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Comparison of Three Commercial Molecular Assays for Detection of Rifampin and Isoniazid Resistance among *Mycobacterium tuberculosis* Isolates in a High-HIV-Prevalence Setting

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In a head-to-head comparison of the MTBDR*plus* version 2.0 (Hain Lifescience), the Xpert MTB/RIF (Cepheid), and the Anyplex MTB/NTM (Seegene) assays, we demonstrated equal sensitivity (59/61; 96.7%) and specificity (53/54; 98.1%) for detecting rifampin resistance with further analysis of discordances. The Xpert assay does not detect isoniazid resistance while the Anyplex assay showed high false positivity.

There are a limited number of commercial molecular assays available for the rapid detection of drug-resistant tuberculosis (DR-TB), and currently only two are endorsed by the WHO for this purpose: the GenoType MTBDR*plus* version 1.0 (Hain Lifescience GmbH, Germany) and the Xpert MTB/RIF (Cepheid, USA) (1–3). The MTBDR*plus* has been further optimized, and this new version (2.0) can now be performed on both smear-positive and smear-negative clinical specimens as well as cultured isolates, according to the manufacturer (4, 5).

The Anyplex MTB/NTM (Seegene, South Korea) assay has not undergone WHO review for use in detecting DR-TB but is widely used. It is a multiplex real-time PCR assay capable of distinguishing between *Mycobacterium tuberculosis* and nontuberculosis mycobacteria (NTM) while allowing for the amplification of drug resistance-related gene (*rpoB*, *katG*, and *inhA*) sequences simultaneously (6, 7).

In this study, we evaluated the performance characteristics of these three molecular assays for the detection of DR-TB by performing a head-to-head comparison of these technologies.

This was a retrospective laboratory-based evaluation study. Cultured isolates were collected from the TB laboratory, Diagnostic Division of the Department of Medical Microbiology, Tshwane Academic Division, National Health Laboratory Services (NHLS). The results from the MTBDR*plus* version 1.0 assay, performed as part of the routine laboratory testing formed the basis of stratification into 50 multidrug-resistant (MDR) isolates, 30 fully susceptible isolates, 20 monoresistant (10 rifampin and 10 isoniazid resistant) isolates, and 20 isolates with undefined mutations. The 20 isolates with undefined mutations, having a wild-type missing with no corresponding mutation band on the MTBDR*plus* version 1.0 assay, were further characterized by means of Sanger sequencing.

These isolates were collected consecutively to make up a total of 120, which was calculated to provide the required sample size for the comparative evaluation. Four isolates from this sample subset were excluded as they were duplicates and another had poor banding repeatedly on the MTBDR*plus* version 2.0 assay. A total of 115/120 (96%) cultured isolates were analyzable on all three systems.

The MTBDR*plus* version 2.0, the Xpert MTB/RIF (GXP) (G3 cartridge), and the Anyplex MTB/NTM (Anyplex) assays were performed according to the manufacturer's instructions. For the GXP, 1 ml of cultured isolate was used, and the sample reagent buffer was added in a 2:1 ratio. The extracted DNA templates of isolates with discordant results and isolates with undefined mutations based on MTBDR*plus* version 1.0 were amplified utilizing the primers as previously described for *rpoB*, *katG*, and *inhA* (8). Cycle sequencing utilizing the BigDye Terminator cycle sequencing ready reaction kit (ABI Prism; Applied Biosystems, USA) was performed according to the manufacturer's instructions. The sequence of the PCR-amplified DNA was then determined utilizing the ABI 3500 XL (Applied Biosystems) genetic analyzer. Single nucleotide polymorphisms and indels were detected using the CLC Genomics Workbench (Qiagen, Germany). All variants were evaluated using the Dream DB database, and high-confidence mutations were regarded as resistant (9). For the comparison of the three molecular assays, we used as a molecular gold standard the original MTBDR*plus* version 1.0 assay mutation results as well as sequencing results as mentioned before with the sequencing result regarded as final.

