Clinical Evaluation of BD MAX MDR-TB Assay for Direct Detection of Mycobacterium tuberculosis Complex and Resistance Markers

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BD MAX MDR-TB assay is a new molecular platform for the detection of Mycobacterium tuberculosis complex (MTBC) in clinical specimens and simultaneous detection of resistance toward isoniazid and rifampicin. This study assessed the assay’s diagnostic accuracy by using pre-characterized MTBC culture-negative (n = 257), smear-negative/MTBC culture-positive (n = 93), and smear-positive/MTBC culture-positive (n = 153) respiratory specimens. Compared with culture, the overall sensitivity and specificity of BD MAX MDR-TB were 86.6% and 100%, respectively; sensitivities for smear-positive and smear-negative samples were 100% and 64.5%. Sensitivity and specificity for isoniazid and rifampicin resistance were 58.3% (biased low due to sample collection strategy in low prevalence setting), 99.3%, 100%, and 98.2%, compared with phenotypic drug resistance testing and 100%, 99.4%, 100%, and 99.4%, compared with GenoType MTBDRplus. In conclusion, BD MAX MDR-TB is an accurate assay for the diagnostic detection of MTBC in respiratory samples and its resistance toward the most important anti-TB drugs isoniazid and rifampicin. Due to its medium to high throughput, good validity, and ease of use, the assay will be of great benefit for medium-sized to large TB diagnostic centers. (J Mol Diagn 2020, 22: 1280–1286; https://doi.org/10.1016/j.jmoldx.2020.06.013)

With 1.5 million deaths, tuberculosis (TB) remains the world’s leading cause of death due to a single infectious agent. 1 Rapid and accurate diagnosis of active TB and fast detection of drug resistance are essential to adequately manage the disease and to control its further transmission. Drug-resistant TB, in particular multidrug-resistant TB [MDR-TB; defined by resistance toward the two most important anti-TB drugs, isoniazid (INH) and rifampicin (RIF)], is one of the most serious threats to global TB control efforts. In 2018, 3.4% of new TB cases and 18% of previously treated cases suffered worldwide from MDR-TB or RIF-resistant TB. Despite some progress in testing and detection, there is still a large gap of unnotified cases. Of the estimated 10.0 million incident cases, only 7.0 million were detected and, of the estimated 484,000 MDR-TB/RIF-resistant TB cases, only one-third were notified in 2018. 1

To close this gap, there is an increased need for easy-to-handle, rapid, reliable molecular assays detecting TB bacteria together with most important resistance markers in clinical specimens. The cartridge-based Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) was the first of such assays. It was endorsed by the World Health Organization in 2010, 2 particularly for application in peripheral laboratories in...
areas that would otherwise not have access to TB diagnostics. The assay has since notably improved TB notification and detection of RIF resistance worldwide.\textsuperscript{5,4} However, Xpert MTB/RIF also has weaknesses. It does not test for INH resistance, which occurs on average in 8\% of RIF-susceptible cases worldwide, with a significant impact on treatment outcomes and development of additional resistances, particularly of MDR-TB.\textsuperscript{5–7} Furthermore, neither Xpert RIF/MTB nor the more sensitive successor test Xpert MTB/RIF Ultra (Cepheid)\textsuperscript{8} allows testing of higher numbers of samples. During recent years, several automated medium- to high-throughput TB assays have been launched, including BD MAX MDR-TB (BD MAX; Becton-Dickinson, Heidelberg, Germany), RealTime MTB and MTB RIF/INH (Abbott, Chicago, IL), cobas MTB and MTB-RIF/INH assays (Roche Diagnostics, Basel, Switzerland), and FluoroType MTBDR (Hain Lifescience GmbH, Nehren, Germany).\textsuperscript{9–11} Compared with the GeneXpert technology, those platforms may be more efficient for application in central hubs. An external laboratory validation conducted by the World Health Organization and the Foundation for Innovative New Diagnostics found high analytic sensitivities for the novel TB test platforms of Becton-Dickinson, Abbott, and Roche, with similar or even better limits of detection for \textit{Mycobacterium tuberculosis} complex (MTBC) compared with Xpert MTB/RIF.\textsuperscript{12}

