Multicenter Study of the Accuracy of the BD MAX™ MDR-TB Assay for Detection of *Mycobacterium tuberculosis* Complex and Mutations Associated with Resistance to Rifampin and Isoniazid

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Summary: In a prospective, multi-center diagnostic accuracy study, the BD MAX MDR-TB assay had high sensitivity and specificity for detection of MTB, and RIF and INH drug resistance and allows for rapid detection of TB and drug resistance.

Abstract

Background: Tuberculosis (TB) control is hindered by absence of rapid tests to identify *Mycobacterium tuberculosis (MTB),* and detect isoniazid (INH) and rifampin (RIF) resistance. We evaluated the accuracy of the BD MAX MDR-TB assay (BD MAX) in South Africa, Uganda, India, and Peru.

Methods: Outpatient adults with signs and/or symptoms of pulmonary TB were prospectively enrolled. Sputum smear-microscopy and BD MAX were performed on a single raw sputum, which was then processed for mycobacterial culture and phenotypic drug susceptibility testing (DST), BD MAX and Xpert MTB/RIF (Xpert).

Results: 1053 participants with presumptive TB were enrolled with median age of 35 (47% female; 32% HIV-infected, and 32% unknown HIV status). In microbiologically-confirmed TB patients, BD MAX sensitivity was 93% (262/282 [95% CI 89, 95]); specificity was 97% (593/610 [96, 98]) among participants with negative cultures on raw sputa. BD MAX sensitivity was 100% (175/175, [98,100]) for smear-positive samples (florescence smear-microscopy), and 81% (87/107, [73,88]) in smear-negative samples. Among participants with both BD MAX and Xpert, sensitivity was 91% (249/274, [87,94]) for BD MAX and 90% (246/274 [86,93]) for Xpert on processed sputa. Sensitivity and specificity for RIF resistance compared to phenotypic DST was 90% (9/10 [60,98]) and 95% (211/222 [91,97]), respectively. Sensitivity and specificity for detection of INH resistance was 82% (22/27 [63,92]) and 100% (205/205 [98,100]), respectively.

Conclusions: The BD MAX MDR-TB assay had high sensitivity and specificity for detection of MTB, and RIF and INH drug resistance and may be an important tool for rapid detection of TB and MDR-TB globally.

Key Words: HIV; acquired immunodeficiency syndrome; tuberculosis; mycobacterium infections; diagnosis; multidrug resistance

Introduction

There were 10 million cases of tuberculosis (TB) in 2017 with 1.6 million deaths; TB is the leading cause of death among HIV-infected persons and the global burden of MDR-TB is high[1]. Despite current efforts, global case-detection of active TB is less than 60% in many endemic settings, with some patients dying before diagnosis[1]. Smear-microscopy, the most widely utilized TB diagnostic modality worldwide, has incomplete sensitivity as a screening tool, particularly in HIV-infected presumptive TB cases [2]. Sputum culture, the reference standard, is costly, takes weeks to provide results, and remains restricted to higher levels of the health infrastructure because of expertise and equipment requirements.

Two rapid molecular tests, namely the Xpert® MTB/RIF assay (Xpert) (Cepheid, Sunnyvale, CA) [3, 4] and the GenoType® MTBDRplus (Hain Lifescience, Nehren, Germany) are commercialized and used in some TB-endemic settings, but each has important limitations in addition to attributes. The World Health Organization (WHO) endorsed Xpert® MTB/RIF assay is fully integrated, automated and appropriate for near-care, and requires relatively little training; however the standard platform for this assay has limited throughput (though larger instruments are now available) and only tests for resistance mutations associated with rifampin (RIF) [3-5]. The GenoType® MTBDRplus test can detect mutations associated with isoniazid (INH) and RIF resistance, but has sub-optimal sensitivity for *Mycobacterium tuberculosis* complex (MTBC) detection, and is not fully integrated or automated [6, 7]. There is a need for products that cover a range of performance profiles suitable for the realities of tiered healthcare systems that include testing at point-of-care as well as in more centralized laboratories[8, 9].