Of the 115 isolates evaluated, 61 were rifampin resistant and 59 were isoniazid resistant based on the molecular gold standard. All three of the assays had exactly the same sensitivity (59/61; 96.7%) and specificity (53/54; 98.1%) for the detection of rifampin resistance. However, the discordances were not the same and are shown in Table 1.

Two cases of rifampin resistance were not detected by either the MTBDR*plus* version 2.0 or the GXP assay; both of these cases

Received 23 June 2015 **Accepted** 23 June 2015

Accepted manuscript posted online 1 July 2015

Citation Strydom K, Ismail F, Matabane MMZ, Onwuegbuna O, Omar SV, Ismail N. 2015. Comparison of three commercial molecular assays for detection of rifampin and isoniazid resistance among *Mycobacterium tuberculosis* isolates in a high-HIV-prevalence setting. J Clin Microbiol 53:3032–3034. doi:10.1128/JCM.01691-15.

Editor: B. A. Forbes

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doi:10.1128/JCM.01691-15

TABLE 1 Resolution of discordant rifampin results by sequencing of the *rpoB* region[a]

Isolate	Result for:			
	MTBDR*plus* version 2.0	Anyplex	GXP	Sequencing
40	S	R	S	Poor sequencing
41[b]	R	S	R	Insertion TTC 432−433
92[c]	R	S	S	Wild type
99[d]	R	S	R	SNP 1862 C>T His>Tyr 445His526Tyr
87[e]	S	R	S	SNP 1184 T>C Leu>Pro 452Leu533Pro
117[f]	S	R	S	SNP 1184 T>C Leu>Pro 452Leu533Pro

[a] R, resistant; S, susceptible; WT, wild type.
[b] Isolate 41: MTBDR*plus* version 2.0 mutation in codon 510−519 (*rpoB* WT 3 missing), no mutation band, GXP probe B not binding.
[c] Isolate 92: MTBDR*plus* version 2.0 S531L mutation, no wild type missing, possibly mixed infection.
[d] Isolate 99: MTBDR*plus* version 2.0 mutation in codon 526−529 (*rpoB* WT 7 missing) and H526Y mutation, GXP probe D not binding.
[e] Isolate 87: Repeat on MTBDR*plus* version 2.0 (later version) mutation in codon 530−533 (*rpoB* WT 8 missing), repeat on GXP G4 cartridge probe E not binding.
[f] Isolate 117: Repeat on MTBDR*plus* version 2.0 (later version) mutation in codon 530−533 (*rpoB* WT 8 missing), repeat on GXP G4 cartridge probe E not binding.

had L533P mutations in the rifampin resistance-determining region upon sequencing. When evaluations were repeated using the most recent release of the version 2.0 assay as well as the GXP (G4 cartridge) assay, these mutations were detected by the MTBDR*plus* version 2.0 assay in codon 530 to 533 of the *rpoB* region (missing wild-type 8 band with no corresponding mutation band) and by the GXP (G4 cartridge) assay (probe E not binding). This mutation has been associated with discordant genotypic and phenotypic susceptibility results, notably when the widely employed broth-based phenotypic susceptibility testing methods are utilized; hence, the L533P mutation was thought to be a silent mutation, with no impact on the efficacy of rifampin (10), and has also been shown to be missed by the G3 cartridge (11, 12). Upon a retrospective review of our laboratory records, these two isolates had tested as rifampin susceptible with the MGIT 960 system. However, recent data have demonstrated that this L533P mutation is associated with low-level resistance, which is clinically significant, and retesting using the latest versions of the assays has addressed this shortcoming (13–15).

One isolate was detected as falsely rifampin resistant by the MTBDR*plus* version 2.0 assay against the molecular gold standard, displaying a S531L mutation and wild-type banding pattern at codon 530 to 533, which suggests a mixed population. This isolate was recorded as rifampin resistant according to MGIT 960 phenotypic drug susceptibility testing performed as part of the routine laboratory testing, further supporting the findings for the line probe assay. However, no mutations were detected utilizing Sanger sequencing. Interestingly the GXP assay showed binding of all probes, but this may be a masking effect of a wild-type strain in a mixed population, which is a limitation of the assay design (16). Both the line probe assay and phenotypic susceptibility testing, on the other hand, are known to be able to detect mixed populations (17), and thus the specificity of the MTBDR*plus* version 2.0 assay may be falsely low in our study.

