

# Non-actionable Results, Accuracy, and Effect of First- and Second-line Line Probe Assays for Diagnosing Drug-Resistant Tuberculosis, Including on Smear-Negative Specimens, in a High-Volume Laboratory

Samantha Pillay,<sup>1,2</sup> Margaretha de Vos,<sup>1</sup> Brigitta Derendinger,<sup>1</sup> Elizabeth Maria Streicher,<sup>1</sup> Tania Dolby,<sup>2</sup> Leeré Ann Scott,<sup>1</sup> Amy Debra Steinhobel,<sup>1</sup> Rob Mark Warren,<sup>1</sup> and Grant Theron<sup>1,6</sup>

<sup>1</sup>DSI-NRF Centre of Excellence for Biomedical Tuberculosis Research, South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Stellenbosch, South Africa; and <sup>2</sup>National Health Laboratory Services, Green Point, Cape Town, South Africa

**Background.** Rapid tuberculosis (TB) drug susceptibility testing (DST) is crucial. Genotype MTBDRsl is a widely deployed World Health Organization (WHO)-endorsed assay. Programmatic performance data, including non-actionable results from smear-negative sputum, are scarce.

**Methods.** Sputa from Xpert MTB/RIF individuals (n = 951) were routinely-tested using Genotype MTBDRplus and MTBDRsl (both version 2). Phenotypic DST was the second-line drug reference standard. Discrepant results underwent Sanger sequencing.

**Findings.** 89% (849 of 951) of individuals were culture-positive (56%, 476 of 849 smear-negative). MTBDRplus had at least 1 nonactionable result (control and/or TB-detection bands absent or invalid, precluding resistance reporting) in 19% (92 of 476) of smear-negatives; for MTBDRsl, 40% (171 of 427) were nonactionable (28%, 120 of 427 false-negative TB; 17%, 51 of 427 indeterminate). In smear-negatives, MTBDRsl sensitivity for fluoroquinolones was 84% (95% confidence interval, 67%–93%), 81% (54%–95%) for second-line injectable drugs, and 57% (28%–82%) for both. Specificities were 93% (89%–98%), 88% (81%–93%), and 97% (91%–99%), respectively. Twenty-three percent (172 of 746) of Xpert rifampicin-resistant specimens were MTBDRplus isoniazid-susceptible. Days-to-second-line-susceptibility reporting with the programmatic advent of MTBDRsl improved (6 [5–7] vs 37 [35–46];  $P < .001$ ).

**Conclusions.** MTBDRsl did not generate a result in 4 of 10 smear-negatives, resulting in substantial missed resistance. However, if MTBDRsl generates an actionable result, that is accurate in ruling-in resistance. Isoniazid DST remains crucial. This study provides real-world, direct, second-line susceptibility testing performance data on non-actionable results (that, if unaccounted for, cause an overestimation of test utility), accuracy, and care cascade impact.

**Keywords.** Genotype MTBDRplus; Genotype MTBDRsl; smear-negative; TB; resistance.

Drug-resistant tuberculosis (DR-TB) is a leading cause of death. Globally, there were half a million rifampicin-resistant (RR) TB cases in 2019; 78% were estimated to be multidrug-resistant (MDR) [1]. Only 59% of RR-MDR individuals started on treatment in 2018 were treated successfully [2], partly due to the underdiagnosis of resistance to drugs other than rifampicin (RIF) such as isoniazid and the fluoroquinolones (FQs) [3, 4].

The Genotype MTBDRplus (Hain Lifesciences, Germany) and MTBDRsl (Hain Lifesciences, Germany) molecular line probe assays (LPAs) are globally used for rapid DR-TB detection. Both are World Health Organization (WHO)-endorsed and commercially available [5]. According to the Western Cape Province Department of Health TB guidelines [6], MTBDRplus is done after Xpert MTB/RIF (Xpert) to check for Xpert-detected false-positive rifampicin resistance and confirm MDR [7]. MTBDRsl is subsequently done to detect second-line resistance. One underappreciated yet important component of these workflows is that, even when an individual is confirmed as TB-positive using Xpert, the downstream reflex test must itself successfully amplify *Mycobacterium tuberculosis* complex (*Mtb*) DNA (LPAs *Mtb* detection is reported as TUB-band positivity). This applies to many reflex technologies and not just LPAs, including new drug susceptibility tests (DSTs) such as Xpert MTB/XDR [8, 9], which have yet to be available at scale.

Received 05 April 2022; editorial decision 24 June 2022; published online 5 July 2022

Correspondence: G. Theron, BMRI Building, 2nd Floor, Room 2035, Faculty of Health Sciences, Department of Molecular and Biology and Human Genetics, Stellenbosch University, Francie van Zijl Drive, Tygerberg Campus, Tygerberg 7505, South Africa (gtheron@sun.ac.za).

Clinical Infectious Diseases® 2023;76(3):e920–e9

© The Author(s) 2022. Published by Oxford University Press on behalf of Infectious Diseases Society of America. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

https://doi.org/10.1093/cid/ciac556

As frontline TB test performance improves, it can outstrip reflex tests' ability to detect TB and do DST (eg, Xpert MTB/RIF is almost always done before the LPAs, despite LPAs being an older technology) [10]. Both MTBDR*plus* and MTBDR*sl* can generate nonactionable results (indeterminate or invalid results) that are critical to report in order to quantify the overall number of drug-resistant cases missed (ie, not just due to imperfect sensitivity for resistance but also due to a failure of the test to detect TB). Such performance data that includes nonactionable results are scarce and a major limitation of the current literature. Despite increased demand for DST due to new oral regimens for RR-MDR TB (with the possibility of new FQ-based first-line regimens), MTBDR*sl* is 1 of only 2 WHO-endorsed rapid tests that can be used to confirm eligibility for these regimens.

The WHO recommends that MTBDR*plus* be used on smear-positive sputum (direct testing) and on culture isolates (indirect testing) for smear-negatives [11]. In contrast, MTBDR*sl* version 2 is recommended for direct smear-negative testing; however, evidence is of “low certainty” [5, 12], and meta-analyses have had insufficient data to create summary point estimates [13–16]. This uncertainty in performance is one reason why LPA uptake for the direct testing is suboptimal. In a global survey of 32 LPA-using laboratories, 66% and 50% tested smear-negative specimens with MTBDR*plus* and MTBDR*sl*,