In a recent validation study, we observed that BD MAX had analytical sensitivity equal to Xpert MTB/RIF Ultra.\textsuperscript{13} However, data with clinical, decontaminated, smear-negative respiratory MTBC specimens and nontuberculous mycobacteria (NTM) remain scarce. The goal of the current study was to assess the diagnostic performance and validity of BD MAX by using pre-characterized cohorts of i) MTBC-negative, ii) paucibacillary, and iii) multibacillary MTBC-positive respiratory secretion samples after decontamination using \textit{N}-acetyl-l-cysteine/sodium hydroxide method with a final sodium hydroxide concentration of 1\%. After centrifugation (20 minutes 3000 \times g), the sediment was resuspended in 1.5 mL of 0.5 mol/L phosphate buffer (pH 6.8) and inoculated for culture in mycobacteria growth indicator tubes (MGIT; Becton-Dickinson) (500 \(\mu\)L) using Löwenstein-Jensen (25 \(\mu\)L) and Stonebrink (25 \(\mu\)L) media. Smears were stained with Auramine O, read under a fluorescence microscope at 400 \(\times\) magnification, and interpreted according to the World Health Organization/International Union Against Tuberculosis and Lung Disease. Cultures were incubated for 8 weeks before a negative result was declared. From positive cultures, species were identified by the use of the line probe assays GenoType CM and GenoType MTBC (Hain Life-Science). RIF and INH resistance markers were determined directly in smear-positive samples or from MTBC-positive cultures using the GenoType MTBDR\textsuperscript{plus} (Hain Life-Science). Phenotypic susceptibility of MTBC isolates toward first-line drugs was tested in MGIT by using the SIRE kit of Becton-Dickinson following the manufacturer’s instructions. Remnants (800 to 1000 \(\mu\)L) of decontaminated samples were stored at \(-20^\circ\text{C}\) for later application to the BD MAX assay.

\section*{Materials and Methods}

\subsection*{Study Design and Specimens}

From October 2018 to September 2019, residuals of decontaminated sputum and tracheal/bronchial secretion samples from routine diagnostics at the mycobacteriology diagnostic center in Gauting, Germany, were preserved at \(-20^\circ\text{C}\) for later analysis on the BD MAX platform provided that: i) the sputum was of good quality, ii) the initial volume of the clinical specimen (sputum or tracheal/bronchial aspirate) was at least 2 mL, iii) the residual material after preparation of smears and inoculation of cultures was at least 800 \(\mu\)L, and iv) the patient was not known to have microbiologically confirmed TB for \(>2\) weeks. A maximum of three samples per patient were collected. All samples that grew MTBC (\(n = 246\)) or NTM (\(n = 52\)) and 205 random samples with negative culture results were taken for the study and anonymized. The final study set comprised 503 samples (326 sputa and 177 tracheal/bronchial aspirates).

\subsection*{Processing of Samples and Mycobacteriology Analysis}

All clinical specimens were decontaminated by using the \textit{N}-acetyl-l-cysteine/sodium hydroxide method with a final sodium hydroxide concentration of 1\%. After centrifugation (20 minutes 3000 \(\times\) g), the sediment was resuspended in 1.5 mL of 0.5 mol/L phosphate buffer (pH 6.8) and inoculated for culture in mycobacteria growth indicator tubes (MGIT; Becton-Dickinson) (500 \(\mu\)L) using Löwenstein-Jensen (25 \(\mu\)L) and Stonebrink (25 \(\mu\)L) media. Smears were stained with Auramine O, read under a fluorescence microscope at 400 \(\times\) magnification, and interpreted according to the World Health Organization/International Union Against Tuberculosis and Lung Disease. Cultures were incubated for 8 weeks before a negative result was declared. From positive cultures, species were identified by the use of the line probe assays GenoType CM and GenoType MTBC (Hain Life-Science). RIF and INH resistance markers were determined directly in smear-positive samples or from MTBC-positive cultures using the GenoType MTBDR\textsuperscript{plus} (Hain Life-Science). Phenotypic susceptibility of MTBC isolates toward first-line drugs was tested in MGIT by using the SIRE kit of Becton-Dickinson following the manufacturer’s instructions. Remnants (800 to 1000 \(\mu\)L) of decontaminated samples were stored at \(-20^\circ\text{C}\) for later application to the BD MAX assay.