The BD MAX™ MDR-TB assay, performed on the BD MAX™ System (both from Becton, Dickinson and Company [BD], Franklin Lakes, NJ), is an automated qualitative *in vitro*

diagnostic test for the direct detection of MTBC DNA in raw induced or expectorated sputum or concentrated sputum sediments from patients for whom there is clinical suspicion of TB and who have not received more than 3 days of anti-tuberculosis therapy in the past six months[10]. The test utilizes real-time polymerase chain reaction (PCR) for the amplification of specific DNA targets and fluorogenic target-specific hybridization probes to detect *M. tuberculosis* complex DNA as well as resistance mutations in the *rpoB* and *katG* genes and the *inhA* promoter region associated with multidrug resistant TB (MDR-TB). The assay is automated and integrated, and requires a stable source of electricity and laboratory technician training; 24 specimens can be tested in one run, and turn-around time from the testing start to result is less than 4 hours. Therefore the BD MAX™ MDR-TB assay is expected to be most suitable for use in central laboratories in which large numbers of specimens are tested and minimal operator hands-on time is desirable. We sought to assess the diagnostic accuracy of the assay in high TB burden, low and middle income settings.

Methods

Study Population

We performed a prospective multicenter diagnostic study in which the accuracy of an investigational *in vitro* molecular diagnostic test (BD MAX™ MDR-TB assay [BD MAX]) performed on sputum was assessed using the reference standard of liquid culture for mycobacteria growth [BD BACTEC™ MGIT™ 960 system (MGIT, BD Sparks, MD)] followed by MTBC identification with BD MGIT™ TBc Identification Test (TBc ID) (BD, Sparks, MD). Xpert MTB/RIF was performed as a commercially available comparator in secondary analysis. Study sites were located in Kampala, Uganda; Cape Town, South Africa; Pune, India; and Lima,

Peru. Participants were recruited consecutively and enrolled between May 2017 and March 2018 into a 'Case-Detection Group' and a 'Drug-Resistant Detection Group', in order to enroll individuals with a higher expected prevalence of drug-resistance. For both groups, inclusion criteria were age >=18 years, written informed consent, and symptoms of pulmonary TB (cough >=2 weeks, and at least one other symptom such as fever, night sweats, or weight loss). Individuals receiving more than two days or doses of TB treatment within the prior six months were excluded from the Case-Detection Group but included in the Drug-Resistant Group if they were suspected or had a history of treatment failure. Non-study directed HIV testing and CD4 testing was recorded, if available. Study-directed testing included one sputum, minimum volume of 3mL, for acid fast bacilli (AFB) smear-microscopy and mycobacterial culture, with *M. tuberculosis* complex (MTBC) identification. Participants unable to spontaneously expectorate the minimum sputum volume were considered early withdrawals.

The study was approved by review committees of the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town, South Africa; Joint Clinical Research Centre, Kampala, Uganda; Uganda National Council for Science and Technology; Universidad Peruana Cayetano Heredia, Comité Institucional de Ética en Investigación; Ethics Committee-B J Medical College & Sassoon General Hospitals, Pune, India; and, Johns Hopkins Medical Institutions, Baltimore, MD.

Laboratory Testing:

All testing was conducted in local laboratories at the study sites (Supplemental Content-Figure 1). The sputum sample was split into two components—Processed and Raw. *Processed*: One portion of the specimen was decontaminated with N-acetyl-L-cysteine-sodium hydroxide (BBL™ MycoPrep™ [BD, Sparks, MD]). After centrifugation, the pellet was suspended in 2ml

buffer. A concentrated auramine-O smear was examined and graded using a fluorescent microscope (FM). Ziehl-Neelsen (ZN) smear was also examined and graded. A 0.5-ml portion of processed sputum sediment was cultured using MGIT, with species identification using TBc ID [11]. A 0.8-mL portion was tested on BD MAX. All positive cultures had phenotypic drug susceptibility testing (DST) for RIF (1 µg/mL) and INH (0.1 µg/mL) using the MGIT system at standard critical concentrations. A 0.5-ml portion of processed sputum specimen was tested with Xpert MTB/RIF. The *Raw* portion of the sputum specimen was not processed and was also tested using ZN and FM, and BD MAX.

