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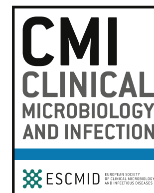

Research note

Diagnostic accuracy of the FluoroType MTB and MTBDR VER 2.0 assays for the centralized high-throughput detection of Mycobacterium tuberculosis complex DNA and isoniazid and rifampicin resistance

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ABSTRACT

Objectives: To evaluate the accuracy of two new molecular diagnostic tests for the detection of drug-resistant tuberculosis, the FluoroType MTB and MTBDR VER 2.0 assays, in combination with manual and automated DNA extraction methods.

Methods: Sputa from 360 Xpert Ultra *Mycobacterium tuberculosis* complex (MTBC)-positive patients and 250 Xpert Ultra MTBC-negative patients were tested. GenoType MTBDR*plus* served as reference for MTBC and drug resistance detection. Sanger sequencing was used to resolve discrepancies.

Results: FluoroType MTB VER 2.0 showed similar MTBC sensitivity compared with FluoroType MTBDR VER 2.0 (manual DNA extraction: 91.6% (294/321) versus 89.8% (291/324); p 0.4); automated DNA extraction: 92.1% (305/331) versus 87.7% (291/332); p 0.05)). FluoroType MTBDR VER2.0 showed comparable diagnostic accuracy to FluoroType MTBDR VER1.0 as previously reported for the detection of MTBC and rifampicin and isoniazid resistance.

Conclusions: The FluoroType MTB and MTBDR VER 2.0 assays together with an automated DNA extraction and PCR set-up platform may improve laboratory operational efficiency for the diagnosis of MTBC and resistance to rifampicin and isoniazid and show promise for the implementation in a centralized molecular drug susceptibility testing model. **Anzaan Dippenaar, Clin Microbiol Infect 2021;27:1351.e1–1351.e4**

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Introduction

Tuberculosis (TB) remains the leading cause of death from an infectious agent, causing an estimated 1.4 million deaths among approximately 10 million TB cases in 2019 [1]. Three million TB cases were estimated to remain undetected in the same year. One solution to close the diagnostic gap for the diagnosis of TB and drug resistance is the use of centralized high-throughput platforms for the detection of *Mycobacterium tuberculosis* complex (MTBC) and molecular drug susceptibility testing. FluoroType (FT) MTB VER 2.0 and MTBDR VER 2.0 (Hain Lifescience GmbH, Nehren, Germany, referred to as FT MTB V2.0 and FT MTBDR V2.0) are simple, rapid, closed-tube molecular tests. Interpretation of FT results is fully automated, thereby removing operator interpretation subjectivity. In addition, the ability of FT MTBDR V2.0 to simultaneously identify a comprehensive array of mutations in the *rpoB* and *katG* and the *inhA* promoter may be useful for guiding individualized treatment regimens [2,3].

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Here, we evaluated the diagnostic performance of FT MTB V2.0 and MTBDR V2.0 using smear-positive and smear-negative sputum specimens. To our knowledge this is the first study to evaluate the performance of these assays using DNA extracts from two methodologies: the automated GenoXtract96 (GXT96) instrument with the GXT96 X2 DNA extraction kit (Hain Lifescience), and the manual FluoroLyse (FL) kit (Hain Lifescience).

Materials and methods

Detailed methodology is described in the Supplementary material (Appendix S1), including an overview of the workflow for the detection of MTBC and resistance to rifampicin and isoniazid using FT MTB V2.0 and MTBDR V2.0 (see Supplementary material, Fig. S1).

Remnant sputum specimens were collected from 610 presumptive TB cases following testing with Xpert MTB/RIF Ultra (Xpert Ultra, Cepheid Inc., Sunnyvale, CA, USA) (on a second specimen) at the National Health Laboratory Services, Green Point, Cape Town, South Africa. MTBC-negative and MTBC-positive sputum sediments, irrespective of the smear status and Xpert Ultra rifampicin-resistance result, were included in the study. As per South African national treatment guidelines, these cases should not have been on TB treatment at the time of sample collection. A unique study number was assigned to each specimen and patient identifiers were removed. A waiver of consent has been granted for this study by the Stellenbosch University Health Research Ethics Committee (Reference: N12/01/001).

