

A performance evaluation of MTBDR_{plus} version 2 for the diagnosis of multidrug-resistant tuberculosis

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SUMMARY

OBJECTIVE: To evaluate the performance of a recently updated rapid molecular diagnostic test, GenoType[®] MTBDR_{plus} version 2, designed to detect drug resistance in both acid-fast bacilli (AFB) smear-negative and -positive specimens.

DESIGN: Sputum samples from 1128 patients at risk for multidrug-resistant tuberculosis (MDR-TB) were tested using MTBDR_{plus} v2 and compared with reference standard MGIT[™] 960[™] drug susceptibility testing. The relationship of participant human immunodeficiency virus (HIV) status, diabetic status, previous treatment, and smear gradation to the likelihood of obtaining an interpretable result was assessed using logistic regression.

RESULTS: The sensitivity and specificity of MTBDR_{plus} v2 for detecting MDR-TB, when compared to a reference

standard, were respectively 96.0% (95%CI 93.5–97.6) and 99.2% (95%CI 97.0–99.9) in AFB smear-positive specimens and 82.8% (95%CI 63.5–93.5) and 98.3% (95%CI 89.9–99.9) in AFB smear-negative specimens. A dose-response relationship was observed between the proportion of interpretable test results and AFB smear bacterial load after adjusting for age, sex, body mass index, HIV status, previous treatment and diabetic status. **CONCLUSION:** While MTBDR_{plus} v2 performs well among both AFB smear-positive and -negative specimens, smear gradation appears to influence both the probability of obtaining an interpretable result and test sensitivity, indicating a significant association between bacillary load and test performance.

KEY WORDS: diagnostic; tuberculosis; multidrug resistance

ALTHOUGH GLOBAL INCIDENCE RATES of tuberculosis (TB) have been decreasing in recent years, multidrug-resistant TB (MDR-TB), defined as resistance to both isoniazid (INH) and rifampicin (RMP), the two most commonly used drugs for first-line treatment, threatens to undermine this recent progress. According to World Health Organization (WHO) estimates, approximately 3.5% of new and 20.5% of previously treated TB cases in 2013 were MDR-TB.¹ These global estimates do not, however provide a complete picture of the MDR-TB epidemic, as subnational rates of MDR-TB have risen to as high as 35% among new cases and 75% among previously treated cases in several eastern European and central Asian countries.¹

Historically, phenotypic drug susceptibility testing (DST) of *Mycobacterium tuberculosis* on solid media took months to produce results.² The advent of broth-based media reduced the time to results to weeks, and the recent introduction of rapid molecular-based diagnostics has further reduced this time to

less than a day.^{3–5} Despite these gains, including WHO approval of the GenoType[®] MTBDR_{plus} line probe assay (Hain Lifescience, Nehren, Germany) and the Xpert[®] MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) in 2008 and 2010, gaps in MDR-TB diagnosis remain. It is estimated that, in 2013, less than half (45%) of all MDR-TB cases were detected globally.¹ Differences in diagnostic test characteristics partially explain this gap in MDR-TB diagnosis: Xpert was developed to detect RMP resistance, but does not assess INH resistance; and although the MTBDR_{plus} version 1 (v1) assay detects resistance to both RMP and INH, given its performance, the WHO recommended its use among acid-fast bacilli (AFB) smear-positive samples only.⁶

In 2012, version 2 of the MTBDR_{plus} assay was released to address performance issues associated with AFB smear status. Several small-scale studies have assessed the performance of this new assay version among homogeneous populations, but a large-scale study in a diverse population has yet to

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Article submitted 19 September 2015. Final version accepted 4 December 2015.

[A version in French of this article is available from the Editorial Office in Paris and from the Union website www.theunion.org]

be performed.^{7–9} Furthermore, the impact of AFB smear status, human immunodeficiency virus (HIV) status, body mass index (BMI), and other clinical factors associated with disease progression on the performance of MTBDR*plus* v2 have yet to be assessed.^{10–13}

Expanding the rapid testing and detection of MDR-TB cases is one of the five priority actions outlined by the WHO in its goals to address the global MDR-TB crisis.¹ Field trials of rapid point-of-care diagnostics provide valuable information for clinical decision making in resource-limited settings.¹⁴

In the present study, we describe the performance of the MTBDR*plus* v2 assay in a large diverse field trial among both AFB smear-positive and -negative specimens.