BD MAX workflow includes a 30-minute incubation step with sample treatment reagent (STR; STR liquefies specimens and reduces viability of MTBC), transfer to BD MAX TB sample tube, and then loading onto the instrument with 24 samples per run (run time less than 4 hours); specimens were batched. The BD MAX assay results for detection of *M. tuberculosis* are categorized as **MTB Detected** (*MTBC* DNA detected); **MTB Not Detected** (No *MTBC* DNA detected and Sample Processing Control detected); **MTB Low POS** (*MTBC* DNA detected but resistance metrics not measurable); **Indeterminate** (due to BD MAX System failure); **Incomplete** (incomplete run) or, **Unresolved** (No *MTBC* DNA detected and no sample processing control detected, indicative of an inhibitory sample or reagent failure). The assay additionally reports detection of mutations associated with RIF and INH resistance, as resistance detected (*MTBC* RIF or INH resistance mutations were detected); not detected (*MTBC* RIF or INH resistant mutations were NOT detected); or unreportable (*MTBC* DNA detected but INH or RIF resistance metrics not measurable).

All index test and reference standard tests were interpreted blinded to any knowledge of clinical information or other tests.

Outcomes determination

Mycobacterial culture followed by MTBC identification was considered the reference standard. Sputa for which the MGIT culture was still contaminated after a second decontamination, or for which the package inserts were not followed were excluded from the primary analysis as not assessable. All other sputa were considered to be negative for tuberculosis. In secondary analysis, we considered a composite microbiological reference standard using Xpert and mycobacterial culture; for the composite, specimens that were positive by either culture or Xpert were considered positive, were considered negative if results of both assays were negative, and were considered non-evaluable if either method was unevaluable (e.g., contaminated) and the other was negative. We evaluated BD MAX results stratified by both ZN and FM status to evaluate the performance among participants with smear-negative TB.

For DST, the reference standard was the results of the phenotypic culture-based DST for INH resistance (0.1 µg/mL) and RIF resistance (1 µg/mL). For all isolates that were discordant by BD MAX assay, Sanger bi-directional sequencing [3500xl Genetic Analyzer and BigDye™ Terminator v3.1 Cycle sequencing kit (ThermoFisher Scientific)] of the *rpoB* RIF resistance-determining region, *katG* and the *inhA* promoter region was performed on the cultured isolate to help resolve any discordance between molecular and phenotypic results. In secondary analysis, we evaluated performance against a composite reference standard consisting of phenotypic DST and Xpert followed by sequencing. In the composite reference standard, specimens with resistance identified by any of the two methods was considered positive for resistance. Specimens were considered negative if results of all assays were negative, or non-evaluable if either method was unevaluable.

Statistical analyses

Student's t-test was used to compare means. Two-sample proportions were compared by χ^2 tests. McNemar's test was used to compare BD MAX and Xpert assays sensitivities. A p-value ≤ 0.05 was considered statistically significant and 95% confidence intervals (CI) were used. Confidence intervals for binary outcomes were obtained using Wilson's score method. Statistical calculations were performed using Stata 14.1 (StataCorp, College Station, Texas,USA), and the R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