DNA was extracted from the sputum sediments and corresponding cultured isolates (if available) using the FL kit and the GXT96 X2 DNA extraction kit (GXT96 instrument) according to the manufacturer's instructions. FT MTB V2.0 and MTBDR V2.0 were performed using the FluoroCycler XT instrument (Hain Lifescience). For DNA extracted using the FL kits, PCR mixes were freshly prepared according to the manufacturer's instructions. Controls included the negative control with no clinical material, prepared during the DNA extraction process, and the positive control, β-C+. For DNA extracted using the GXT96 instrument, PCR mixes were set up by the instrument.

For MTBC detection, FT MTB V2.0 and MTBDR V2.0 results were compared with the TUB band from the GenoType (GT) MTBDR*plus* result obtained from the corresponding cultured isolates. In a secondary analysis, FT MTB V1.0 was carried out on the FL extract for all samples with discrepant (false-positive) results for MTBC detection. In the secondary analysis, we considered all discrepant samples with positive FT MTB V1.0 results as true MTBC positives. In case of invalid or indeterminate results (automatically scored as such by the analyser module of the FluoroSoftware XT-IVD because of the absence of the amplification control, or inability to discriminate between susceptibility/resistance, respectively), the FT assays were repeated, and the second result was used for the final analysis.

For the detection of rifampicin and isoniazid resistance, FT MTBDR V2.0 results were compared with the GT MTBDR*plus* results obtained from the corresponding cultured isolates, as not all specimens had a valid GT MTBDR*plus* result from a sputum sediment. For discrepant drug resistance results, GT MTBDR*plus* results obtained from the sputum sediments were used as the reference method for a head-to-head comparison between GT MTBDR*plus* and FT MTBDR V2.0. If no valid GT MTBDR*plus* result occurred from sputum sediment or GT MTBDR*plus* failed to differentiate specific mutations, Sanger sequencing was used as reference method. In case of invalid or indeterminate results, FT MTBDR V2.0 was repeated and the second result was used for the final analysis. Samples that showed indeterminate FT MTBDR V2.0 results in resistance detection were not included in the diagnostic parameter calculation but were used to calculate inhibition rate (number of samples with no actionable resistance result over total number tested).

Statistical analysis (two-sample test of proportions, https://www.stata.com/manuals/rprtest.pdf) was performed using Stata statistical software, release 14.0 (StataCorp Inc., College Station, TX, USA).

Results

Of the 610 sputum specimens included, 360 sputum specimens from Xpert Ultra MTBC-positive patients included 119 sputum smear-positive and 241 sputum smear-negative specimens. From the 360 sputum specimens from Xpert Ultra MTBC-positive patients, 36 were Mycobacteria growth indicator tube (MGIT) culture negative and 324 were MGIT culture-confirmed MTBC positive by GT MTBDR*plus*. In addition, 250 sputa were collected from patients diagnosed as Xpert Ultra MTBC-negative, of which eight were found to be MGIT culture positive with a confirmatory MTBC-positive result by GT MTBDR*plus* (see Supplementary material, Fig. S2).

Both FT MTB V2.0 and MTBDR V2.0 showed a sensitivity and specificity of 100% for the detection of MTBC in smear-positive specimens irrespective of the DNA extraction method used. The sensitivity and specificity of FT MTB V2.0 for the detection of MTBC in smear-negative specimens were 86.8% (95% CI 81.4%–91.1%) and 93.8% (95% CI 90.3%–96.4%), and 87.8% (95% CI 82.6%–91.9%) and 94.4% (95% CI 91.0%–96.8%), from FL and GXT96 X2 extracts, respectively. The sensitivity and specificity of FT MTBDR V2.0 for the detection of MTBC in smear-negative specimens were 84.4% (95% CI 78.7%–89%) and 97.5% (95% CI 94.8%–99%), and 80.8% (95% CI 74.9%–85.9%) and 97.1% (95% CI 94.4%–98.7%) from FL and GXT96 X2 extracts, respectively (Table 1). The discrepancy analysis using FT MTB V1.0 for both FT assays and a detailed layout of the results are described in the Supplementary material (Appendix S1, Table S1).