\subsection*{BD MAX Assay}

Frozen samples were thawed and treated with Sample Treatment Reagent (Becton-Dickinson) for inactivation and liquefaction in a final Sample Treatment Reagent—to-sample ratio of 2:1, shaken 10 times, and shaken again after 5 minutes’ incubation at room temperature. After an additional 25 minutes, 2.5 mL of the Sample Treatment Reagent—treated sample was transferred to a labeled sample tube and then transferred to the BD MAX system. Sample tube preparation and system operation followed the manufacturer’s instructions. In brief, after manually loading samples, reagent strips, and PCR cartridges on the BD MAX machine, all further processes (sample preparation, target PCR amplification and detection, and interpretation of results) ran fully automatically. Each BD MAX extraction tube contained a Sample Processing Control (monitoring the efficacy of DNA extraction and DNA target amplification and detection), which was processed together with the sample DNA. If the Sample Processing Control failed, the result of the sample was reported as unresolved unless the target PCR was positive. The amplified targets were identified by hybridization to probes labeled with specific fluorophores for Sample Processing Control, MTBC DNA, and RIF and INH resistance markers. BD MAX determines RIF resistance—associated mutations in the 81 bp \textit{rpoB} gene.
INH resistance–associated mutations were determined in the katG gene and the inhA promoter region. The BD MAX system software version V5.00A automatically reported MTBC results as either “MTBC DNA detected” or “No MTBC DNA detected” and resistance markers, if reportable, as “MTBC RIF resistant DNA detected,” “No MTBC RIF resistant DNA detected,” “MTBC INH resistant DNA detected (katG Mut detected and/or inhAPr Mut detected),” or “No MTBC INH resistant DNA detected.” The total run time was <4 hours for 24 samples.

Discrepant Results

When BD MAX indicated resistances that were phenotypically not confirmed, or vice versa when phenotypic resistances were not confirmed by BD MAX, genetic resistance markers were investigated according to Sanger sequencing of the BD MAX targeted regions. Primers were used were rpoB-F 5′-GGGAGCGGATGACCACCCA-3′ and rpoB-R 5′-GCCGTACGGGTTGATGACCTTCTG-3′ for the RIF resistance—determining region of the rpoB gene, katG-F 5′-TGGCCGGCCGTCGACATT-3′ and katG-R 5′-CCAGCAGGGCTCTTCGTAACC-3′ for the katG gene, and inhA-pr-F 5′-ACAAAACGTCACGAGCGTAACC-3′ and inhA-pr-R 5′-GTTGGCGTTGATGACCTTCTG-3′ for the inhA promoter.

Data Analysis

The performance of BD MAX with regard to the detection of MTBC in clinical samples was evaluated compared with culture and smear microscopy; its performance with regard to the detection of resistances was compared with phenotypic DST (pDST) in MGIT using the BACTEC MGIT 960 device (Becton-Dickinson) and with the line probe assay GenoType MTBDRplus. The VassarStats calculator was used to determine 95% CIs, and \( \chi^2 \) calculations were determined by using Social Science Statistics. \( P < 0.05 \) was considered statistically significant.

Ethical Considerations

The study has been positively evaluated by the ethics committees of the Ludwig-Maximilian-University of Munich (study number 17-761).

Results

BD MAX was applied to 503 pre-characterized, decontaminated respiratory samples. Of these, 48.9% (246 of 503) grew MTBC (244 M. tuberculosis, 2 M. africana) and 10.3% (52 of 503) NTM (2 M. abscessus, 18 M. avium, 1 M. celatum, 1 M. chelonae, 1 M. fortuitum, 19 M. intracellularare, 5 M. kansasii, 2 M. malmoense, and 3 M. xenopi); 40.8% (205 of 503) were culture negative. Of the MTBC culture-positive samples, 37.8% (93 of 246) were negative and 62.2% (153 of 246) were positive in smear microscopy (Table 1). Forty-one of the 52 samples that grew NTM (78.8%) were smear positive.