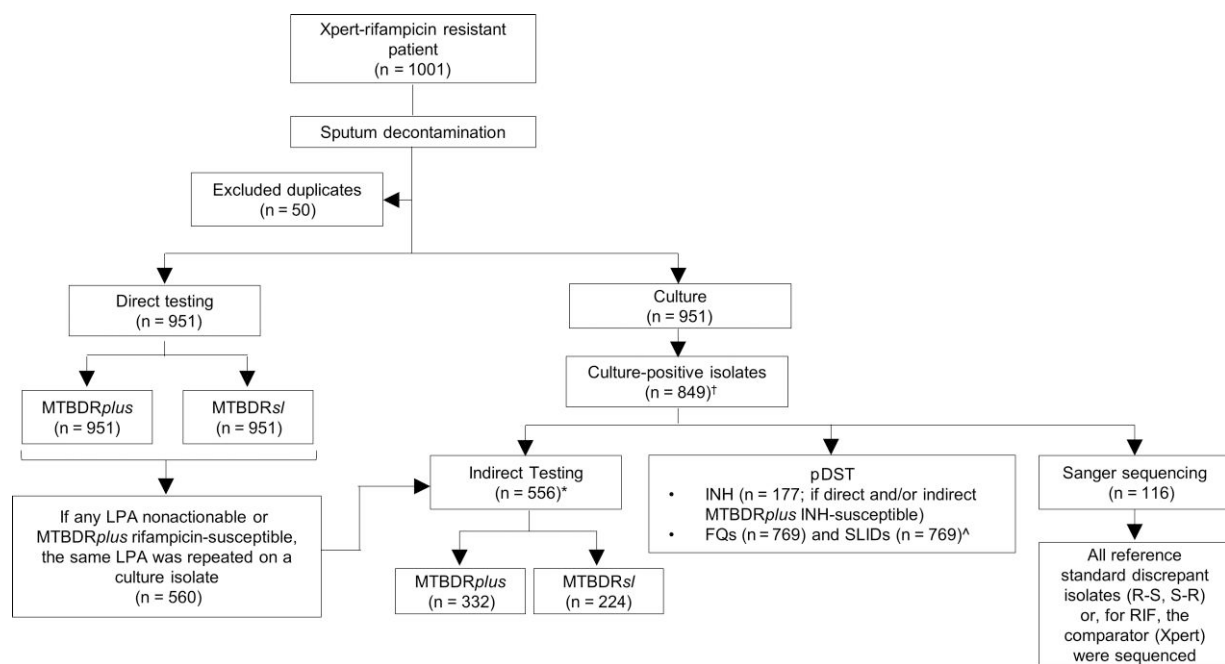
respectively [17], despite the positive WHO recommendation. Critically, more data are therefore needed.

Our overarching aim was to evaluate MTBDR*plus* (version 2) and MTBDR*sl* (version 2) performance, including in smear-negative specimens, and describe the nonactionable result rate. Importantly, we did this in a programmatic context that relies on affordable existing diagnostic tools to help guide therapeutic decisions. This approach enabled us to evaluate the association between the expansion of direct second-line DST and time to treatment and compare this to the period prior to the advent of direct second-line DST. Our intention was to provide data for laboratories and clinicians diagnosing and treating drug-resistant TB in resource-constrained settings where programmatic laboratory decisions and policies related to rapid diagnostic testing follow WHO guidance.

## METHODS

### Study Design

This study was performed in a programmatic context following the TB diagnostic algorithm in the Western Cape, South Africa (Figure 1). Direct testing was performed initially using MTBDR*plus* and MTBDR*sl* on sputum consecutively tested with no study-specific criteria between 1 June 2016 and 30 September 2019. MTBDR*plus* was performed on specimens of all smear status, defined below as the “after period.” All valid results were reported



**Figure 1.** Testing flow diagram showing direct and indirect testing using MTBDR*plus* and MTBDR*sl* and the use of reference standard phenotypic testing for second-line drugs, irrespective of the LPA result. Prior to the study, the flow of tests were the same except MTBDR*sl* was not used and MTBDR*plus* was only done directly if the specimen was smear-positive. \*4 direct nonactionables were culture-negative and unable to be tested indirectly. <sup>†</sup>102 Xpert-positives were not culture-positive and hence did not have an isolate available. <sup>^</sup>80 isolates were contaminated upon regrowth for FQ and SLID pDST. Abbreviations: FQ, fluoroquinolones; INH, isoniazid; LPA, line probe assay; MDR, multidrug-resistant; pDST, phenotypic drug susceptibility testing; R, resistant; RIF, rifampicin; S, susceptible; SLID, second-line injectable drugs; Xpert, Xpert MTB/RIF.

and reflexed for MTBDR<sub>sl</sub> testing. All TUB-band negative, indeterminate for 1 or both drugs were reported as invalid (MTBDR<sub>plus</sub>/MTBDR<sub>sl</sub>); rifampicin-susceptible results were reported as discrepant and reflexed for indirect testing using a confirmed culture-positive isolate. All culture isolate results, except Sanger sequencing, formed part of indirect diagnostic workflows including Genotype MTBDR<sub>plus</sub>, MTBDR<sub>sl</sub>, and phenotypic drug susceptibility testing (pDST), and all valid results were reported immediately. Phenotypic DST was done on specimens with valid direct and indirect LPA results. All discrepant results for MTBDR<sub>plus</sub>/MTBDR<sub>sl</sub> with reference standard pDST were resolved with repeat testing on the cultured isolate. For discrepancies that remained even after repeat testing, sequencing was performed (Figure 1).

### Sputum Collection and Preparation

In the Western Cape Province, 2 sputum samples were collected upfront for screening of presumptive TB per local guidelines [6]. Sputum processing and testing was done at the National Health Laboratory Service Green Point reference laboratory in Cape Town, South Africa. Pretreatment individuals who were first tested using Xpert MTB/RIF (version 4.3; Xpert) formed part of the then standard-of-care algorithm [18]. A paired sputum specimen from Xpert-RR individuals (n = 1001) was decontaminated using *n*-acetyl-L-cysteine-sodium hydroxide (final concentration, 1%) and the sediment resuspended in 2 mL phosphate buffer [19]. Auramine microscopy was performed. From decontaminated sputum, 0.5 mL was inoculated into a mycobacteria growth indicator tube (MGIT; Becton Dickinson) and incubated in a BACTEC MGIT960 instrument for ≤35 days (our programmatic standard of care due to space limitations).

### DNA Extraction and Line Probe Assay Testing

DNA extracted per manufacturer's guidelines [20, 21] from resuspended sputum sediments was tested directly with MTBDR<sub>plus</sub> and MTBDR<sub>sl</sub> (version 2 of both) in parallel by a single operator irrespective of smear status. The GT blot (Hain Lifesciences) and Genoscan software (GS-001, Hain Lifesciences) were used to analyze results followed by operator visual confirmation. All invalid tests (direct testing) were repeated as recommended (the repeat result was reported in analyses). For specimens (direct testing) that were TB-negative per LPAs (ie, TUB-band negative), indeterminate for at least 1 locus, or with an LPA DST result discrepant with pDST, the corresponding isolate was tested using the same LPA (indirect testing). A total of 332 and 224 isolates were tested using MTBDR<sub>plus</sub> and MTBDR<sub>sl</sub>, respectively. The manufacturer-recommended 2.2°C/s ramp rate [17, 22] and ISO15189 standards were used. Results were interpreted per Supplementary Table 1.

### TB and Phenotypic Drug Susceptibility Testing Reference Standards

MGIT960 culture positivity with MTBDR<sub>plus</sub> TUB-positivity was used for the detection of TB. Rifampicin pDST was not done. pDST was done programmatically for isoniazid, FQs,

and second-line injectable drugs. Per the algorithm, only MTBDR<sub>plus</sub> RR, isoniazid-susceptible isolates received isoniazid pDST to ensure resistance was not excluded (we are hence unable to calculate MTBDR<sub>plus</sub>'s sensitivity, specificity, and positive predictive value (PPV) for isoniazid resistance). If direct MTBDR<sub>plus</sub> was nonactionable or isoniazid susceptible, indirect MTBDR<sub>plus</sub> testing was done and, only based on this result, was isoniazid pDST done (hence, only the negative predictive value (NPV) of indirect MTBDR<sub>plus</sub> for resistance was calculable). See the Supplementary Methods for more information.

### Discrepant Analysis

Sanger sequencing was used as the composite reference standard to resolve discrepancies involving LPAs, pDST, and Xpert RR and MTBDR<sub>plus</sub> rifampicin-susceptible specimens (Supplementary Methods, Supplementary Table 6).