For rifampicin resistance detection, FT MTBDR V2.0 from FL extracts showed an initial sensitivity and specificity of 100% (95% CI 85.8%–100%) and 97.8% (95% CI 92.2%–99.7%) in smear-positive specimens; and 100% (95% CI 92%–100%) and 96.9% (95% CI 92.4%–99.2%) in smear-negative specimens, respectively. From GXT96 X2 extracts the assay showed a sensitivity and specificity of 100% (95% CI 85.8%–100%) and 97.9% (95% CI 92.5%– 99.7%) in smear-positive specimens; and 97.6% (95% CI 87.1%–99.9%) and 99.2% (95% CI 95.6%–100%) in smear-negative specimens, respectively (Table 2). For isoniazid resistance detection, FT MTBDR V2.0 showed an initial sensitivity and specificity of 100% in smear-positive specimens, irrespective of the DNA extraction method. In smear-negative specimens, the assay showed a sensitivity and specificity of 93.8% (95% CI 82.8%–97.7%) and 97.4% (95% CI 92.6%–99.5%), and 90.9% (95% CI 78.3%–97.5%) and 99.1% (95% CI 95%–100%), from FL and GXT96 X2 extracts, respectively. The discrepancy analysis for both smear-positive and smear-negative specimens by Sanger sequencing are described in the Supplementary material (Appendix S1, Table S2 and S3). FT MTBDR V2.0 was able to detect specific mutations in *rpoB*, *katG* and *inhA* promoter with an accuracy of at least 98.9% for all three targets, irrespective of the DNA extraction method (see Supplementary material, Appendix S1, Table S4).

Discussion

This is the first study to perform a head-to-head assessment of FT MTB V2.0 and FT MTBDR V2.0 using two DNA extraction methods; the manual FL kit and the automated GXT96 instrument with the GXT96 X2 DNA extraction kit.

Table 1
Diagnostic performance of FT MTB V2.0 and FT MTBDR V2.0 for the detection of MTBC from smear-positive and smear-negative sputum specimens

	Overall	Sputum smear-positive	Sputum smear-negative
	% (95% CI) *n/N*	% (95% CI) *n/N*	% (95% CI) *n/N*
FT MTB V2.0			
FluoroLyse			
No. of invalid tests	13	2	11
Sensitivity	91.6 (87.9–94.3) 294/321, p 0.44[d]	100 (96.9–100) 116/116	86.8 (81.4–91.1) 178/205, p 0.74[c]
Specificity	93.8 (90.1–96.3) 259/276, p 0.04[d]	100 (2.5–100) 1/1	93.8 (90.3–96.4) 258/275 p 0.04[c]
GXT96			
No. of invalid tests	9	0	9
Sensitivity	92.1 (88.6–94.7) 305/331, p 0.05[d]	100 (96.9–100) 118/118	87.8 (82.6–91.9) 187/213, p 0.76[b]; p 0.04[c]
Specificity	94.4 (90.8–96.8) 255/270, p 0.72[d]	100 (2.5–100) 1/1	94.4 (91.0–96.8) 254/269, p 0.71[b]; p 0.12[c]
FT MTBDR V2.0			
FluoroLyse			
No. of invalid tests	9	5	4
Sensitivity	89.8 (85.9–92.8) 291/324	100 (96.8–100) 113/113	84.4 (78.7–89) 178/211
Specificity	97.5 (94.6–98.9) 270/277	100 (2.5–100) 1/1	97.5 (94.8–99) 269/276
GXT96			
No. of invalid tests	0	0	0
Sensitivity	87.7 (83.5–90.9) 291/332	100 (96.9–100) 118/118	80.8 (74.9–85.9) 173/214 p 0.34[a]
Specificity	96.8 (93.7–98.4) 269/278	0 (0–97.5) 0/1	97.1 (94.4–98.7) 269/277 p 0.80[a]

Abbreviations: FT, FluoroType; MTBC, *Mycobacterium tuberculosis* complex.
[a] p values comparing the two DNA extractions methods for FT MTBDR V2.0 in smear-negative specimens.
[b] p values comparing the two DNA extractions methods for FT MTB V2.0 in smear-negative specimens.
[c] p values comparing FT MTBDR V2.0 and FT MTB V2.0 within DNA extraction method for smear-negative specimens.
[d] p values comparing FT MTBDR V2.0 and FT MTB V2.0 within DNA extraction method for all specimens.

