twenty-seven percent of MDR strains and 54% of INH mono-resistant strains were detected as INH resistant by a mutation in the *inhA* gene only. These strains would not have been detected as MDR by the previous version of the assay (Genotype MTBDR), which only tested for mutations in the *katG* gene (20–22). In this setting, the presence of probes for *inhA* substantially increased the sensitivity for detection of INH resistance (and MDR TB). The prevalence of mutations in the *inhA* and *katG* genes seems to vary widely in different geographic locations. For example, *katG* mutations were found in 97% (77/79) and *inhA* mutations in 24% (19/79) of INH-resistant isolates from KwaZulu-Natal (23), whereas van Rie and colleagues reported *katG* mutations in 72% of INH-resistant isolates (41/57) and mutation in the *inhA* gene in only 2% (1/57) isolates in the Western Cape province of South Africa (18). Studies from other countries have confirmed this variability in the contribution of different mutations to INH resistance (24, 25). Mutations in *inhA* were rarely reported in Germany (11). A high prevalence of *katG* mutations has been reported to account for a high proportion of INH resistance in high-TB-prevalence countries and for a much lower proportion in lower TB prevalence settings, presumably due to ongoing transmission of these strains in high-burden settings (25).

A high proportion of interpretable results (80%) was obtained from smear-negative, culture-positive specimens tested by the MTBDR*plus* assay. One hundred percent correlation was seen for specimens in which MTBDR*plus* and conventional DST results were available. However, because only 25% of smear-negative specimens were culture-positive in this laboratory, the overall yield of interpretable results would be low (14–16%) if routine screening of all smear-negative specimens were implemented. Nevertheless, the ability to obtain rapid MDR screening results on smear-negative sputum specimens in a high-HIV-prevalence setting such as South Africa, where a large proportion of patients with HIV who are coinfecting with TB (i.e., at risk of MDR TB) are smear negative, is advantageous.

Receipt of specimens and decontamination of sputum for conventional culture and DST were performed according to usual NHLS procedures by the main TB laboratory staff. This laboratory is a high-volume facility, with over 600 specimens per day set up for culture and approximately 1,000–1,200 specimens per day received for smear microscopy. The high correlation of the GenoType MTBDR*plus* results with conventional results reflects well on the performance of the test in this high-volume laboratory. Although a trained molecular biologist was responsible for the molecular testing, the decontamination of specimens and all conventional testing was performed by the general TB laboratory technical staff. The facility for PCR testing was separate from the routine TB laboratory and had strictly controlled and limited access, enabling PCR contamination to be avoided during this study. These results suggest that this test can be successfully implemented in a high-volume

routine TB diagnostic laboratory with well-qualified technical staff. The MTBDR*plus* assay is rapid, reliable, and easy to interpret.

Although the MTBDR*plus* assay can produce results within 8 hours, in this high-volume facility a longer turnaround time of 2 working days was routinely reported from the time of selection of specimens for testing based on positive smear results because the limitations of general laboratory space for DNA extraction and reading of sputum smears did not permit the whole MTBDR*plus* procedure to be completed on a single day. This equates to a total turnaround time of less than 7 days when specimen transport time, time to perform smear microscopy, and reporting of results is included. This is a substantial reduction compared with conventional culture and DST.

Calls from the WHO and other international agencies to urgently expand access to culture and DST in response to the dual challenges that HIV and MDR TB (and XDR TB) pose significant challenges to TB control programs and TB laboratory services (7). The cost and complexity of establishing culture and DST capacity to meet the anticipated need, especially in low-income countries where these services are not generally available, present overwhelming challenges. Consequently, other diagnostic methods, and in particular molecular testing, should be considered as alternatives. This study shows that it is feasible to perform molecular-based rapid MDR screening with the GenoType MTBDR*plus* test routinely in a high-volume laboratory with no previous experience of routine implementation of molecular assays. The high degree of accuracy, the substantial reduction in reporting time, and the possibility for high throughput with substantial cost savings suggest that molecular testing has the potential to revolutionize the diagnosis of MDR TB. On the basis of FIND-negotiated prices, the cost of the molecular assay is less than 50% of that for conventional liquid culture and DST for INH and RIF. Simplification of specimen processing for molecular testing may allow for easier referral of specimens to central laboratories and may contribute to large-scale drug-resistance surveillance studies. The impact of such rapid assays on patient outcomes is highly dependent on the quality of the rest of the TB control program, including the specimen transport system, reporting of results, and patient follow-up. Strengthening of the whole program is required if such new technologies are to benefit patients (26). Strengthening the capacity of laboratories is also necessary to enable full benefit to be drawn from emerging new TB diagnostic technologies.