The Anyplex assay detected one case of false rifampin resistance and two cases of false rifampin susceptibility. Of the two falsely susceptible cases, one had a H526Y mutation and the other an insertion TTC at codon 514 on sequencing. The insertion TTC at codon 514 of the *rpoB* gene has been described in various studies (18–22) although at low frequency: 2.5% of rifampin-resistant isolates in one study and 4% in another (18, 23). This mutation has been associated with various levels of rifampin resistance with corresponding rifampin MICs of 16 to >256 μg/ml (20, 22). The second mutation that was not detected by the Anyplex assay was an H526Y mutation. This is a commonly occurring mutation associated with drug resistance and thus has important treatment implications if not detected (24). The case of false rifampin resistance could not be resolved by means of Sanger sequencing as this isolate displayed poor sequencing despite repeat attempts; however, the isolate remained wild type upon repeat testing with both the MTBDR*plus* version 2.0 and the GXP assays.

No comparison for the detection of isoniazid resistance was possible for the GXP assay as this does not form part of the test. The MTBDR*plus* version 2.0 and the Anyplex assays both had sensitivities of 100% for the detection of isoniazid resistance mutations at codon 315 for the *katG* gene and *inhA* promoter region. The Anyplex assay had poor specificity of 82.4% due to falsely reporting 10 isolates as having mutations present in the *inhA* and/or *katG* regions. These isolates were defined as wild type by the MTBDR*plus* version 1.0 assay. All 10 isolates with discordant isoniazid results were wild type on sequencing and also had all wild-type bands present on the MTBDR*plus* version 2.0 assay.

The three molecular assays under evaluation in this study performed well for the detection of rifampin resistance. The MTBDR*plus* version 2.0 and GXP (G3 cartridge) assays had good performances and the later versions of both addressed the shortcomings of the earlier versions. The MTBDR*plus* version 2.0 assay was superior to the Anyplex assay overall for the diagnosis of MDR-TB. The main drawback of the GXP assay is the lack of isoniazid susceptibility testing.

The strengths of this study are the large numbers of resistant isolates evaluated and the direct comparison between all three systems. The limitations of this study are the restrictive genotypic standard which is expected to miss 10 to 15% of isoniazid resistance (25), the lack of sequencing and phenotypic drug susceptibility data for all isolates, and the fact that the assays were not performed on direct samples. The Anyplex assay has the advantage of detecting NTM, but this was not assessed. In the South African context, this is of lesser concern but may be of importance in specialized populations.

Molecular assays can detect nonviable mycobacteria, which can lead to problems in interpretation by clinicians; however, they do offer faster clinically actionable results. The three assays showed overall good performance for the detection of DR-TB, with each having its own strengths and weaknesses.

ACKNOWLEDGMENTS

We acknowledge the National Health Laboratory Services for financial support.

We thank Seegene and Hain Lifesciences for their generosity in providing the kits.

REFERENCES

1. **World Health Organization.** 2008. Molecular line probe assays for rapid screening of patients at risk of multi-drug resistant tuberculosis (MDR-