### Implementation and Effect of Programmatic MTBDR<sub>plus</sub> and MTBDR<sub>sl</sub> Testing

We compared the diagnostic care cascade in the “before algorithm” (2 January 2012–30 December 2015) vs the “after algorithm” (1 June 2016–30 September 2019) periods. In the before algorithm period, programmatic DST for isoniazid, FQs, and amikacin was done phenotypically. MTBDR<sub>plus</sub> (includes v1) was done routinely for both rifampicin and isoniazid directly in smear-positives or on culture isolates. In the after algorithm period, MTBDR<sub>plus</sub> and MTBDR<sub>sl</sub> (both version 2) were implemented programmatically and reported for potential patient management (see the Supplementary Methods for more detail on these periods).

### Statistical Analyses

GraphPad Prism (version 6; GraphPad Software) and Stata (version 14.0; StataCorp; 2 sample proportion test and McNemar test) were used. *P* values ≤.05 were significant.

### Ethics

This study was done in accordance with relevant guidelines and regulations approved by the Health Research Ethics Committee of Stellenbosch University and the Western Cape Province Department of Health. Permission was granted to access anonymized residual specimens collected as part of routine diagnostic practice, and informed consent waived.

## RESULTS

### Cohort Characteristics

Of 1001 Xpert RR sputa, 95% (951) were from unique patients, 89% (849) were confirmed culture-positive (93 were culture-negative and 10 culture-contaminated), and 81% (769) had a usable second-line pDST result (8%; 80 contaminated; Figure 1). Most individuals were male with smear-negative TB (Supplementary Table 2). In individuals with a known

human immunodeficiency virus (HIV) status, 50% (203 of 404) were with HIV. Those with HIV were more likely to be sputum smear-negative than those not with HIV (59%, 120 of 203 vs 48%, 110 of 230;  $P = .018$ ).

### Smear Microscopy, Culture, and Phenotypic DST Results

Among the culture-positives, 44% (373 of 849) and 56% (476 of 849) were sputum smear-positive and smear-negative, respectively. Using MTBDR<sub>plus</sub>, 21% (177 of 849) and 60% (509 of 849) were classified as rifampicin-monoresistant and MDR (Figure 2). Using MTBDR<sub>sl</sub>, 5% (42 of 769), 1% (11 of 769), and 2% (19 of 769) were FQ-resistant, second-line injectable drug (SLID)-resistant, or both FQ- and SLID-resistant, respectively (Figure 3).

### MTBDR<sub>plus</sub>

#### Nonactionables

Three percent (11 of 373) and 19% (92 of 476) of sputum smear-positives and smear-negatives had nonactionable results, respectively; of those, 70% (521 of 746) were phenotypically isoniazid resistant (Figure 2). Of the sputum smear-negative nonactionables, 18% (88 of 476) were due to a false-negative TB result and 1% (4 of 476) were due to an indeterminate call (Figure 2). Nonactionable results from indirect testing are provided in Supplementary Figure 1. No MTBDR<sub>plus</sub> invalid results occurred.

### MTB

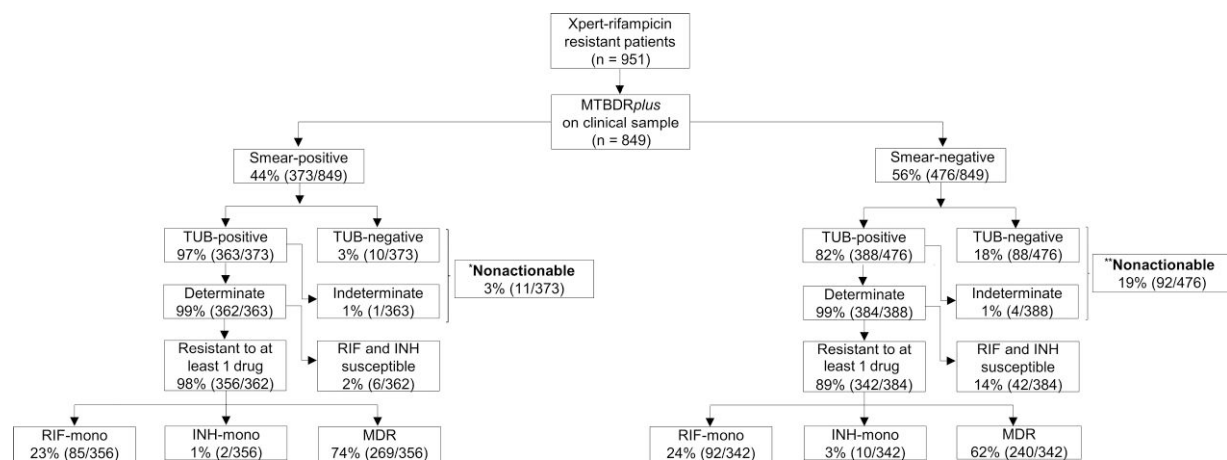
The sensitivity of MTBDR<sub>plus</sub> was 97% (363 of 373) and 82% (388 of 476;  $P < .001$ ) for sputum smear-positive and smear-negative TB, respectively (Table 1).

### Rifampicin

Ninety-one percent (686 of 746) of the Xpert RR patients whose direct MTBDR<sub>plus</sub> was actionable were MTBDR<sub>plus</sub> RR (24% [177 of 746] had MTBDR<sub>plus</sub>-defined rifampicin mono-resistance). In a head-to-head comparison of direct MTBDR<sub>plus</sub> and Xpert actionable results, 8% (60 of 746) were Xpert-resistant MTBDR<sub>plus</sub>-susceptible, with most discrepant in smear-negative TB rather than in smear-positive TB (Figure 2). Overall, of the discrepant successfully sequenced (9 culture-contaminated, 3 nonamplifiable), 85% (22 of 26) resolved in favor of Xpert (Table 2). Indirect MTBDR<sub>plus</sub> results are provided in Supplementary Figure 1.