The BD MAX assay reported MTBC-positive results with 213 (42.3%) and MTBC-negative results with 290 (57.7%) samples (Table 1). Compared with mycobacterial culture as the gold standard, sensitivity of BD MAX was 86.6% (95% CI, 81.5% to 90.4%), 100% (95% CI, 96.9% to 100%), and 64.5% (95% CI, 53.8% to 74%) with all, smear-positive, and smear-negative samples, respectively. Sensitivity rates with sputa and tracheal/bronchial aspirates were equal among smear-positive (100% each) and almost equal among smear-negative (62.8% and 65.1%) samples. Thirty-three smear-negative samples yielded negative BD MAX results although culture was positive with MTBC. Twenty-three (69.7%) of those 33 were retested from the same sample tubes, which had been stored overnight at 2°C to 28°C; nine of them became positive in the second run, suggesting an impact of the longer incubation time with Sample Treatment Reagent. Together with these results, the proportion of TB-positive BD MAX tests with smear-negative samples increased to 74.2% (95% CI, 63.9% to 82.4%) (Table 1).

Specificity was 100% with both culture-negative samples (95% CI, 99.7% to 100%) and samples growing NTM (95% CI, 93.1% to 100%) (Table 1).

There was only one invalid result (1 of 503 [0.2%]) due to failure of the Sample Processing Control, suggesting inhibition or reagent failure. The test was repeated and yielded a valid negative result in the second run.

Of 213 samples tested MTBC positive in the first run, 76.1%, 4.7%, and 19.2% yielded complete resistance profiles for both INH and RIF, partial results for either INH or RIF, and no resistance results at all, respectively (Table 2). Unreportable resistance results were clearly associated with low bacterial loads: the percentage of smear-negative samples increased from 9.6% for samples with complete resistance profiles provided by BD MAX to 82.9% for samples with unreportable resistance results \( P < 0.00001 \).

BD MAX detected resistance markers for INH but not for RIF in six (2.8%) of 213 samples, with two samples each yielding a katG mutation, one sample an inhA mutation, and three samples both katG and inhA mutations. MDR-TB (with concurring mutations in katG and rpoB) was found in two samples [2 of 213 (0.9%)]. rpoB mutations without concomitant INH resistance were reported for four samples [4 of 213 (1.9%)]. A total of 150 samples indicated wild-type katG, inhA, and rpoB genes [150 of 213 (70.4%)].

Using pDST as reference, sensitivity and specificity of BD MAX to detect INH resistance were 58.3% (95% CI, 28.6% to 83.5%) and 99.3% (95% CI, 95.9% to 100%), respectively (Table 3). Five samples with phenotypically INH-resistant isolates were tested susceptible according to BD MAX. However, BD MAX results were in agreement with those from the line probe assay MTBDRplus, which targets resistance-associated mutations in katG and the inhA promoter region, as well as with Sanger sequencing, which also...
yielded wild-type target sequences in katG and 

inhA promoter. We assume that those five isolates harbor resistance-associated mutations outside the katG and inhA promoter target regions of BD MAX and MTBDRplus. Notably, the five isolates originated only from two patients. One additional sample was tested INH susceptible according to pDST but resistant (inhA promoter mutation) by BD MAX (Table 3). INH resistance was reproduced by BD MAX retesting, whereas Sanger sequencing of the inhA promoter region as well as MTBDRplus only identified wild-type sequences. There was no evidence of heteroresistance (ie, presence of both wild-type and mutated organisms).

Sensitivity and specificity of BD MAX to detect RIF resistance were 100% (95% CI, 31% to 100%) and 98.2% (95% CI, 94.4% to 99.5%), respectively (Table 3). Three BD MAX results (1.8%) of 166 were discrepant RIF resistant. With one of those, BD MAX could not reproduce RIF resistance in a retest from the same sample tube, suggesting an erroneous result related to a low initial concentration of MTBC bacteria. For the other two samples, the presence of rpoB resistance markers were confirmed by MTBDRplus, which found missing wild-type signals for codons 526 to 529; Sanger sequencing identified rpoB mutation H526S, which belongs to the so-called disputed rpoB mutations that are tested RIF susceptible according to automated pDST in MGIT but may exhibit increased minimal inhibitory concentrations resulting in low-level resistance.14 Thus, BD MAX correctly identified genetic RIF resistance markers in the two discordant cases that escaped detection by automated pDST in MGIT due to low-level RIF resistance.