**Conflict of Interest Statement:** None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript. R.O. is employed by the Foundation for Innovative New Diagnostics (FIND), and H.A. is a paid consultant to FIND, a nonprofit agency that has a contractual agreement with Hain Lifescience GmbH, the manufacturer of the GenoType MTBDR*plus* assay. This agreement calls on FIND to conduct demonstration studies of this assay. In turn, Hain Lifescience has agreed to provide the assay at a substantially reduced price to the public sector of developing countries. FIND is currently undertaking, in collaboration with NHLS and the South Africa Medical Research Council, a large-scale demonstration project of the MTBDR*plus* assay in South Africa.

**Acknowledgment:** FIND (Foundation for Innovative New Diagnostics), Geneva, Switzerland, provided MTBDR*plus* test kits and the required consumables. Hain Lifescience and Davies Diagnostics provided training and technical support but had no role in the study. The authors thank the NHLS TB laboratory staff, Greenpoint, Cape Town, for their technical assistance.

## References

1. World Health Organization. Global tuberculosis control: surveillance, planning, financing. Geneva, Switzerland: The Organization; 2007. WHO/HTM/TB/2007.376.
2. Department of Health. DOTS-Plus for standardised management of multidrug-resistant tuberculosis in South Africa. Policy guidelines. Pretoria, South Africa: South Africa Department of Health; 2004.