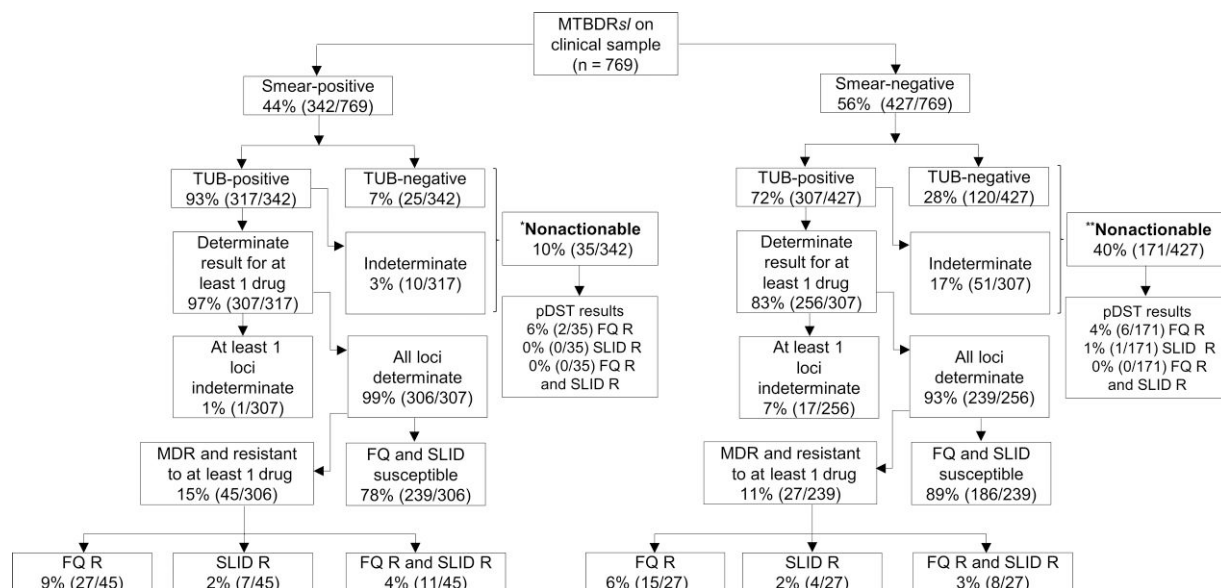
### Isoniazid

Sixty-eight percent (509 of 746) of Xpert RR patients whose direct MTBDR<sub>plus</sub> was actionable had, per MTBDR<sub>plus</sub>, MDR and 2% (12 of 746) isoniazid mono-resistance (the remainder were rifampicin-monoresistant). A total of 328 received indirect MTBDR<sub>plus</sub> testing, and 53% (177 of 328) were MTBDR<sub>plus</sub> RR, isoniazid-susceptible (Supplementary Figure 1). There were 17% (30 of 177) that were phenotypically resistant. We could only calculate MTBDR<sub>plus</sub>'s NPV for isoniazid resistance when done indirectly, which was 83% (147 of 177). When discrepant isoniazid results (indirect MTBDR<sub>plus</sub>-susceptible, pDST-resistant,  $n = 30$ ) were analyzed, 80% (24 of 30) had usable sequences. Seventy-nine percent (19 of 24), all of which were sequencing wild-type, resolved in favor of MTBDR<sub>plus</sub> (Table 2), resulting in NPV increasing to 97% (166 of 171).



**Figure 2.** Direct MTBDR<sub>plus</sub> testing of sputum is successful in almost all smear-positives and most smear-negatives; however, it fails to generate a susceptibility result in a significant minority of smear-negatives (1 in 5), indicating that a failure to detect tuberculosis is the primary cause of drug resistance being missed (ie, nonactionable results). Furthermore, a significant minority of Xpert RIF-resistant patients do not have MDR per MTBDR<sub>plus</sub>, suggesting a continued role for isoniazid drug susceptibility testing. Importantly, in patients with actionable MTBDR<sub>plus</sub> results, sensitivity and specificity for resistance did not differ by smear status. Resistance classifications on the bottom 2 rows of boxes are per direct MTBDR<sub>plus</sub>. Of the 951 Xpert rifampicin-resistant patients, only 849 were confirmed culture-positive. \*Indirect smear-positive MTBDR<sub>plus</sub> results: MDR ( $n = 7$ ), RIF-mono ( $n = 0$ ), INH-mono ( $n = 1$ ), fully susceptible ( $n = 3$ ), and nonactionable ( $n = 0$ ). \*\*Indirect smear-negative MTBDR<sub>plus</sub> results: MDR ( $n = 69$ ), RIF-mono ( $n = 0$ ), INH-mono ( $n = 3$ ), fully susceptible ( $n = 20$ ), and nonactionable ( $n = 0$ ). Abbreviations: INH, isoniazid; mono, mono-resistant; MDR, multi-drug-resistant; RIF, rifampicin; TUB, TUB-band; Xpert, Xpert MTB/RIF.





**Figure 3.** Although direct MTBDRs/ testing of sputum is successful in most patients, it results in relatively high proportions of nonactionable results in smear-positives and especially in smear-negatives. MTBDRs/ failed in 4 of 10 smear-negative patients with Xpert-diagnosed rifampicin resistance. As seen for MTBDR*plus*, a failure to generate an actionable result on smear-negatives was the primary cause of missed resistance (as opposed to a false-negative susceptible result). Resistance classifications on the bottom 2 rows of boxes are per direct MTBDRs/. Of the 849 culture-positive patients, only 769 had usable pDST (80-contaminated). \*Indirect smear-positive MTBDRs/ results: FQ-R (n = 3), SLID-R (n = 0), FQ-R and SLID-R (n = 0), fully susceptible (n = 33), and nonactionable (n = 0). \*\*Indirect smear-negative MTBDRs/ results: FQ-R (n = 7), SLID-R (n = 4), FQ-R and SLID-R (n = 2), fully susceptible (n = 175), and nonactionable (n = 0). Abbreviations: FQ, fluoroquinolones; MDR, multidrug-resistant; pDST, phenotypic drug susceptibility testing; R, resistant; SLID, second-line injectable drug; TUB, TUB-band.

## MTBDRs/

### Nonactionable

When done directly, 10% (35 of 342) of sputum smear-positives and 40% (171 of 427) of smear-negatives were nonactionable (Figure 3). In addition, 4% (8 of 206), 0% (1 of 206), and 0% (0 of 206) of nonactionables were phenotypically resistant to FQs, SLIDs, or both FQs and SLIDs, respectively. Like MTBDR*plus* on sputum smear-negatives, most MTBDRs/ smear-negative results were nonactionable due to a false-negative TB result (28%, 120 of 427) or an indeterminate result (17%, 51 of 427; Figure 3). A total of 28 MTBDRs/ results were initially invalid prior to pDST (1%, 2 of 373 for sputum smear-positives vs 5%, 26 of 476 for sputum smear-negatives;  $P < .001$ ; Supplementary Table 3), but all resolved upon retesting (and were hence ultimately not nonactionable). No indirect nonactionable results occurred (Supplementary Figure 2).

### MTB

Sensitivity was 93% (347 of 373) and 73% (349 of 476;  $P < .001$ ) for sputum smear-positive and smear-negative specimens, respectively (Table 1), and less than MTBDR*plus* in the same individuals (97%; 95% confidence interval [CI], 94%–98% vs 93%; 95% CI, 90%–95%;  $P < .001$ ) for sputum smear-positives and (82%; 95% CI, 77%–84% vs 73%; 95% CI, 69%–77%;  $P < .001$ ) for smear-negatives.

## Fluoroquinolones

For direct sputum smear-positive and smear-negative testing, sensitivities were 89% (40 of 45) and 84% (31 of 37;  $P = .105$ ) and specificities were 92% (180 of 195) and 93% (117 of 126;  $P = .855$ ), respectively (Table 1, Figure 4). For indirect testing, sensitivity was 92% (12 of 13) and specificity was 100% (211 of 211; Supplementary Table 4). When discrepant FQ results from direct testing were analyzed (MTBDRs/-resistant pDST-susceptible,  $n = 24$ ; MTBDRs/-susceptible pDST-resistant,  $n = 11$ ), 83% (29 of 35) generated usable sequences. Sixty-nine percent (20 of 29) of discrepancies favored MTBDRs/ and 31% (9 of 29) favored pDST (Table 3). MTBDRs/ falsely reported 2 specimens with *gyrA* S95T natural polymorphisms [24] as resistant through the absence of a wild-type band (WT3, MUT3C). After following discrepant analysis reclassification, sensitivities and specificities increased (Figure 4, Supplementary Table 5).