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Results

Characteristics of the study population

Of the 1102 participants screened, 1053 participants met the enrollment criteria of the case detection group, had adequate sputa and were enrolled, of which 69 were excluded due to lack of an interpretable mycobacterial culture for a microbiological reference standard (e.g., MGIT culture contamination), and 92 (9%) raw sputa had non-evaluable BD MAX test results (Figure 1, Supplemental Content-Figure 2); ten additional individuals were enrolled into the 'drug resistance detection' group and contributed data only to analyses pertaining to drug resistance determination. Characteristics of the study population are shown in Table 1. HIV test results were primarily available in South Africa and Uganda. Among the 712 participants with a known HIV status, 47% (333/712) were positive with a median CD4 count of 367 (IQR 228-536). TB was microbiologically confirmed on liquid culture in 314/984 (32%) and differed between study sites (31/320 [10%] South Africa, 81/259 [31 %] Uganda, 54/136 [40%] India, 148/269 [55%] Peru, $p<0.01$). 670/984 (68%) had no positive cultures and were classified as 'Not TB'.

BD MAX MDR-TB test performance among all participants

Table 2 shows overall BD MAX results for the detection of MTB against the microbiological reference standard on raw sputum (Supplemental Content-Table 1 for processed sputum). BD MAX test sensitivity was 93% (262/282, 95% confidence interval [CI] 89,95) from raw sputum in participants with confirmed TB. Among individuals categorized as "not TB", specificity was 97% (593/610 [96,98]). Among these discordant participants (positive BD MAX, negative cultures), all were smear-negative (if available), 9/17 (53%) were low positive, and 6/17 (35%) had a prior history of TB. When using a composite microbiological reference standard of mycobacterial culture and/or Xpert, BD MAX sensitivity was 93% (275/297 [89, 95]) from raw sputum. Specificity was to 99% (584/592 [97, 99]). Specificity estimates were similar against a composite reference standard, comparing those with and without a prior history of TB (147/150 [98%] versus 437/442 [99%], respectively). Positive predictive value (PPV) for confirmed TB and negative predictive value (NPV) were, respectively, 94% (91,96) and 97% (95,98) among all participants.

Among ZN and FM smear-positive, culture-positive participants, BD MAX sensitivity was 100% (148/148 [98,100]) and 100% (175/175 [98,100]) from raw sputum specimens, respectively. Among ZN smear-negative specimens, sensitivity was 85% (114/134,[78,90]; among FM smear-negative specimens, sensitivity was 81% (87/107, [73,88]). Overall, 7% (19/282) of participants with confirmed TB were low positive on BD MAX; among these, 100% were smear-negative.

Comparison to Xpert MTB/RIF for detection of MTB

We compared the performance of BD MAX and Xpert MTB/RIF assays for the detection of MTB on processed sputa (Supplemental Content-Table 2). The sensitivity was similar between the two assays at 91% (249/274 [87,94]) and 90% (246/274, [86,93]) for the BD MAX and Xpert MTB/RIF assays, respectively. Specificity was 96% (588/615 [94, 97]) and 98% (604/615 [97,99]) for BD MAX and Xpert MTB/RIF, respectively. When stratified by smear status, the BD MAX assay sensitivity was 65% (44/68, [53,75]) compared to 59% for Xpert (40/68, [47,70]) among FM smear-negative samples.

Detection of drug resistance

Among the 297 TB microbiologically confirmed on liquid culture in both enrollment groups (i.e. case-detection and drug-resistance detection groups), resistance results were available by the BD MAX test in 232 (78%) participants, from which 230 participants had reportable results for both RIF and INH resistance. Overall, 202 (87%) had drug-susceptible TB on phenotypic testing, and 29 (13%) had resistance to either INH and/or RIF.

RIF resistance detection

Among 10 microbiologically confirmed TB patients with RIF resistance on phenotypic DST, sensitivity of BD MAX for detection of RIF resistance was 90% (9/10 [60, 98]). Specificity of BD MAX for RIF susceptibility among 222 TB participants without detection of RIF resistance by phenotypic DST was 95% (211/222 [91, 97]) (Table 3; Supplemental Content-Table 3). Among 11 participants with resistance detected by BD MAX but not phenotypic DST, bidirectional sequencing found 6 with true resistance mutations (2 D435Y, 1 D435F, 2 L430P, 1

L452P) and 2 silent mutations (F433F). Another specimen gave a phenotypic DST error, but Xpert and bidirectional sequencing (H445N) were RIF resistant. Each of these resistance mutations has been previously associated with treatment failure[12-17]. When examined against a composite reference standard inclusive of Xpert and bidirectional sequencing, sensitivity of the BD MAX assay was 94% (16/17, [73,99]) and specificity was 98% (200/205, [94,99]; 2 silent mutations were regarded as susceptible).