3. World Health Organization. Anti-tuberculosis drug resistance in the world: third global report. Geneva, Switzerland: World Health Organization; 2004. WHO/HTM/TB/2004.343.
4. Centers for Disease Control and Prevention. Emergence of *Mycobacterium tuberculosis* with extensive resistance to second-line drugs worldwide, 2000–2004. *MMWR Morb Mortal Wkly Rep* 2006;55:301–305.
5. Centers for Disease Control and Prevention. Notice to readers: revised definition of extensively drug-resistant tuberculosis. *MMWR Morb Mortal Wkly Rep* 2006;55:1176.
6. Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, Zeller K, Andrews J, Friedland G. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet* 2006;368:1575–1580.
7. World Health Organization. The global MDR-TB and XDR-TB response plan. Geneva, Switzerland: World Health Organization; 2007. WHO/HTM/TB/2007.387.
8. Stop TB Partnership; World Health Organization. Global plan to stop TB, 2006–2015. Geneva, Switzerland: World Health Organization; 2006. WHO/HTB/STB/2006.35.
9. Drobniewski FA, Hoffner S, Rusch-Gerdes S, Skenders G, Thomsen V; WHO European Laboratory Strengthening Task Force. Recommended standards for modern tuberculosis laboratory services in Europe. *Eur Respir J* 2006;28:903–909.
10. GenoType MTBDRplus, version 1.0 [product insert]. [Internet] Feb 2007 [updated 2007 Apr 3; accessed 2007 May 9]. Nehren, Germany: Hain Lifescience, GmbH. Available from: [http://www.hain-lifescience.com/pdf/304xx\\_pbl.pdf](http://www.hain-lifescience.com/pdf/304xx_pbl.pdf)
11. Hilleman D, Rüsch-Gerdes S, Richter E. Evaluation of the Genotype MTBDRplus Assay for rifampicin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and in clinical specimens. *J Clin Microbiol* 2007;45:2635–2640.
12. Barnard M, Albert H, O'Brien R, Coetzee G, Bosman M. Assessment of the MTBDRPlus assay for the rapid detection of multidrug-resistant tuberculosis from smear-positive sputa. *Int J Tuberc Lung Dis* 2007;11:S229.
13. Kent PT, Kubica GP. Public health mycobacteriology: a guide for the level III laboratory. Atlanta, GA: Centers for Disease Control; 1985.
14. Enarson DA, Rieder HL, Arnadottir T, Trebucq A. Management of tuberculosis: a guide for low income countries. Paris, France: International Union Against Tuberculosis and Lung Disease; 2000.
15. Allen BW. Tuberculosis bacteriology in developing countries. *Med Lab Sci* 1984;41:400–409.
16. Laszlo A, Rahman M, Espinal M, Raviglione M; WHO/IUATLD Network of Supranational Reference Laboratories. Quality assurance programme for drug susceptibility testing of *Mycobacterium tuberculosis* in the WHO/IUATLD Supranational Reference Laboratory Network: five rounds of proficiency testing, 1994–1998. *Int J Tuberc Lung Dis* 2002;6:748–756.
17. Telenti A, Imboden P, Marchesi F, Lowrie D, Cole S, Colston MJ, Matter L, Schopfer K, Bodmer T. Detection of rifampicin-resistance mutations in *Mycobacterium tuberculosis*. *Lancet* 1993;341:647–650.
18. Van Rie A, Warren R, Mshanga I, Jordaan A, van der Spuy GD, Richardson M, Simpson J, Gie RP, Enarson DA, Beyers N, et al. Analysis for a limited number of gene codons can predict drug resistance of *Mycobacterium tuberculosis* in a high-incidence community. *J Clin Microbiol* 2001;39:636–641.
19. Bártfai Z, Somoskövi A, Ködmön C, Szabó N, Puskás E, Kosztolányi L, Faragó E, Mester J, Parsons LM, Salfinger M. Molecular characterization of rifampin-resistant isolates of *Mycobacterium tuberculosis* from Hungary by DNA sequencing and line probe assay. *J Clin Microbiol* 2001;39:3736–3739.
20. Miotto P, Piana F, Penati V, Canducci F, Migliori GB, Cirillo DM. Use of genotype MTBDR assay for molecular detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* clinical strains isolated in Italy. *J Clin Microbiol* 2006;44:2485–2491.
21. Hilleman D, Rüsch-Gerdes S, Richter E. Application of the Genotype MTBDR assay directly on sputum samples. *Int J Tuberc Lung Dis* 2006;10:1057–1059.
22. Somoskovi A, Dormandy J, Mitsani D, Rivenberg J, Salfinger M. Use of smear-positive samples to assess the PCR-based genotype MTBDR assay for rapid, direct detection of the *Mycobacterium tuberculosis* complex as well as its resistance to isoniazid and rifampin. *J Clin Microbiol* 2006;44:4459–4463.
23. Kiepiela P, Bishop KS, Smith AN, Roux L, York DF. Genomic mutations in the *katG*, *inhA* and *aphC* genes are useful for the prediction of isoniazid resistance in *Mycobacterium tuberculosis* isolates from Kwa-Zulu Natal, South Africa. *Tuber Lung Dis* 2000;80:47–56.
24. Baker LV, Brown TJ, Maxwell O, Gibson AL, Fang Z, Yates MD, Drobniewski FA. Molecular analysis of isoniazid-resistant *Mycobacterium tuberculosis* isolates from England and Wales reveals the phylogenetic significance of the *ahpC*-46A polymorphism. *Antimicrob Agents Chemother* 2005;49:1455–1464.
25. Mokrousov I, Narvskaya O, Otten T, Limenschenko E, Steklova L, Vyshnevskiy B. High prevalence of *katG* Ser315Thr substitution among isoniazid-resistant *Mycobacterium tuberculosis* clinical isolates from Northwestern Russia, 1996–2001. *Antimicrob Agents Chemother* 2002;46:1417–1424.
26. Yagui M, Perales MT, Asencios L, Vergara L, Suarez C, Yale G, Salazar C, Saavedra M, Shin S, Ferrousier O, et al. Timely diagnosis of MDR-TB under program conditions: is rapid drug susceptibility testing sufficient? *Int J Tuberc Lung Dis* 2006;10:838–843.