METHODS

Study design

This prospective (diagnostic) cohort study was conducted as part of the Global Consortium for Drug-resistant TB Diagnostics (GCDD), a collaboration between the University of California San Diego and study sites in India, Moldova, and South Africa. Informed consent was provided by all patients prior to study enrollment.

The study was approved by institutional review boards at the University of California, San Diego, CA, USA, as well as the participating institutions at the three study sites: the Hinduja National Hospital, Mumbai, India, the Institute of Phthisiopneumology, Chisinau, Moldova, and Stellenbosch University, Cape Town, South Africa.

Details of patient recruitment and selection have been reported previously.^{15,16} In brief, between 2012 and 2013, TB patients with risk factors for drug-resistant TB were screened at the participating study sites. Patients were eligible if they were aged ≥ 5 years and had a smear-positive AFB or positive Xpert result in the previous 14 days. In addition, patients had to meet at least one of the following criteria for suspected MDR-TB: received >1 month of treatment for a previous TB episode; failed anti-tuberculosis treatment, with positive sputum smear or culture after ≥ 3 months of standard anti-tuberculosis treatment; close contact with a known drug-resistant TB case; newly diagnosed with MDR-TB in the previous 30 days; or previously diagnosed with MDR-TB and failed anti-tuberculosis treatment, with a positive sputum smear or culture after ≥ 3 months of a standard MDR-TB treatment regimen. Patients were excluded if they were unable to provide at least 7.5 ml combined sputum or had received second-line DST results in the previous 3 months.

Specimen collection and processing

The initial sputum sample was collected by study staff at the clinic site during enrolment and the second sample by the participant themselves on waking the following morning. These two samples were pooled and used for multiple diagnostic tests, including AFB smear, MTBDR*plus* v2 line-probe assay and MGIT 960 culture (the inoculum for MGIT 960 DST). All tests were performed at the local study site laboratories according to the manufacturer's instructions or according to standard procedures.¹⁷ *M. tuberculosis* culture-positive specimens were tested for susceptibility to RMP and INH at concentrations of 1.0 and 0.1 $\mu\text{g/ml}$, respectively.¹⁸

MTBDR*plus* v2

The updated MTBDR*plus* v2 assay strip contains 27 hybridization probes. Two probes, the conjugate and amplification probes, ensure test function. Four control probes confirm the presence of *M. tuberculosis* and identify wild-type (wt) locus sequences for the *rpoB*, *katG*, and *inhA* promoter gene regions. Eight *rpoB* wt probes overlap gene regions between codons 505 and 533. Four *rpoB* mutant probes identify the specific mutations D516V, H526Y, H526D, and S531L. One wt probe is specific for the *katG*315 codon, and two mutant probes identify S315T mutations at this codon. Two *inhA* wt probes cover the *inhA* promoter from position -15 to -8 , and four mutant probes identify specific nucleic acid changes in the *inhA* promoter region (C-15T, A-16G, T-8C, and T-8A). The absence of one or more wt probes for a given resistance-associated gene region, or the absence of one or more wt probes in addition to the presence of one or more mutant probes for a given gene region, is considered indicative of resistance. Assay results are classified as indeterminate if either of the test function probes are absent; if the *M. tuberculosis* or gene loci probes are absent; or if all wt probes, in addition to mutation probes, are present (GenoType MTBDR*plus* version 2.0 instruction manual, Hain, 2012).

Data analysis

The performance of MTBDR*plus* v2 was described by computing sensitivity, specificity, positive likelihood ratio (LR+) and negative likelihood ratio (LR–) for RMP resistance, INH resistance, and MDR-TB; 95% confidence intervals (CIs) for all proportions were calculated using the Wilson Score method.¹⁹ Logistic regression was used to determine if any demographic or clinical data were associated with obtaining valid MTBDR*plus* v2 results. Analyses were performed using Stata, v 13 (StataCorp, College Station, TX, USA).

Table 1 Detection of RMP and INH resistance using MTBDRplus v2 vs. MGITTM 960TM

MTBDRplus v2	MGIT results				Total
	Resistant	Susceptible	Culture-negative	Null/not done	
RMP					
Resistant	462	7	9	0	478
Susceptible	16	324	32	3	375
Indeterminate	62	36	173	4	275
Total	540	367	214	7	1128
INH					
Resistant	478	1	5	0	484
Susceptible	31	280	33	3	347
Indeterminate	83	34	176	4	298
Total	592	315	214	7	1128

RMP = rifampicin; INH = isoniazid.