INH resistance detection

Among 27 microbiologically confirmed TB patients with INH resistance on phenotypic DST, BD MAX assay sensitivity was 82% (22/27, [63, 92]; 4 *inhA* promoter and 16 *katG* gene mutations, 2 with both) (Table 3; Supplemental Table 3). Specificity of the assay among 205 TB participants without detection of INH resistance by phenotypic DST was 100% (205/205 [98, 100]).

BD MAX MDR-TB test performance stratified by HIV-infection

BD MAX test sensitivity and specificity, stratified by HIV-infection status, are shown in Supplemental Content-Table 4-5. Among 273 HIV-infected participants with evaluable results, assay sensitivity was 86% (44/51 [74,93]), and assay specificity was 98% (217/222 [95,99]). When stratified by ZN smear microscopy status, sensitivity of the BD MAX assay for detection of HIV-associated TB was 100% (22/22, [85, 100]) for smear-positive, and 76% (22/29, [58, 88]) in smear-negative HIV/TB patients. Among TB patients with both BD MAX and Xpert test results, sensitivity was 82% (41/50, [69, 90]) for both assays.

Discussion

For the diagnosis of active TB in diverse low and middle-income settings, the BD MAX MDR-TB test had a sensitivity of 93% for confirmed pulmonary TB cases, with accuracy that appeared comparable to Xpert MTB/RIF. Of note, Xpert® MTB/RIF Ultra was not commercially available at the time of the study. While sample size was limited, BD MAX had high sensitivity and specificity for detection of both rifampin and isoniazid resistance, consistent with current targets for development of new tools for rapid drug susceptibility tests for TB[18]. The BD MAX assay may therefore represent a new diagnostic tool in the armamentarium for rapid identification of TB globally.

Globally, the burden of MDR-TB, INH-mono-resistant, and RIF-mono-resistant TB remains high and developing tools that accurately identify both INH and RIF resistance are increasingly important in allowing rapid, individualized therapy. Among the potential benefits of the BD MAX assay is detection of mutations in *inhA* promoter, *katG*, in addition to *rpoB*, in contrast to other commonly used molecular assays that focus on initial identification of RIF resistance alone. While the distribution of INH mutations has been less well mapped globally, WHO estimates suggest nearly 8% of TB patients worldwide have rifampin-susceptible, isoniazid resistant TB; some parts of the world may have rates of mono-resistance in excess of 10-20%, with poorer treatment outcomes when treated with standard first line regimens[19-21]. Failure to identify INH monoresistance may also lead to suboptimal treatment that can select for further acquired resistance[22, 23]. Consequently, in 2018, the WHO issued the first treatment guidelines for Hr-TB (INH-resistance with RIF susceptibility), which indicates provisions for a regimen that includes RIF, levofloxacin, pyrazinamide, and ethambutol for six months[21, 24].

Empirical treatment of Hr-TB is not currently suggested. Rapid detection of INH resistance is therefore an important consideration for patient treatment and for TB control programs.

In reference laboratories, the ability to batch or conduct increased numbers of tests simultaneously may improve efficiency in labs with very high volumes as countries adopt hub-and-spoke models of sample referral. Given the relatively straightforward workflow of the BD MAX assay with a short incubation step and run time of less than 4 hours, high volume labs could prepare the next group of samples during the BD MAX system run time. This may offer benefits over alternative platforms with more limited throughput.