Table 2
Diagnostic performance of FT MTBDR V2.0 and FT MTB V2.0 for the detection of rifampicin and isoniazid resistance from smear-positive and smear-negative sputum specimens

	Overall	Sputum smear-positive	Sputum smear-negative
	% (95% CI) *n/N*	% (95% CI) *n/N*	% (95% CI) *n/N*
FluoroLyse			
Rifampicin			
No. of ind tests	3	0	3
Sensitivity	100 (93.3–100) 68/68	100 (85.8–100) 24/24	100 (92–100) 44/44, p 0.30[b]
Specificity	97.3 (93.9–98.9) 214/220	97.8 (92.1–99.7) 87/89	96.9 (92.4–99.2) 127/131, p 0.72[a]; p 0.20[b]
Isoniazid			
No. of ind tests	14	0	14
Sensitivity	95.9 (87.8–98.9) 71/74	100 (86.8–100) 26/26	93.8 (82.8–98.7) 45/48, p 0.19[a]; p 0.61[b]
Specificity	98.5 (95.4–99.6) 200/203	100 (95.8–100) 87/87	97.4 (92.6–99.5) 113/116, p 0.13[a]; p 0.34[b]
GXT96			
Rifampicin			
No. of ind tests	8	0	8
Sensitivity	98.5 (90.6–100) 64/65	100 (85.8–100) 24/24	97.6 (87.1–99.9) 40/41, p 0.44[a]
Specificity	98.6 (95.7–99.6) 215/218	97.9 (92.5–99.7) 92/94	99.2 (95.6–100) 123/124, p 0.41[a]
Isoniazid			
No. of ind tests	19	0	19
Sensitivity	94.3 (85.3–98.2) 66/70	100 (86.8–100) 26/26	90.9 (78.3–97.5) 40/44, p 0.11[a]
Specificity	99.5 (96.8–100) 201/202	100 (96.1–100) 92/92	99.1 (95–100) 109/110, p 0.36[a]

Abbreviations: FT, FluoroType; ind, indeterminate; MTBC, *Mycobacterium tuberculosis* complex.
[a] p values comparing different smear groups for each DNA extraction method.
[b] p values comparing different DNA extraction methods in smear-negative specimens.

The diagnostic accuracy of FT MTBDR V2.0 for the detection of MTBC and rifampicin and isoniazid resistance was highly concordant with that of GT MTBDR*plus*. Performance of FT MTBDR V2.0 is similar to what has been reported for V1.0 [4,5]. This demonstrates its suitability as a replacement assay for GT MTBDR*plus*, without the subjectivity of visually interpreting the hybridization patterns. FT MTB V2.0 performed on GXT96 X2 DNA extracts had a higher diagnostic accuracy for the detection of MTBC compared with FT MTBDR V2.0 in smear-negative specimens (p 0.04). This may be explained by FT MTB V2.0 targeting a multi-copy region, IS*6110*, whereas FT MTBDR V2.0 targets *rpoB* only. No significant difference was, however, observed when FL DNA extracts were used.

This study was limited by the use of Xpert Ultra as sample selection criteria, and the quality between the sample used for Xpert Ultra testing and the second sample used for FT MTB V2.0 and FT MTBDR V2.0 in this study may have been different. Although the study did not assess the diagnostic accuracy of the two FT assays in a prospectively enrolled cohort, the large number of smear-negative culture-positive specimens tested should allow for a fair representation of severe pauci-bacillary specimens. This study also did not use phenotypic drug susceptibility testing as the reference method for resistance detection and the results of the study were only compared with a combination of GT MTBDR*plus* and limited Sanger sequencing of the resistance targets. As heteroresistance was not specifically investigated, this cannot be ruled out as a cause for the remaining discordant results between the FT MTBDR V2.0 and the reference method.

The high diagnostic accuracy of FT MTBDR and MTB V2.0 assays in combination with an automated DNA extraction and PCR set-up method may shorten turnaround-time for the diagnosis of MTBC and resistance to isoniazid and rifampicin when implemented in high-throughput centralized laboratories. Centralized molecular drug susceptibility testing at district-level or province-level with a supporting sample transport network can result in cost sharing across health disciplines, consolidate maintenance of high-level infrastructure in centralized facilities, and ensure increased testing volumes to achieve economies of scale [6]. This centralized model has been used in South Africa via the National Health Laboratory Service and shows promise for the implementation of new diagnostic assays [7].

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Transparency declaration

AD has received conference and salary support from Hain Lifescience GmbH. Kits and reagents were provided by Hain Lifescience GmbH. All other authors have no conflicts of interest to declare.

Author contributions

AD performed experiments, analysed the data, interpreted results, and wrote the manuscript. BD performed experiments, and edited and approved the manuscript. TD provided access to specimens, and edited and approved the manuscript. PDvH edited and approved the manuscript. GT and RMW conceptualized the study, interpreted the results, and edited and approved the manuscript. MdV conceptualized the study, performed experiments, analysed the data, interpreted results and wrote the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.04.022.

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