RESULTS

A total of 1128 participants were enrolled in the study. Nine hundred and fourteen (81%) were *M. tuberculosis* culture-positive, 540 (59%) of whom were phenotypically RMP-resistant and 592 (65%) were phenotypically INH-resistant. Among the 540 phenotypically RMP-resistant specimens, 462 (86%) were resistant, 16 (3%) susceptible, and 62 (11%) indeterminate on MTBDRplus v2. Among the 592 phenotypically INH-resistant specimens, 478 (82%) were classified as resistant, 31 (5%) were classified as susceptible, and 83 (14%) were indeterminate on MTBDRplus v2 (Table 1). Subanalysis revealed that among six phenotypically RMP-monoresistant specimens, two were resistant, one susceptible, and three indeterminate on MTBDRplus v2; among 58 phenotypically INH-monoresistant specimens, 32 (55%) were resistant, 15 (26%) susceptible, and 11 (19%) indeterminate on MTBDRplus v2. Culture-negative specimens were also assessed using the MTBDRplus v2 assay: 42 (20%) specimens were classified as either INH- and/or RMP-resistant or susceptible.

Overall, the sensitivity and specificity of MTBDRplus v2 when compared to culture for the detection of *M. tuberculosis* were respectively 91.8%

(95%CI 89.9–93.5) and 76.2% (95%CI 69.8–81.6). Among specimens with both valid phenotypic DST and valid MTBDRplus v2 results, the sensitivity and specificity of MTBDRplus v2 were respectively 96.7% (95%CI 94.5–98.0) and 97.9% (95%CI 95.5–99.1) for the detection of RMP resistance, 93.9% (95%CI 91.4–95.8) and 99.6% (95%CI 97.7–100.0) for INH resistance, and 95.1% (95%CI 92.6–96.9) and 99.1% (95%CI 97.1–99.8) for MDR-TB. Performance characteristics were also stratified by AFB status (Table 2) and study site (Table 3). Point estimates for both sensitivity and specificity were consistently lower for AFB smear-negative specimens than for AFB smear-positive specimens. MTBDRplus v2 sensitivity and specificity were consistent across study sites, with the exception of INH and RMP sensitivity estimates in South Africa, which were significantly lower than in India and Moldova.

Of the 914 *M. tuberculosis* culture-positive specimens, 102 (11%) were classified as indeterminate for RMP resistance and 121 (13%) as indeterminate for INH resistance on MTBDRplus v2. The absence of the *M. tuberculosis* control probe was the primary (~70%) cause of indeterminate classification. The

Table 2 Performance parameters of MTBDRplus v2 in detecting *Mycobacterium tuberculosis* RMP, INH, and MDR-TB compared to conventional DST, stratified by smear status

Drug resistance smear status	Isolates* <i>n</i>	Sensitivity % (95%CI)	Specificity % (95%CI)	LR+ % (95%CI)	LR- % (95%CI)
<i>M. tuberculosis</i>	1128	91.8 (89.8–93.5)	76.2 (69.8–81.6)	4 (3–5)	0.11 (0.09–0.14)
AFB+	826	95.6 (93.8–96.9)	74.1 (60.7–84.4)	4 (2–6)	0.06 (0.04–0.09)
AFB-	302	71.9 (63.8–78.9)	76.9 (69.4–83.1)	3 (2–4)	0.37 (0.28–0.48)
RMP-resistant	809	96.7 (94.5–98.0)	97.9 (95.5–99.1)	46 (22–95)	0.03 (0.02–0.06)
AFB+	712	97.1 (94.9–98.4)	98.5 (96.0–99.5)	65 (25–173)	0.03 (0.02–0.05)
AFB-	97	91.4 (75.8–97.8)	95.2 (85.6–98.7)	19 (6–57)	0.09 (0.03–0.27)
INH-resistant	790	93.9 (91.4–95.8)	99.6 (97.7–100)	264 (37–1867)	0.06 (0.04–0.09)
AFB+	699	94.9 (92.4–96.6)	100 (97.9–100)	∞	0.05 (0.03–0.08)
AFB-	91	81.6 (65.1–91.7)	98.1 (88.6–99.9)	43 (6–303)	0.19 (0.10–0.37)
MDR-TB	775	95.1 (92.6–96.9)	99.1 (97.1–99.8)	102 (33–316)	0.05 (0.03–0.07)
AFB+	686	96.0 (93.5–97.6)	99.2 (97.0–99.9)	126 (32–502)	0.04 (0.03–0.06)
AFB-	89	82.8 (63.5–93.5)	98.3 (89.9–99.9)	50 (7–349)	0.18 (0.08–0.39)

* Samples that did not have both interpretable phenotypic and genotypic results were excluded from the analysis.