BD MAX results exhibited strong agreement of 100% and 99.4% with MTBDRplus for both INH/RIF resistance and susceptibility, respectively (Table 3). In particular, all resistance markers detected by MTBDRplus for INH (4 katG 315T and 3 katG 315T plus inhA-1ST) and RIF (2 rpoB D516V, 1 rpoB H526D, and 2 rpoB H526S) were also indicated by BD MAX. Among samples tested susceptible to INH/RIF by MTBDRplus, all but two were also reported susceptible by BD MAX.

**Discussion**

BD MAX is one of the novel generations of closed and automated TB assays that use medium- to high-throughput platforms and allow for rapid detection of MTBC and potential resistance toward RIF and/or INH. BD MAX has thus far been clinically evaluated in a larger multicenter study and two rather small single-center studies. The multicenter trial prospectively evaluated the performance of BD MAX in a setting of high TB burden using both raw and processed but a rather small number of smear-negative, sputum samples.9 The two small-scale studies reported performance with pulmonary and extrapulmonary samples under conditions of routine diagnostics.15,16 The current study aimed to evaluate the diagnostic performance of BD MAX with a larger number of decontaminated respiratory samples under low-prevalence, low—MDR-TB conditions of a diagnostic laboratory in a Central European country. In industrialized countries with strong private sectors, the vast majority of respiratory samples are decontaminated and cultured to exploit the advantage of the high sensitivity of

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**Table 1** Diagnostic Accuracy of BD MAX Using Cx as Reference Method

<table>
<thead>
<tr>
<th>Cx results</th>
<th>BD MAX (only first test results)</th>
<th>BD MAX (including second run)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative, Positive, % Sensitivity</td>
<td>% Specificity</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>$n$</td>
</tr>
<tr>
<td>MTBC positive ($n = 246$)</td>
<td>33</td>
<td>213</td>
</tr>
<tr>
<td>sm negative ($n = 93$)</td>
<td>33</td>
<td>60</td>
</tr>
<tr>
<td>sm positive ($n = 153$)</td>
<td>0</td>
<td>153</td>
</tr>
<tr>
<td>MTBC negative ($n = 257$)</td>
<td>257</td>
<td>0</td>
</tr>
<tr>
<td>Cx negative ($n = 205$)</td>
<td>205</td>
<td>0</td>
</tr>
<tr>
<td>Cx NTM ($n = 52$)</td>
<td>52</td>
<td>0</td>
</tr>
<tr>
<td>Total ($N = 503$)</td>
<td>290</td>
<td>213</td>
</tr>
</tbody>
</table>

*n = 23 retested samples. The recalculation of specificity and the proportion of positive results including results from a second run are biased by the fact that only discrepant samples were retested and are, therefore, for information only.

BD MAX, BD MAX MDR-TB assay; Cx, culture; MTBC, Mycobacterium tuberculosis complex; NTM, nontuberculous mycobacteria; sm, smear microscopy.

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**Table 2** Performance of Resistance Testing by BD MAX

<table>
<thead>
<tr>
<th>BD MAX resistance results</th>
<th>$n$ (%)</th>
<th>sm negative, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete results</td>
<td>162 (76.1)</td>
<td>9.6</td>
</tr>
<tr>
<td>INH S, RIF S</td>
<td>150 (70.4)</td>
<td>12.7</td>
</tr>
<tr>
<td>INH R, RIF S</td>
<td>6 (2.8)</td>
<td>25.0</td>
</tr>
<tr>
<td>INH S, RIF R</td>
<td>4 (1.9)</td>
<td>12.5</td>
</tr>
<tr>
<td>INH R, RIF R</td>
<td>2 (0.9)</td>
<td>0.0</td>
</tr>
<tr>
<td>Partial results</td>
<td>10 (4.7)</td>
<td>50.0</td>
</tr>
<tr>
<td>INH S, RIF NR</td>
<td>3 (1.4)</td>
<td>66.7</td>
</tr>
<tr>
<td>INH NR, RIF S</td>
<td>7 (3.3)</td>
<td>42.9</td>
</tr>
<tr>
<td>Unreportable results</td>
<td>41 (19.2)</td>
<td>82.9</td>
</tr>
<tr>
<td>INH NR, RIF NR</td>
<td>41 (19.2)</td>
<td>82.9</td>
</tr>
<tr>
<td>Total</td>
<td>213 (100)</td>
<td>28.2</td>
</tr>
</tbody>
</table>