## Second-Line Injectable Drugs

For direct testing in sputum smear-positives and smear-negatives, sensitivities were 86% (19 of 22) and 81% (13 of 16;  $P = .011$ ), respectively, and specificities were 97% (205 of 212) and 88% (112 of 127;  $P = .002$ ), respectively (Table 1, Figure 3). For indirect testing, sensitivity was 100% (6 of 6) and specificity was 100% (218 of 218; Supplementary Table 4, Figure 4). When direct MTBDRs/-pDST discrepant results (MTBDRs/-resistant

**Table 1. Accuracy of Direct MTBDR<sub>plus</sub> and MTBDR<sub>s/l</sub> Testing for Tuberculosis and Phenotypic Second-line Drug Resistance in Sputum of Xpert-Positive Rifampicin-Resistant Patients**

Assay		Overall		Smear-Positive		Smear-Negative	
		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
MTBDR <sub>plus</sub>	Tuberculosis	88 (751/849) 86–90	43 (40/93) 32–53	97 (363/373) 94–98	36 (4/11) 10–69	82 (388/476) 77–84 <sup>a</sup> <b>P &lt; .001</b>	44 (36/82) 32–54 <sup>a</sup> P = .635
MTBDR <sub>s/l</sub>	Tuberculosis	82 (696/849) 79–84 <sup>b</sup> <b>P &lt; .001</b>	51 (47/93) 32–54 <sup>b</sup> P = .303	93 (347/373) 90–95 <sup>b</sup> P = .006	73 (8/11) 39–93 <sup>b</sup> P = .086	73 (349/476) 69–77 <sup>a</sup> <b>P &lt; .001</b> <sup>b</sup> P = .002	48 (39/82) 36–58 <sup>a</sup> P = .117 <sup>b</sup> <b>P &lt; .001</b>
	Fluoroquinolones	87 (71/82) 77–93	93 (297/321) 90–96	89 (40/45) 75–96	92 (180/195) 88–96	84 (31/37) 67–93 <sup>a</sup> P = .105	93 (117/126) 89–98 <sup>a</sup> P = .855
	Second-line injectable drugs	84 (32/38) 68–93 <sup>c</sup> P = .720	94 (317/339) 90–95 <sup>c</sup> P = .820	86 (19/22) 65–97 <sup>c</sup> P = .001	97 (205/212) 93–98 <sup>c</sup> P = .108	81 (13/16) 54–95 <sup>a</sup> P = .011 <sup>c</sup> P = .821	88 (112/127) 81–93 <sup>a</sup> P = .002 <sup>c</sup> P = .052
	Fluoroquinolone and second-line injectable drugs	70 (19/27) 69–98	97 (257/264) 94–98	85 (11/13) 54–98	97(165/169) 94–99	57 (8/14) 28–82 <sup>a</sup> P = .118	97 (92/95) 91–99 <sup>a</sup> P = .701

Data are % (n/N), 95% confidence interval. All P values which were statistically significant appeared in bold.

<sup>a</sup>Within-row comparisons between smear statuses.

<sup>b</sup>Within-column comparisons for MTBDR<sub>s/l</sub> vs MTBDR<sub>plus</sub>.

<sup>c</sup>Within-column comparisons for second-line injectable drugs vs fluoroquinolones.

pDST-susceptible, n = 22 and MTBDR<sub>s/l</sub>-susceptible pDST-resistant, n = 6) were analyzed, 43% (12 of 28) had sequenceable isolate DNA. In contrast to FQs, most discrepancies (67%, 8 of 12)

resolved in favor of pDST (Table 3, Supplementary Table 7). Following reclassification, sensitivity and specificity increased (Figure 4, Supplementary Table 5).

**Table 2. Sequencing of MTBDR<sub>plus</sub> Targets (*rpoB*, *katG*, *inhA* Promoter Region) Done to Resolve Discrepant Results Either Between MTBDR<sub>plus</sub> and Xpert (Rifampicin) or MTBDR<sub>plus</sub> and Phenotype (Isoniazid)**

Locus	MTBDR <sub>plus</sub>	Comparator Result	Mutation	No. of Isolates	Sequencing			Resolved in Favor of
					No. With Heteroresistance	Susceptibility Result	Probe Assay or Comparator	
Rifampicin <i>rpoB</i> <sup>a</sup> (n = 29)	S	R	S531L	8	1	R	Xpert	
	...	...	H526Y	2	0	R	Xpert	
	...	...	D516V	3	1	R	Xpert	
	...	...	Q513P	1	0	R	Xpert	
	...	...	L511P <sup>b</sup>	8 (1 Double mutant with D485N)	1	R	Xpert	
	...	...	WT	4	0	S	MTBDR <sub>plus</sub>	
...	...	NR	3	...	...	...		
Discrepant resolution by sequencing				85% (22/26) resistant (resolved in favor of Xpert) 15% (4/26) susceptible (resolved in favor of MTBDR <sub>plus</sub> )				
Isoniazid <i>katG</i> <sup>c</sup> (n = 24)	S	R	G312C	1	...	R	pDST	
	...	...	S315T	3	...	R	pDST	
	...	...	WT	19	...	S	MTBDR <sub>plus</sub>	
	...	...	NR	1	NR	NR	NR	
<i>inhA</i> promoter <sup>c</sup> (n = 24)	S	R	-8 T/C WT	1 23	...	R, S	pDST MTBDR <sub>plus</sub>	
	Discrepant resolution by sequencing				21% (5/24) resistant (resolved in favor of pDST) 79% (19/24) susceptible (resolved in favor of MTBDR <sub>plus</sub> )			

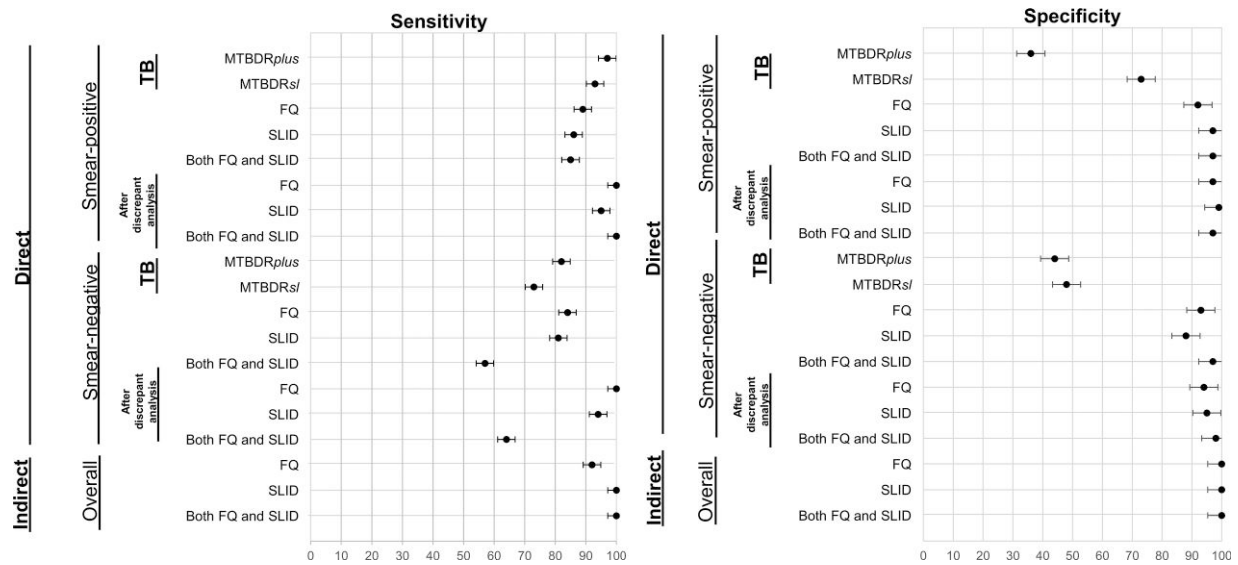
Sequencing suggested Xpert is more sensitive for rifampicin resistance than MTBDR<sub>plus</sub>. MTBDR<sub>plus</sub> detected mutations known to cause isoniazid resistance better than pDST. See Supplementary Methods for how line probe assay results were categorized as discrepant.