RMP = rifampicin; INH = isoniazid; MDR-TB = multidrug-resistant tuberculosis; CI = confidence interval; LR = likelihood ratio; + = positive; - = negative; AFB = acid-fast bacilli.

Table 3 Performance parameters of MTBDR*plus* v2 in the detection of RMP, INH, and MDR-TB compared to conventional drug susceptibility testing stratified by study site

Study site	Resistance	Isolates <i>n</i>	Sensitivity % (95%CI)	Specificity % (95%CI)
India	RMP	438	98.0 (95.8–99.1)	98.8 (92.5–99.9)
	AFB+	422	98.0 (95.7–99.1)	98.7 (91.9–99.9)
	AFB–	16	100 (62.9–100)	100 (56.1–100)
	INH	433	97.3 (94.9–98.6)	100 (93.3–100)
	AFB+	418	97.5 (95.1–98.9)	100 (92.9–100)
	AFB–	15	90.9 (57.1–99.5)	100 (39.6–100)
	MDR-TB	426	96.2 (93.4–97.9)	98.8 (92.5–99.9)
	AFB+	411	96.4 (93.6–98.0)	98.7 (92.0–99.9)
Moldova	AFB–	15	88.9 (50.7–99.4)	100 (51.7–100)
	RMP	204	97.0 (90.9–99.2)	98.1 (92.5–99.7)
	AFB+	166	98.7 (92.0–99.9)	97.8 (91.4–99.6)
	AFB–	38	91.7 (71.5–98.5)	100 (73.2–100)
	INH	193	92.7 (85.7–96.6)	100 (94.5–100)
	AFB+	161	94.4 (86.8–97.9)	100 (93.7–100)
	AFB–	32	85.7 (62.6–96.2)	100 (67.9–100)
	MDR-TB	190	94.5 (87.1–98.0)	99.0 (93.7–99.9)
South Africa	AFB+	158	98.6 (91.5–99.9)	98.8 (92.8–99.9)
	AFB–	32	78.9 (53.9–93.0)	100 (71.7–100)
	RMP	167	72.2 (49.6–88.4)	97.2 (92.6–99.1)
	AFB+	124	75.0 (50.6–90.4)	99.0 (94.0–99.9)
	AFB–	43	50.0 (2.7–97.3)	92.7 (79.0–98.1)
	INH	164	61.8 (43.6–77.3)	99.2 (95.2–100)
	AFB+	120	64.3 (44.1–80.7)	100 (95.0–100)
	AFB–	44	50.0 (13.9–86.1)	97.4 (84.6–99.9)
	MDR-TB	159	77.8 (51.9–92.6)	99.3 (95.5–100)
	AFB+	117	76.5 (49.8–92.2)	100 (95.4–100)
	AFB–	42	100 (5.5–100)	97.6 (85.6–99.9)

RMP = rifampicin; INH = isoniazid; MDR-TB = multidrug-resistant tuberculosis; CI = confidence interval; AFB = acid-fast bacilli; + = positive; – = negative.

majority of the remaining specimens were classified as indeterminate due to the presence of uninterpretable banding patterns. The proportion of indeterminates did not vary significantly by study site.