Our study has limitations. We conducted the study using a single sputum sample. Consequently, specificity of the BD MAX assay may be underestimated in situations where there was molecular detection of MTB by the BD MAX assay, but no detection by culture on a single sputum specimen. Nonetheless, our results show high specificity for detection of MTB, as well as drug resistance, and our results are strengthened by inclusion of a composite reference standard inclusive of Xpert testing and sequencing. Five individuals had phenotypic INH resistance not detected by BD MAX, reducing sensitivity for INH resistance detection. Sequencing of the targeted regions did not find mutations within *katG* or *inhA* promotor for three isolates, suggesting mutations outside the targeted regions. For 1/5, sequencing suggested heteroresistance in which a wild-type strain and a strain with *inhA* promoter mutation may have been present. For 1/5, sequencing suggested heteroresistance in which a wild-type strain and a strain with *katG* mutation may have been present. Geographic regions with higher prevalence of INH resistance mediated by mutations outside *katG* or *inhA* promotor regions may consider inclusion of phenotypic drug susceptibility testing within diagnostic algorithms.

On the other hand, our study has several important strengths. We are the first to report on the diagnostic accuracy of the BD MAX assay on a prospective cohort in high burden, low and middle income settings where the test may be most useful. The study was done in multiple representative settings that included sites in Asia and South America, in addition to sub-Saharan Africa. We provide results on both HIV-infected and uninfected individuals, and included a comparison with Xpert, which is widely used in similar settings. Our results suggest that the BD MAX assay has similar performance to Xpert, and offers the added advantage of providing results for INH resistance in addition to RIF.

In conclusion, there is a need for new diagnostic tools to combat the global burden of tuberculosis. For many high-burden settings with a high-volume of testing, the BD MAX assay may represent an important automated tool for rapid detection of both MTB and drug resistance.

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Potential Conflict of Interest

M.S., B.K., D.A., S.E.D., JB, and Y.C.M. have no conflicts of interest related to this study, its findings, or this manuscript. The JHU team oversaw all data analysis including independent statistical analysis and data verifications, results reporting, and made the decision to publish a manuscript. S.P, D.K., C.E.M., and C.C.C. are employed by Becton, Dickinson and Company (BD), which donated BD MAX instruments and BD MAX MDR-TB kits and trained laboratory staff. BD also assisted with the study concept, design, and implementation. S.A. and K.H. are shareholders in Loke Diagnostics (Aarhus, Denmark). E.H.P. has been a consultant to Fujisawa Healthcare, Inc., Gilead Sciences, Novartis, and GlaxoSmithKline and is a member of the speakers' bureaus for Pharmacia and Novartis. J.A.S. has received research funding from Bayer and Pharmacia, has been a consultant for Bayer and Pfizer, and has been on the speakers' bureau for Pfizer and Ortho McNeil. M.P.N. reports grants from National Institutes of Health. All other authors have no conflicts to disclose.

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FIGURE LEGENDS

Figure 1: Study population and study flow diagram
Abbreviations: INC, incomplete; IND, indeterminate; UNR, unresolved; INV/EC, Invalid/External Control; RIF, rifampin; INH, isoniazid

Table 1: Study demographics

		N (%)
Total		984
Age	Median(IQR)	34 (27-44)
Gender	Male	528 (53.7%)
Race	Asian	136 (14%)
	Black	579 (59%)
	Other/More than one race	269 (27%)
Site		
	India	136 (14%)
	Peru	269 (27%)
	South Africa	320 (33%)
	Uganda	259 (26%)
HIV status	HIV-positive	303 (31%)
	HIV-negative	347 (35%)
	HIV unknown	334 (34%)
CD4 (among HIV positive)[a]	Median (IQR)	365 (231-538)

[a]CD4 data available in 113 individuals based on routine testing for clinical purposes at the study sites.