Abbreviations: NR, not reportable (did not amplify for sequencing); pDST, phenotypic drug susceptibility testing; R, resistant; S, susceptible; WT, wild type; Xpert, Xpert MTB/RIF.

<sup>a</sup>Only Xpert rifampicin-resistant and MTBDR<sub>plus</sub> rifampicin-susceptible discrepant drug were sequenced from the isolate.

<sup>b</sup>L511P is considered borderline by the World Health Organization, which recommends that people found with this mutation be classified as resistant [23].

<sup>c</sup>Discrepant isolates sequenced included only MTBDR<sub>plus</sub>-susceptible that were phenotypic-resistant (due to contemporaneous programmatic algorithm).



**Figure 4.** Selected summary forest plots showing sensitivity and specificity estimates (with 95% confidence intervals) for MTBDR*plus* and MTBDR*sl*. Importantly, only patients first detected as TB positive (top 2 rows) can generate an actionable line probe assay drug susceptibility testing result. Estimates for smear-negatives were lower than for smear-positives and, overall, estimates for SLIDs were lower than for FQs. All estimates improved in favor of LPAs after discrepant resolution. Abbreviations: FQ, fluoroquinolones; SLID, second-line injectable drugs; TB, tuberculosis.

**Table 3. Sequencing of MTBDR*sl* Targets (*gyrA*, *rrs*) to Resolve Results Discrepant With Phenotypic Drug Susceptibility Testing**

	Locus	MTBDR <i>sl</i>	pDST	Mutation	No. of Isolates	Susceptibility Result	Sequencing	
							Resolved in Favor of Line Probe Assay or pDST	
Fluoroquinolones	<i>gyrA</i> (n = 11)	S	R	G81C <sup>a</sup>	1	S	MTBDR <i>sl</i>	
	...	...	...	A88T	1	R	pDST	
	...	...	...	WT	9	S	MTBDR <i>sl</i>	
	(n = 24)	R	S	A88T	1	R	MTBDR <i>sl</i>	
	...	...	...	C86T	1	R	MTBDR <i>sl</i>	
	...	...	...	D89N	1	R	MTBDR <i>sl</i>	
	...	...	...	A90V	4	R	MTBDR <i>sl</i>	
	...	...	...	S91P	1	R	MTBDR <i>sl</i>	
	...	...	...	D94G	2	R	MTBDR <i>sl</i>	
	...	...	...	S95T <sup>b</sup>	2	S	pDST	
...	...	...	WT	6	S	pDST		
...	...	...	NR	6	...	...		
Discrepant resolution by sequencing					69% (20/29) in favor of MTBDR <i>sl</i> 31% (9/29) in favor of pDST			
Second-line injectable drugs	<i>rrs</i> (n = 6)	S	R	WT	3	...	S	MTBDR <i>sl</i>
	...	...	...	NR	3	...	...	...
	(n = 22)	R	S	WT	8	...	S	pDST
	...	...	...	A1401G	1	...	R	MTBDR <i>sl</i>
	...	...	...	NR	13	...	...	...
	...	...	...	...	...	...	...	...
Discrepant resolution by sequencing					33% (4/12) in favor of MTBDR <i>sl</i> 67% (8/12) in favor of pDST			

Most fluoroquinolone discrepant results resolved in favor of MTBDR*sl*, whereas most second-line injectable drug discrepant results resolved in favor of pDST.

Abbreviations: NR, number of specimens that did not amplify for sequencing; pDST, phenotypic drug susceptibility testing; R, resistant; S, susceptible; WT, wild-type.

<sup>a</sup>G81C, silent mutation.

<sup>b</sup>S95T, does not cause resistance [23, 24].

**Table 4. Comparison of Key Care Cascade Gaps for the Diagnosis of Drug Resistance Before and After the Implementation of Improved Molecular Diagnostics for Resistance Beyond Rifampicin**

...	Retrospective Period MTBDR <sub>plus</sub> Only on Smear-Positives Second-line DST by pDST Only			Prospective Period MTBDR <sub>plus</sub> and MTBDRs/ Irrespective of Smear Status Second-line pDST Still Done		
	Overall (n = 2938)	Smear-Positive (n = 1674)	Smear-Negative (n = 1264)	Overall (n = 799)	Smear-positive (n = 416)	Smear-negative (n = 383)
On treatment without receiving any second-line DST	23 (668/2938)	21 (357/1674)	25 (311/1264) <sup>a</sup> P = .358	5 (40/799) <sup>b</sup> P < .001	2 (7/416) <sup>b</sup> P < .001	9 (33/383) <sup>b</sup> P < .001
MTBDR <sub>plus</sub> direct testing	N/A	100 (1674/1674)	N/A	100 (799/799)	100 (416/416)	100 (383/383)
With an actionable result	N/A	79 (1317/1674)	N/A	99 (797/799)	100 (416/416)	99 (381/383) <sup>a</sup> P = .140
Without an actionable result	N/A	21 (357/1674)	N/A	0 (2/799)	0 (0/416)	1 (2/383)
MTBDRs/ direct testing	N/A	N/A	N/A	100 (799/799)	100 (416/416)	100 (383/383)
With an actionable result	N/A	N/A	N/A	78 (622/799)	91 (380/416)	63 (242/383) <sup>a</sup> P < .001
Without an actionable result	N/A	N/A	N/A	22 (177/799)	9 (36/416)	37 (141/383) <sup>a</sup> P < .001
Days to result (actionable or nonactionable)	N/A	N/A	N/A	6 (5–7)	6 (5–7)	6 (5–7) <sup>a</sup> P < .001
MTBDRs/ indirect testing	N/A	N/A	N/A	22 (177/177)	9 (36/36)	37 (141/141)
With an actionable result	N/A	N/A	N/A	22 (177/177)	9 (36/36)	37 (141/141)
Without an actionable result	N/A	N/A	N/A	0	0	0
Days to result (actionable or nonactionable)	N/A	N/A	N/A	22 (16–26)	16 (13–22)	22 (18–27) <sup>a</sup> P = .081
pDST	77 (2270/2938)	79 (1317/1674)	75 (953/1264) <sup>a</sup> P = .358	94 (750/799) <sup>b</sup> P < .001	96 (400/416) <sup>b</sup> P < .001	91 (350/383) <sup>a</sup> P = .500 <sup>b</sup> P < .001
Days to result (interquartile range)	37 (35–46)	33 (29–38)	42 (36–50) <sup>a</sup> P < .001	30 (27–36) <sup>b</sup> P < .001	28 (25–35) <sup>b</sup> P < .001	34 (30–40) <sup>a</sup> P < .001 <sup>b</sup> P < .001
Overall, second-line DST	...	...	...	...	...	...
Patients who required second-line DST on isolates (indirect MTBDRs/ or pDST) when direct MTBDRs/ was nonactionable	0	0	0	22 (177/799)	9 (36/416)	37 (141/383) <sup>a</sup> P < .001
Days to first actionable second-line DST result (direct MTBDRs/, indirect MTBDRs/, or pDST)	37 (35–46)	33 (29–38)	42 (36–50) <sup>a</sup> P < .001	6 (5–7)	6 (5–7)	6 (5–7) <sup>a</sup> P < .001

Implementation of first-line MTBDR<sub>plus</sub> testing on Xpert rifampicin-resistant sputum to include smear-negatives and MTBDRs/ testing on all sputum resulted in a greater proportion of patients receiving second-line DST, reduced reliance on culture, and reduced turnaround time. The Supplementary Methods section contains more information on these periods. Data are median (interquartile range) or % (n/N). All P values which were statistically significant appeared in bold.