BD MAX, BD MAX MDR-TB assay; INH NR, isoniazid resistance not reportable; INH R, isoniazid resistance detected; INH S, isoniazid resistance not detected; RIF NR, rifampicin resistance not reportable; RIF R, rifampicin resistance detected; RIF S, rifampicin resistance not detected; sm, smear microscopy.
Table 3  Accuracy of BD MAX to Predict Resistance Toward INH and RIF

<table>
<thead>
<tr>
<th>Phenotypic and genotypic resistance results</th>
<th>BD MAX resistance results</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>INH</td>
<td>S</td>
<td>R</td>
<td>RIF</td>
</tr>
<tr>
<td>pDST</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant, n</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Susceptible, n</td>
<td>153</td>
<td>152</td>
<td>1*</td>
<td>166</td>
</tr>
<tr>
<td>Sensitivity % (95% CI)</td>
<td>58.3 (28.6—83.5)</td>
<td></td>
<td></td>
<td>100 (31—100)</td>
</tr>
<tr>
<td>Specificity, % (95% CI)</td>
<td>99.3 (95.9—100)</td>
<td></td>
<td></td>
<td>98.2 (94.4—99.5)</td>
</tr>
<tr>
<td>MTBDRplus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant, n</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Susceptible, n</td>
<td>158</td>
<td>157</td>
<td>1*</td>
<td>164</td>
</tr>
<tr>
<td>Sensitivity, % (95% CI)</td>
<td>100 (56.1—100)</td>
<td></td>
<td></td>
<td>100 (31—100)</td>
</tr>
<tr>
<td>Specificity, % (95% CI)</td>
<td>99.4 (96—100)</td>
<td></td>
<td></td>
<td>99.4 (96—100)</td>
</tr>
</tbody>
</table>

*Resistance marker in inhA promoter region detected by BD MAX.

†Two discrepant RIF-resistant samples harbored rpoB mutation H526S associated with low-level RIF resistance. These cases escaped detection by pDST in mycobacteria growth indicator tubes and were correctly detected RIF resistant by BD MAX.

‡One discrepant RIF-resistant sample did not exhibit any resistance markers according to MTBDRplus. The RIF-resistant result could not be reproduced in a second BD MAX run (data not shown).

Sensitivity of BD MAX to detect resistance was calculated compared with resistant cases detected according to MTBDRplus; specificity of BD MAX to detect susceptibility was calculated compared with susceptible cases according to MTBDRplus.

BD MAX, BD MAX MDR-TB; INH, isoniazid; pDST, phenotypic drug susceptibility testing; R, resistance markers; RIF, rifampicin; S, no resistance markers.

culture and the option of pDST. The sediment of decontaminated specimens might, however, perform differently in PCRs than fresh sputum.

We especially focused on test sensitivity with paucibacillary, smear-negative samples and samples growing NTM. Particularly in fully industrialized countries, NTM are increasingly isolated from clinical specimens and reported as cause of opportunistic infections. Previous studies observed unspecific low positive results of Xpert MTB/RIF and Abbott RealTime MTB in the presence of NTM in high concentrations. Modern assays need to reliably differentiate between infections with either MTBC or NTM, and BD MAX performed well in this respect.