Correlates of test performance

The distribution of demographic and clinical factors among valid (resistant or susceptible) vs. invalid

(indeterminate) MTBDR*plus* v2 assay results are presented in Table 4. Univariate analysis was used to assess any significant difference in variable distribution. AFB smear grade 1+ or greater was significantly associated with valid results for both RMP and INH. BMI appeared borderline significantly inversely associated with obtaining a valid result for INH, and significantly inversely associated with

Table 4 Distribution of patient demographic and clinical characteristics by validity of MTBDR*plus* v2 assay results for RMP and INH

	RMP results			INH results		
	Valid (<i>n</i> = 812) <i>n</i> (%)	Invalid (<i>n</i> = 102) <i>n</i> (%)	<i>P</i> value	Valid (<i>n</i> = 793) <i>n</i> (%)	Invalid (<i>n</i> = 121) <i>n</i> (%)	<i>P</i> value
Smear grade						
Negative (reference)	98 (12.1)	48 (47.1)		92 (11.6)	54 (44.6)	
Rare or scanty	58 (7.1)	17 (16.7)	0.12	55 (6.9)	20 (16.5)	0.13
Few (1+)	171 (21.1)	14 (13.7)	<0.001	166 (20.9)	19 (15.7)	<0.001
Many (2+)	168 (20.7)	7 (6.9)	<0.001	165 (20.8)	10 (8.3)	<0.001
TNTC (3+)	317 (39.0)	16 (15.7)	<0.001	315 (39.7)	18 (14.9)	<0.001
Age, years, median [IQR]	33 [24–45]	33 [24–47]	0.39	33 [24–45]	33 [24–46]	0.63
Male	525 (64.7)	61 (59.8)	0.34	514 (64.8)	72 (59.5)	0.26
HIV-positive (vs. negative/unknown)	99 (12.2)	14 (13.7)	0.66	97 (12.2)	16 (13.2)	0.75
Previous treatment	629 (77.5)	78 (76.5)	0.82	619 (78.1)	88 (72.7)	0.20
BMI, kg/m ² , median [IQR]	18.0 [16.0–20.4]	19.6 [17.3–21.8]	0.005	18.0 [16.0–20.4]	18.8 [16.9–21.3]	0.06
Diabetes	42 (5.2)	7 (6.9)	0.49	43 (5.4)	6 (5.0)	0.83
Site						
India (reference)	439 (53.8)	53 (52.0)		433 (54.6)	59 (48.8)	
Moldova	204 (25.1)	22 (21.6)	0.67	193 (24.3)	33 (27.3)	0.33
South Africa	169 (21.4)	27 (26.5)	0.27	167 (21.1)	29 (24.0)	0.32

RMP = rifampicin; INH = isoniazid; TNTC = too numerous to count; IQR = interquartile range; HIV = human immunodeficiency virus; BMI = body mass index.

Table 5 Logistic regression of demographic and clinical characteristics and the odds of obtaining a valid (resistant or susceptible) vs. an invalid (indeterminate) MTBDRplus v2 result

Characteristics	Model 1 OR (95%CI)	Model 2 OR (95%CI)	Model 3 OR (95%CI)
Rifampicin resistance			
Smear grade			
Negative (reference)	1.7 (0.9–3.2)	1.6 (0.9–3.1)	1.8 (0.9–3.4)
Rare	6.0 (3.1–11.4)*	6.6 (3.0–11.1)*	6.6 (3.4–12.7)*
Few	11.8 (5.1–27.0)*	11.6 (5.1–26.7)*	12.4 (5.4–28.8)*
Many	9.7 (5.3–17.8)*	8.9 (4.8–16.6)*	10.6 (5.6–20.1)*
TNTC			
BMI		0.9 (0.9–1.0)	0.9 (0.9–1.0)
Previously treated			0.6 (0.4–1.1)
HIV status			1.3 (0.7–2.7)
Diabetic status			0.7 (0.3–1.7)
Isoniazid resistance			
Smear grade			
Negative (reference)			
Rare	1.6 (0.9–3.0)	1.6 (0.9–3.0)	1.7 (0.9–3.2)
Few	5.1 (2.9–9.2)*	5.1 (2.8–9.1)*	5.3 (2.9–9.6)*
Many	9.7 (4.7–20.0)*	9.6 (4.7–19.8)*	10.0 (4.8–20.6)*
TNTC	10.3 (5.7–18.4)*	10.0 (5.5–17.9)*	10.6 (5.8–19.5)*
BMI		1.0 (0.9–1.0)	0.9 (0.9–1.0)
Previously treated			0.9 (0.5–1.4)
HIV status			1.3 (0.7–2.5)
Diabetic status			1.0 (0.4–2.5)

* *P* values meeting significance criterion with $\alpha < 0.05$.