Abbreviations: DST, drug susceptibility testing; N/A, nonapplicable; pDST, phenotypic drug susceptibility testing.

<sup>a</sup>Comparisons within rows and between columns by same smear status.

<sup>b</sup>Comparisons within rows in retrospective vs prospective periods.

### Joint FQ and SLID Resistance

For sputum smear-positives and smear-negatives, direct sensitivities were 85% (11 of 13) and 57% (8 of 14;  $P = .118$ ), respectively, and specificities were 97% (165 of 169) and 97% (92 of 95;  $P = .701$ ), respectively (Table 1, Figure 3). Indirect testing sensitivity and specificity were very high (Supplementary Table 4, Figure 4). Like that observed for the individual drug classes, after discrepancy resolution, MTBDRs/ sensitivity and specificity increased (Supplementary Table 5).

### Diagnosis Care Cascade Gaps in Before and After Periods

We compared programmatic data from the period immediately preceding the study (before period when MTBDR<sub>plus</sub> was the only LPA done directly, only on sputum smear-positives, and the only second-line testing was pDST) to a similar period after

the start of study testing (after period; both LPAs were done, at a minimum, directly and reported for routine patient management). With MTBDRs/ implementation, the proportion of individuals on treatment without second-line DST results decreased from 23% (668 of 2938) to 5% (40 of 799;  $P < .001$ ; Table 4), and second-line DST results were available more quickly (33 [29–38] to 16 [13–22] days for smear-positives and 42 [36–50] to 22 [18–27] days for smear-negatives), even after factoring in many smear-negatives with direct nonactionable results that required subculture for further testing compared with smear-positives (37%, 143 of 383 vs 9%, 36 of 416;  $P < .001$ ; Table 4).

### DISCUSSION

There are limited data on nonactionable results, accuracy, and effect of rapid molecular assays for the diagnosis of resistance



beyond rifampicin, especially on smear-negative sputum. To address this, we performed a large-scale evaluation of the newest-generation LPAs in a routine programmatic setting, did comprehensive reference standard testing, and compared care cascade data before and after. Definitive data on MTBDRs/ performance on smear-negative specimens is essential as the need for FQ susceptibility testing increases and new tools such as Xpert MTB/XDR remain expensive (cost per cartridge \$19.80, at least \$3860 to upgrade existing modules [25]).

Our key findings include that 19% and 40% of smear-negative individuals tested by MTBDRplus and MTBDRsl were nonactionable, respectively, resulting in many individuals with resistance missed; about 25% of Xpert RR patients have MTBDRplus-defined isoniazid susceptibility; and deployment of direct LPA testing was associated with improvements in days to diagnosis, more individuals receiving DST, and reduced culture reliance.

MTBDRsl had almost double the nonactionable result rate of MTBDRplus in smear-negatives for TB detection, causing diagnostic and treatment delays. Our data highlight the suboptimal ability of reflex DSTs to detect TB even in individuals already identified as TB-positive by frontline tests. This information loss will persist as the limit of detection of new frontline tests outstrips that of reflex tests (Xpert MTB/RIF Ultra vs Xpert MTB/XDR). We recommend that all studies that evaluate reflex test report this key metric (nonactionable results).

In Xpert RR specimens that were MTBDRplus rifampicin-susceptible, Xpert was correct more frequently than MTBDRplus [26, 27]. Possible reasons include heteroresistance and variants not included in MTBDRplus. These findings question diagnostic algorithms that use MTBDRplus to confirm Xpert-detected rifampicin resistance [7, 27, 28].

Importantly, MTBDRplus has value for isoniazid-susceptibility detection. Our data suggest that isoniazid is likely effective in 25% of Xpert RR individuals. In agreement with that observed in the Democratic Republic of the Congo [29] and Iran [30], we recommend that RR TB not be automatically assumed to be MDR and all Xpert RR individuals receive isoniazid DST (which should always be done as isoniazid resistance prevalence is globally in excess of that of rifampicin [31]).

Fluoroquinolones are key components of new regimens, and SLIDs such as amikacin remain important. Although important new tools such as Xpert MTB/XDR are emerging [32], MTBDRsl is already established in many laboratories worldwide. The sensitivity and specificity for FQ on smear-negatives were 84% and 93%, respectively. High MTBDRsl sensitivity (81%) was observed on smear-negatives for SLID; however, specificity was less (88%); both improved after discrepant analysis. Importantly, in contrast to FQs, most SLID MTBDRsl-pDST discrepant results resolved in favor of pDST-confirmed susceptibility.

In the after period, we found significant improvements in the proportion of people who had any second-line DST results

(such individuals are thus more likely to start effective treatment) and time to result. Such real-world data regarding the programmatic impact of TB diagnostics are scarce but important. With the scale-up of second-line LPAs, individual with smear-negative TB still suffered from unacceptably long times to diagnosis. This subset of individuals should be targeted for interventions in order to accelerate treatment initiation, such as new expensive assays such as Xpert MTB/XDR or Deeplex Myc-TB (Genoscreen) [8, 33].

A strength and limitation of our study is the programmatic context of the study, permitting it to be large and the results reported for potential patient management within the South African care cascade. However, this meant that the study was constrained by contemporary diagnostic algorithms, which affected specimen and meta-data availability given the suboptimal quality of care common in high-volume resource-scarce settings.

Time to DST results associated with LPA scale-up may vary across other provinces within South Africa as, unlike in the Western Cape, only 1 specimen is collected initially for presumptive TB and a second sputum specimen is dependent on an individual returning to a clinic (this may affect generalizability). We were unable to do pDST for rifampicin and isoniazid; however, our primary objective was to evaluate LPA performance for second-line drugs. We also did targeted sequencing rather than whole-genome sequencing, and discrepant analyses may have missed rare non-canonical variants; however, WHO-recommended second-line pDST was done in all isolates [34].

LPA use in our programmatic laboratory was associated with improvements in the care cascade, and patient-important outcomes remained suboptimal. Until next-generation reflex DSTs are widely available, expanded LPA testing remains key to the successful scale-up of new regimens, despite important paucibacillary specimen performance caveats.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

**Author Contributions.** S. P., M. dV., G. T., and R.W. conceptualized the experiments. T. D., S. P., B. D., and R.V. assisted with data curation. S.P. performed formal analysis and methodology and wrote the original draft. A. D. S., L. A. S., and E. S. assisted S.P. with the investigation. All authors reviewed and edited the manuscript.

**Acknowledgments.** The authors thank the National Health Laboratory and Hain Lifesciences. The authors thank Dr Rouxjeane Venter for help with patient data collection.

**Disclaimer.** Hain Lifesciences donated MTBDRsl kits but had no role in study design or results interpretation.

**Financial support.** This work was supported by the Stellenbosch University Faculty of Health Sciences, the National Research Foundation, and Harry Crossley. R. M. W. acknowledges funding from the South

African Medical Research Council. G. T. acknowledges funding from the EDCTP2 Programme supported by the European Union (SF1401, OPTIMAL DIAGNOSIS), and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (U01AI152087).