Among samples that grew MTBC in culture, the sensitivity of BD MAX was 100% (95% CI, 96.9% to 100%) and 64.5% (95% CI, 53.8% to 74%) with smear-positive and smear-negative samples, respectively. The sensitivity for smear-negative TB samples was almost identical to that reported from a recent multicenter study (65%) and in the range (41% to 75%) of other commercial assays such as Abbott RealTime MTB, Xpert MTB/RIF, and FluoroType MTB. Sensitivity of BD MAX with smear-negative specimens is assumed to be higher when testing unprocessed specimens instead of processed specimens, as suggested by Shah et al, who reported a sensitivity of 81% with raw smear-negative sputa. In line with this hypothesis, we recently observed an analytical sensitivity of BD MAX of 2.1 cfu/mL with unprocessed sputa being equal to that of Xpert MTB/RIF Ultra.

High specificity rates of molecular TB assays are of particular importance in low TB prevalence settings to reach high positive predictive values and to reliably discriminate between MTBC and NTM in smear-positive cases. In this respect, BD MAX seemed to be superior over Abbott RealTime MTB and Xpert MTB/RIF Ultra. Given an estimated rate of 4% (range, 2% to 6%) of MTBC-positive respiratory samples (with equal proportions of smear-negative and smear-positive samples) in laboratories of low-incidence countries, positive and negative predictive values of BD MAX would be calculated to 100% and 98.6% (range, 97.8% to 99.3%), respectively, based on the sensitivity and specificity values determined in this study. For comparison, Shah et al reported slightly lower positive and negative predictive values of 94% and 97% for fresh samples in high-prevalence countries.

It is of increasing importance to accurately identify resistance markers directly in clinical specimens to allow for rapid and adequate therapy and control of further transmission. One of the greatest assets of BD MAX is its ability to detect both INH and RIF resistance. Non-MDR INH-resistant TB accounts for 8% of patients worldwide; failure to identify it increases the risk of developing further resistance. This study showed that BD MAX accurately detected RIF and INH resistance markers of the targeted sequences. BD MAX resistance results were in nearly full concordance (99.4%) with those of MTBDRplus and Sanger sequencing. Only two specimens exhibited discrepancies, one with a reproducible discrepant INH resistance and another one with a not-reproducible discrepant RIF resistance indicated by BD MAX. For the latter, the initially false resistant result may have resulted from delayed hybridization of individual rpoB wild-type probes due to low concentrations of MTBC bacteria, as also described for other assays such as Xpert MTB/RIF. Regarding detection of RIF resistance by BD MAX, an additional aspect must be considered: as with Xpert MTB/RIF or Abbott RealTime MTB, BD MAX does not indicate the
specific rpoB mutation. RIF-resistant results might, therefore, be confirmed by use of other genotypic tests (ie, sequencing) and pDST to avoid unnecessary treatment in case of silent mutations and to detect disputed mutations often causing low-level resistance.

This study had some limitations. First, frozen N-acetyl-L-cysteine/sodium hydroxide sediments were used. Freezing might have negatively influenced the sensitivity of the assay, in particular with paucibacillary samples. Second, the study was performed in a low-resistance setting, which did not allow investigating the assay with a broader range of mutations. Third, inclusion of up to three samples per patient might have created an atypical distribution of resistance genotypes. For example, mutations outside the katG and inhA regions targeted by BD MAX are expected in <10% of INH-resistant isolates, although with large geographical variations from 5% to 30%.27–29 In this study, the five (of 12) samples with INH-resistant MTBC isolates that did not exhibit katG or inhA mutations were collected from only two patients. Thus, the relatively low sensitivity of 58.3% we reported for INH resistance cannot be considered representative.

In conclusion, our study showed that BD MAX can be applied to decontaminated respiratory specimens to detect MTBC with high sensitivity and specificity and INH/RIF resistance markers with high reliability. We consider this test as a great contribution to larger TB laboratories on an intermediate or reference level with the need for medium- to high-throughput testing.

Acknowledgments

We thank Anja Speiser for technical support and the technical team of the mycobacteriology laboratory SYNLAB Gauting for support.

Author Contributions

S.H.-T. and H.H. designed the study and wrote the manuscript; S.H.-T. additionally supervised the study and collected and analyzed the data; S.P. and M.M. performed the experiments; M.B. implemented and supervised the assays on the BD MAX platform and wrote the manuscript; and M.H.-N. and K.A. collected and provided clinical samples and data.

References