Table 2: Diagnostic Accuracy of the BD MAX assay for detection of MTB among raw specimens against a microbiological reference standard of mycobacterial culture

Analysis	BD MAX result	Microbiological culture		Total (95% CI)
		Positive	Negative	
Overall	Positive	262	17	279 (PPV 94% [91,96])
	Negative	20	593	613 (NPV 97% [95,98])
	Total	282	610	892
	Sensitivity			93% (89,95)[a]
	Specificity			97% (96,98)[a]
Smear Stratified[b]				
FM smear positive	Positive	175	1	176 (PPV 99% [97,100])
	Negative	0	0	0
	Total	175	1	176
	Sensitivity			100% (98,100)
	Specificity			--
FM smear negative	Positive	87	15	102 (PPV 85% [77,91])
	Negative	20	591	611 (NPV 97% [95,98])
	Total	107	606	713
	Sensitivity			81% (73, 88)
	Specificity			98% (96, 99)
ZN smear positive	Positive	148	0	148 (PPV 100% [98,100])
	Negative	0	0	0 (NPV 100% [21, 100])
	Total	148	0	148
	Sensitivity			100% (98,100)
	Specificity			--
ZN smear negative	Positive	114	16	130 (PPV 88% [81,92])
	Negative	20	592	612 (NPV 97% [95,98])
	Total	134	608	742
	Sensitivity			85% (78 ,90)
	Specificity			97% (96,98)

[a]Using a composite reference standard consisting of Xpert and mycobacterial culture, sensitivity was 93% (275/297, [89,95]) and specificity was 99% (584/592, [97,99]).

[b]Smears performed from raw specimens. Two (2) specimens had a smear unknown status for ZN and three (3) specimens had a smear status unknown for FM.

Table 3: Performance of the BD MAX assay for the detection of drug resistance among raw specimens

Analysis	BD MAX result	Microbiological culture based DST		Total (95% CI)
		Positive	Negative	
ANY DRUG RESISTANCE (INH or RIF) [a]	Positive	24	7	31 (PPV 77%)
	Negative	5	195	200 (NPV 95%)
	Total	29	202	231
	Sensitivity			83% (66,92)
	Specificity			97% (93,98)
INH Resistance				
	Positive	22[b]	0	22 (PPV 100%)
	Negative	5 [d]	205	210 (NPV 98%)
	Total	27	205	232
	Sensitivity			82% (63 , 92)
	Specificity			100% (98 ,100)
RIF Resistance				
	Positive	9	11[c]	20 (PPV 45%)
	Negative	1	211	212 (NPV 99.5%)
	Total	10	222	232
	Sensitivity			90% (60, 98)
	Specificity			95% (91, 97)

[a]Cases where BD MAX gave a RIF or INH Not Detected result or a RIF or INH Unreportable result were excluded. 230 samples had reportable results for both RIF and INH resistance; one additional sample without INH results available is included which had RIF resistance detected by BD MAX. 8 samples were resistant both for RIF and INH based on the DST. The BD MAX assay detected the dual resistance for 7/8 samples.

[b]Among 22 INH resistant isolates detected by BD MAX assay, mutations were detected in both *inhA* promoter and *katG* gene for 2 specimens; in *inhA* promoter alone for 4 specimens, and in *katG* gene alone for 16 specimens.

[c]Eleven samples were resistant with BD MAX assay and sensitive by phenotypic DST. Among these 11, 6 were positive for RIF resistance by Xpert and bi-directional sequencing, and 2 were found to have silent mutations. Another specimen gave a phenotypic DST error, but Xpert and bidirectional sequencing were resistant. When examined against a composite reference standard

inclusive of Xpert and bidirectional sequencing (222 samples), sensitivity was 94% (16/17, [73,99]) and specificity was 98% (200/205, [94,99])

[d]Among 5 isolates phenotypically resistant to INH but negative by BD MAX, sequencing of the targeted regions did not find mutations within *katG* or *inhA* promotor suggesting resistance due to mutations outside the targeted regions for three isolates. For 1/5, sequencing suggested heteroresistance with a wild-type strain and a strain with a mutation in the *inhA* promoter may have been present. For 1/5, sequencing suggested heteroresistance with a wild-type strain and a strain with a mutation in the *katG* gene may have been present.

Figure 1