OR = odds ratio; CI = confidence interval; TNTC = too numerous to count; BMI = body mass index; HIV = human immunodeficiency virus.

obtaining a valid result for RMP, indicating that those with a higher BMI were more likely to have an invalid test result. The likelihood of obtaining a valid test was not significantly associated with age, sex, HIV status, previous treatment, diabetic status, or study site.

Three models were generated to determine the relationship between clinical and demographic factors and the likelihood of a valid or successful test (Table 5). The first model included only AFB smear gradation, the only significant factor associated with valid results for both RMP and INH in the univariate analysis. The second model included BMI to account for any residual association of bacilli load and test performance based on the assumption that individuals with low BMI would likely produce sputum with larger amounts of bacilli due to disease progression. The third model included three more variables, HIV status, a known confounder of test performance, and previous anti-tuberculosis treatment and diabetic status, both associated with disease progression. The models were compared using the LR test; Models 2 and 3 were not statistically superior to Model 1. After controlling for smear status, BMI was no longer significantly associated with valid results in any of the models. HIV status, previous TB treatment status, BMI and diabetic status were not significantly associated with test performance (valid results). However, AFB smear grade was significantly associated with both RMP and INH test performance. Higher amounts of bacteria in the sputum, as defined by AFB smear grade, were associated with significantly more valid test results. A dose-response relationship was evident; interpretable test results

increased with each progressive AFB smear grade, culminating in an AFB smear 3+ being approximately 10 times more likely to result in an interpretable MTBDRplus v2 result than smear-negative specimens.

DISCUSSION

Overall, MTBDRplus v2 performed well among both AFB smear-positive and -negative specimens. However, the performance of the test, particularly sensitivity, was affected by AFB smear status. Sensitivity for the detection of MDR-TB among AFB smear-positive specimens was 96.0% (95%CI 93.5–97.6) compared to 82.8% (95%CI 63.5–93.5) among AFB smear-negative specimens. This difference in sensitivity between AFB smear-negative and -positive specimens was more pronounced for INH resistance (81.6%, 95%CI 65.1–91.7 vs. 94.9%, 95%CI 92.4–96.6) than for RMP resistance (91.4%, 95%CI 75.8–97.8 vs. 97.1%, 95%CI 94.9–98.4). Furthermore, the proportion of results that were valid was strongly correlated with AFB smear gradation. After adjusting for BMI, HIV status, diabetic status, and history of previous anti-tuberculosis treatment, AFB smear 3+ specimens were approximately 10 times more likely to result in a valid result than AFB smear-negative specimens.

Of the 914 culture-positive specimens, <1% of the MTBDRplus v2 test results were indeterminate due to test failure. Our observed rates of indeterminate assay results (11–13%) among culture-positive specimens were similar to those reported by the WHO (10–

14%).⁶ If the study protocol had included repeat testing for indeterminate results, indeterminate rates would most likely have been comparable to those reported by Luetkemeyer et al. among culture-positive specimens (3–5%).²⁰

M. tuberculosis was detected, as evidenced by hybridization of the *M. tuberculosis* probe, in 92% of all culture-positive specimens (both AFB smear-negative and -positive). This result is slightly higher than two smaller studies evaluating MTBDR*plus* v2 among a mix of AFB smear-positive and -negative specimens that reported sensitivities to *M. tuberculosis* of 73% and 88%.^{7,8} When comparing performance among only AFB smear-negative specimens, *M. tuberculosis* sensitivity for the present study, 74%, was lower than the 79–80% reported by Crudu et al. and higher than the 57% reported by Bernard et al.^{7,8} As a comparison, MTBDR*plus* v1, although not recommended for use on AFB smear-negative samples, had previously reported *M. tuberculosis* detection rates among exclusively AFB smear-negative specimens of 46%, 48% and 65%.^{20–22} When compared to Xpert, as reported by the Cochrane Review, the sensitivity of MTBDR*plus* v2 to *M. tuberculosis* among AFB smear-positive specimens of 92% was lower than the pooled Xpert sensitivity of 98%; however, the sensitivity of MTBDR*plus* v2 to *M. tuberculosis* among AFB smear-negative specimens of 74% was higher than the Xpert sensitivity of 67%.²³

The MTBDR*plus* v2 assay also detected *M. tuberculosis* among 24% of culture-negative specimens in the current study. Probe hybridization in these culture-negative cases may have been caused by the presence of non-viable bacteria in the specimen, or may indicate that bacteria were present in amounts too small to sustain culture growth.