**Potential conflicts of interest.** The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

## References

1. World Health Organization. Global Tuberculosis Report. Geneva, Switzerland: 2020.
2. World Health Organization. Global Tuberculosis Report. Geneva, Switzerland: 2021.
3. Naidoo P, Theron G, Rangaka MX, et al. The South African tuberculosis care cascade: estimated losses and methodological challenges. *J Infect Dis* 2017; 216: S702–S13.
4. Cox H, Dickson-Hall L, Ndjeka N, et al. Delays and loss to follow-up before treatment of drug-resistant tuberculosis following implementation of Xpert MTB/RIF in South Africa: a retrospective cohort study. *PLoS Medicine* 2017; 14:e1002238.
5. World Health Organization. The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: policy guidance. Geneva, Switzerland: 2016.
6. Director AC, Kruger MJ. Clinical Guidelines & Standard Operating Procedure for the Implementation of the Short & Long DR-TB regimens for Adults, Adolescents and Children. Published by National Department of Health. Available from: [https://www.westerncape.gov.za/assets/departments/health/tuberculosis\\_-\\_dr\\_tb\\_clinical\\_guidelines\\_2018.pdf](https://www.westerncape.gov.za/assets/departments/health/tuberculosis_-_dr_tb_clinical_guidelines_2018.pdf).
7. Beylis N, Ghebrekristos Y, Nicol M, et al. Management of false-positive rifampicin resistant Xpert MTB/RIF. *Lancet Microbe* 2020; 1:e238.
8. World Health Organization. World Health Organization consolidated guidelines on tuberculosis: module 3: diagnosis-rapid diagnostics for tuberculosis detection: web annex 4: evidence synthesis and analysis. Geneva, Switzerland: 2021.
9. Penn-Nicholson A, Georghiou SB, Ciobanu N, et al. Detection of isoniazid, fluoroquinolone, ethionamide, amikacin, kanamycin, and capreomycin resistance by the Xpert MTB/XDR assay: a cross-sectional multicentre diagnostic accuracy study. *Lancet Infect Dis* 2022;22:242–9.
10. García-Basteiro AL, DiNardo A, Saavedra B, et al. Point of care diagnostics for tuberculosis. *Pulmonology* 2018; 24:73–85.
11. Samra Z, Kaufman L, Bechor J, et al. Comparative study of three culture systems for optimal recovery of mycobacteria from different clinical specimens. *Eur J Clin Microbiol Infect Dis* 2000; 19:750–4.
12. World Health Organization. WHO consolidated guidelines on drug-resistant tuberculosis treatment. Geneva, Switzerland: World Health Organization; 2016.
13. Theron G, Peter J, Richardson M, et al. The diagnostic accuracy of the GenoType® MTBDRsl assay for the detection of resistance to second-line anti-tuberculosis drugs. *Cochrane Database Syst Rev* 2014; CD010705.
14. Bai Y, Wang Y, Shao C, et al. Genotype MTBDRplus assay for rapid detection of multidrug resistance in *Mycobacterium tuberculosis*: a meta-analysis. *PLoS One* 2016; 11:e0150321.
15. Drobniowski F CM, Jordan J, et al. Systematic review, meta-analysis and economic modelling of molecular diagnostic tests for antibiotic resistance in tuberculosis. Southampton (UK): NIHR Journals Library. (Health Technology Assessment, No. 19.34.) Chapter 3, Systematic review. Available from: <https://www.ncbi.nlm.nih.gov/books/.NBK293793>. 2015.
16. Theron G, Peter J, Richardson M, et al. Genotype® MTBDRsl assay for resistance to second-line anti-tuberculosis drugs. *Cochrane Database Syst Rev* 2016; 9: CD010705.
17. Derendinger B, De Vos M, Nathavitharana R, et al. Widespread use of incorrect PCR ramp rate negatively impacts multidrug-resistant tuberculosis diagnosis (MTBDR plus). *Sci Rep* 2018; 8:3206.
18. National Institute for Communicable Diseases Division of National Health Laboratory Service. South African Tuberculosis Drug Resistant Survey. 2015 [cited 2022 July 14]. Available from: [http://www.nicd.ac.za/assets/files/K-12750%20NICD%20National%20Survey%20Report\\_Dev\\_V11-LR.pdf](http://www.nicd.ac.za/assets/files/K-12750%20NICD%20National%20Survey%20Report_Dev_V11-LR.pdf).
19. Kent PT, Kubica GP, et al. Public health mycobacteriology: a guide for the level III laboratory. Atlanta, GA: Centers for Disease Control and Prevention; 1985.
20. Hain Lifescience GmbH. Genotype MTBDRplus version 2.0: instruction manual. Germany: Hain Lifescience GmbH, Nehren, 2012.
21. Hain Lifescience GmbH. Genotype MTBDRsl version 2.0: instruction manual. Germany: Hain Lifescience GmbH, Nehren; 2015.
22. Derendinger B, de Vos M, Pillay S, et al. Frequent suboptimal thermocycler ramp rate usage negatively impacts MTBDRsl performance for second-line drug resistant tuberculosis diagnosis. *J Mol Diagn* 2022; 24:494–502.
23. World Health Organization, Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance. World Health Organization Geneva. 2021.
24. Miotto P, Tessema B, Tagliani E, et al. A standardised method for interpreting the association between mutations and phenotypic drug resistance in *Mycobacterium tuberculosis*. *Eur J Respir J* 2017; 50:1.
25. Naidoo K, Dookie N, et al. Can the GeneXpert MTB/XDR deliver on the promise of expanded, near-patient tuberculosis drug-susceptibility testing? *Lancet Infect Dis* 2022; 22:e121–7.
26. Van Rie A, Whitfield MG, De Vos E, et al. Discordances between molecular assays for rifampicin resistance in *Mycobacterium tuberculosis*: frequency, mechanisms and clinical impact. *J Antimicrob Chemother* 2020; 75:1123–9.
27. Ngabonziza JCS, Decroo T, Migambi P, et al. Prevalence and drivers of false-positive rifampicin-resistant Xpert MTB/RIF results: a prospective observational study in Rwanda. *Lancet Microbe* 2020; 1:e74–83.
28. Ghebrekristos Y. Characterization of *Mycobacterium tuberculosis* isolates with discordant rifampicin susceptibility test results. University of Cape Town, South Africa. 2018. Available at: <https://open.uct.ac.za/handle/11427/29248>.
29. Bisimwa BC, Nachega JB, Warren RM, et al. Xpert MTB/RIF-detected Rifampicin Resistance is a Sub-Optimal Surrogate for Multidrug Resistant Tuberculosis in Eastern Democratic Republic of the Congo: Diagnostic and Clinical Implications 2020.
30. Nasiri M, Zamani S, Pormohammad A, et al. The reliability of rifampicin resistance as a proxy for multidrug-resistant tuberculosis: a systematic review of studies from Iran 2018; 37:9–14.
31. Dean Anna S, Zignol Matteo, Cabibbe Andrea Maurizio, et al. Prevalence and genetic profiles of isoniazid resistance in tuberculosis patients: A multicountry analysis of cross-sectional data. *PLoS Medicine* 2020; 17(1):e1003008.
32. Bainomugisa A, Gilpin C, Coulter C, et al. New Xpert MTB/XDR: added value and future in the field. *European Respiratory Journal* 2020; 56.
33. Feuerriegel S, Kohl TA, Utpatel C, et al. Rapid genomic first- and second-line drug resistance prediction from clinical *Mycobacterium tuberculosis* specimens using deeplex-MycTB. *Eur Respir J* 2021.57.
34. Georghiou S B, Schumacher S G, Rodwell T C, et al. Guidance for studies evaluating the accuracy of rapid tuberculosis drug-susceptibility tests. *J Infect Dis* 2019;220): S126–35.