Direct comparison of assay results by smear status to previously published MTBDR*plus* v2 results was difficult, as all previous studies had assessed only small numbers of drug-resistant specimens. However, the sensitivity and specificity of the test for RMP resistance in this study, stratified by smear status, was similar (within 95% CIs) to those reported by Crudu et al.⁷ The only evident difference in INH resistance performance measures between the current study and the study by Crudu et al. was the sensitivity and specificity of the test in the case of AFB smear-negative specimens. INH sensitivity and specificity among AFB smear-negative specimens in this study were respectively 81.6% (95%CI 65.1–91.7) and 98.1% (95%CI 88.6–99.9), compared to 93.5% and 82.3% reported by Crudu et al. N'guessan et al. reported RMP sensitivity and specificity only among AFB smear-positive specimens, and the only evident difference in assay performance was the sensitivity of RMP resistance (73.2%), which was significantly lower than observed in the current study (97.1%,

95%CI 94.9–98.4). The sensitivity and specificity of MTBDR*plus* v2 reported by Barnard et al. were 100% for both RMP and INH resistance, higher than both estimates from the current study.⁸

Given the absence of large diverse studies assessing the overall performance of MTBDR*plus* v2, the sensitivity and specificity for RMP and INH could only be compared to the performance of MTBDR*plus* v1, as reported by the WHO Expert Group Report and a meta-analysis of MTBDR*plus* v1 by Ling et al.^{6,24} Overall, the sensitivity for RMP resistance observed in this study (96.7%, 95%CI 94.5–98.0) was slightly lower than reported by the WHO (98.4%) and by Ling et al. (also 98.4%).^{6,24} In contrast, overall sensitivity for INH resistance in this study (93.9%, 95%CI 91.4–95.8) was slightly higher than reported by the WHO (91.4%) and by Ling et al. (88.7%). The specificities of both RMP and INH resistance in the current study were similar to those reported by the WHO and Ling et al.^{6,24}

Smear gradation was the strongest predictor for valid results (either susceptible or resistant) for either RMP and INH, even after adjusting for age, sex, BMI, previous history of anti-tuberculosis treatment, HIV status, and diabetic status. As smear positivity increased, so did the odds of obtaining a valid assay result. This correlates with both the WHO findings for version 1 of the MTBDR*plus* assay and those reported by Singhal et al. for both version 1 and 2 of the MTBDR*plus* assay, where a significant association was found between smear gradation and the proportion of interpretable or valid results.⁶

One notable limitation of this study was the lack of repeat testing of indeterminate MTBDR*plus* v2 assay results. This limitation likely contributed to a higher rate of indeterminate results than may otherwise have been reported had indeterminate MTBDR*plus* v2 assay results been repeated.

CONCLUSIONS

The MTBDR*plus* v2 assay performed well among both AFB smear-positive and -negative specimens in the current study. The sensitivity and specificity for the detection of RMP resistance, INH resistance, and MDR-TB using MTBDR*plus* v2 were respectively 96.7% (95%CI 94.5–98.0) and 97.9% (95%CI 95.5–99.1), 93.9% (95%CI 91.4–95.8) and 99.6% (95%CI 97.7–100.0), and 95.1% (95%CI 92.6–96.9) and 99.1% (95%CI 97.1–99.8). Smear gradation appeared to influence both the probability of obtaining an interpretable result and test sensitivity and specificity, indicating a significant association between bacillary load and test performance. MTBDR*plus* v2 does, however, appear to consistently provide accurate results for both RMP and INH resistance.

Acknowledgements

The authors would like to thank R L Jackson, N Hillery, and K Eisenach for their contribution to the study of laboratory data quality assurance and specimen validation.

Specimens, data and funding for this project were provided by the Global Consortium for Drug-resistant TB Diagnostics (<http://gcd.ucs.edu>). The project was funded by the National Institute of Allergy and Infectious Diseases, Bethesda, MD (National Institutes of Health [NIH] U01-AI082229, Principal Investigator A Catanzaro). MS received funding through the National Heart, Lung, and Blood Institute, Bethesda, MD (NIH T32 HL098062-05). TCR received funding through National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA (P30 AI036214-20) and received salary support from the Foundation for Innovative New Diagnostics (FIND, Geneva, Switzerland), a non-profit organization. The terms of this arrangement have been reviewed and approved by the University of California, San Diego, CA, USA, in accordance with its conflict of interest policies. TCV acknowledges support from the South African Medical Research Council Centre for TB Research, Department of Science and Technology/National Research Foundation Centre of Excellence for Biomedical Tuberculosis Research, Division of Molecular Biology and Human Genetics, Stellenbosch University (Tygerberg, South Africa) for infrastructure support.

Conflicts of interest: no other conflicts declared.

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RESUME

OBJECTIF : Evaluer la performance d'un test de diagnostic moléculaire, GenoType® MTBDRplus version 2, récemment mis à jour et conçu pour détecter la pharmacorésistance sur les frottis négatifs et positifs pour les bacilles acido-alcoolo-résistants (BAAR).

SCHEMA : Les échantillons de crachats de 1128 patients à risque de tuberculose multirésistante (TB-MDR) ont été testés par MTBDRplus v2 et comparés au standard de référence des tests de pharmacosensibilité MGIT™ 960™. Une régression logistique a permis d'évaluer la relation du statut pour le virus de l'immunodéficience humaine (VIH) du participant, de la présence d'un diabète, d'un traitement préalable et du grade du frottis avec la probabilité d'obtenir un résultat interprétable.

RÉSULTATS : La sensibilité et la spécificité de MTBDRplus v2 dans la détection de la TB-MDR, comparées à un standard de référence, ont été de 96,0%

(IC95% 93,5–97,6) et 99,2% (IC95% 97,0–99,9) pour les échantillons à frottis positif pour BAAR et de 82,8% (IC95% 63,5–93,5) et 98,3% (IC95% 89,9–99,9) pour les échantillons à frottis négatif pour BAAR, respectivement. Une relation dose-réponse a été observée entre la proportion de résultats de tests interprétables et la charge bactérienne des frottis BAAR après ajustement sur l'âge, le sexe, l'indice de masse corporelle, le statut VIH, un traitement préalable et la présence d'un diabète.

CONCLUSION : Si le MTBDRplus v2 fonctionne aussi bien parmi les frottis BAAR positifs et négatifs, la gradation des frottis paraît influencer à la fois la probabilité d'obtenir un résultat interprétable et la sensibilité du test, indiquant une association significative entre la charge bacillaire et la performance du test.

RESUMEN

OBJETIVO: Evaluar el rendimiento diagnóstico de una actualización reciente de la prueba molecular rápida GenoType® MTBDRplus, versión 2, diseñada con el fin de detectar la farmacorresistencia de *Mycobacterium tuberculosis* en muestras con resultados positivos y negativos de la baciloscopia (AFB) de esputo.

MÉTODO: Se practicó la prueba MTBDRplus v2 en muestras provenientes de 1128 pacientes en riesgo de presentar tuberculosis multidrogorresistente (TB-MDR) y se compararon los resultados con la prueba MGIT™ 960™ de referencia. Mediante un análisis de regresión logística, se examinó la relación de la situación de los pacientes frente al virus de la inmunodeficiencia humana (VIH), la diabetes, el antecedente de tratamiento y el grado de la AFB, con la posibilidad de obtener un resultado interpretable de la prueba MTBDRplus v2.

RESULTADOS: Con respecto al criterio de referencia, la prueba MTBDRplus v2 ofreció una sensibilidad de 96,0% (IC95% 93,5–97,6) y una especificidad de

99,2% (IC95% 97,0–99,9) en la detección de la TB en las muestras con AFB positiva y en las muestras con AFB negativa la sensibilidad fue 82,8% (IC95% 63,5–93,5) y la especificidad fue 98,3% (IC95% 89,9–99,9). Se observó una relación entre dosis y respuesta de la proporción de resultados interpretables de la prueba y el grado de la AFB, una vez corregidos los datos en función de la edad, el sexo, el índice de masa corporal, la situación frente al VIH, la diabetes y el antecedente de tratamiento.

CONCLUSIÓN: Si bien la prueba MTBDRplus v2 ofrece un buen rendimiento diagnóstico cuando se practica en muestras de esputo con AFB positiva o negativa, el grado de positividad de la AFB parece influir sobre la posibilidad de obtener un resultado interpretable y también sobre la sensibilidad de la prueba, lo cual habla en favor de una asociación significativa entre la carga bacilar y el rendimiento diagnóstico de la prueba.