TB). Policy statement.WHO, Geneva, Switzerland. http://www.who.int/tb/features_archive/policy_statement.pdf.
2. **World Health Organization.** 2011. Rapid implementation of the Xpert MTB/RIF diagnostic test: technical and operational 'how to' practical considerations. WHO, Geneva, Switzerland. http://whqlibdoc.who.int/publications/2011/9789241501569_eng.pdf.
3. **World Health Organization.** 2013. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary tuberculosis in adults and children. WHO, Geneva, Switzerland. www.who.int/iris/bitstream/10665/112472/1/9789241506335_eng.pdf.
4. **Hain Lifesciences GmbH.** 2012. GenoType MTBDR*plus*, version 2.0 product insert. Hain Lifescience, Nehren, Germany.
5. **Crudu V, Stratan E, Romancenco E, Allerheiligen V, Hilleman A, Moraru N.** 2012. First evaluation of an improved assay for improved molecular genetic detection of tuberculosis as well as rifampin and isoniazid resistances. J Clin Microbiol **50:**1264–1269. http://dx.doi.org/10.1128/JCM.05903-11.
6. **Seegene Inc.** 2010. Anyplex plus MTB/NTM real-time detection user manual. Seegene, Seoul, South Korea.
7. **Perry MD, White LP, Ruddy M.** 2014. Potential for use of the Seegen Anyplex MTB/NTM real-time detection assay in a regional reference laboratory. J Clin Microbiol **52:**1708–1710. http://dx.doi.org/10.1128/JCM.03585-13.
8. **Campbell PJ, Morlock GP, Sikes RD, Dalton TL, Metchock B, Starks AM, Hooks DP, Cowan LS, Plikaytis BB, Posey JE.** 2011. Molecular detection of mutations associated with first and second-line drug resistance compared with conventional drug susceptibility testing of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother **55:**2032–2041. http://dx.doi.org/10.1128/AAC.01550-10.
9. **Sandgren A, Strong M, Muthukrishnan P, Weiner BK, Church GM, Murray MB.** 2009. Tuberculosis drug resistance mutation database. PLoS Med **6:**e2. http://dx.doi.org/10.1371/journal.pmed.1000002.
10. **Hilleman D, Rusch-Gerdes S, Richter E.** 2007. Evaluation of the GenoType MTBDR*plus* assay for rifampicin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and clinical specimens. J Clin Microbiol **45:**2635–2640. http://dx.doi.org/10.1128/JCM.00521-07.
11. **Somoskovi A, Deggim V, Ciardo D, Bloemberg GV.** 2013. Diagnostic implications of inconsistent results obtained with the Xpert MTB/RIF assay in detection of *Mycobacterium tuberculosis* isolates with an *rpoB* mutation associated with low-level rifampicin resistance. J Clin Microbiol **51:**3127–3129. http://dx.doi.org/10.1128/JCM.01377-13.
12. **Rufai AB, Kumar P, Singh A, Prajapati S, Balooni V, Singh S.** 2014. Comparison of Xpert MTB/RIF with line probe assay for detection of rifampicin-monoresistant *Mycobacterium tuberculosis*. J Clin Microbiol **52:**1846–1852. http://dx.doi.org/10.1128/JCM.03005-13.
13. **Van Deun A, Barrere L, Bastian I, Fattorini L, Hoffmann H, Kam KM, Rigouts L, Rusch-Gerdes S, Wright S.** 2009. *Mycobacterium tuberculosis* strains with highly discordant rifampicin susceptibility test results. J Clin Microbiol **47:**3501–3506. http://dx.doi.org/10.1128/JCM.01209-09.
14. **Van Deun A, Aung KJM, Bola V, Lebeke R, Hossain MA, de Rijk WB, Rigouts L, Gumusboga A, Torrea G, de Jong BC.** 2013. Rifampicin drug resistance tests for tuberculosis: challenging the gold standard. J Clin Microbiol **51:**2633–2640. http://dx.doi.org/10.1128/JCM.00553-13.
15. **Williamson DA, Roberts SA, Bower JE, Vaughan R, Newton S, Lowe O, Lewis CA, Freeman JT.** 2012. Clinical failures associated with *rpoB* mutations in phenotypically occult multidrug-resistant *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis **16:**216–220. http://dx.doi.org/10.5588/ijtld.11.0178.
16. **Lawn SD, Nicol MP.** 2011. Xpert MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol **6:**1067–1082. http://dx. http://dx.doi.org/10.2217/fmb.11.84.
17. **Folkvardsen DB, Thomsen VO, Rigouts L, Rasmussen EM, Bang D, Bernaerts G, Werngren J, Toro JC, Hoffner S, Hillemann D, Svensson E.** 2013. Rifampicin heteroresistance in *Mycobacterium tuberculosis* cultures detected by phenotypic and genotypic drug susceptibility test methods. J Clin Microbiol **51:**4220–4222. http://dx.doi.org/10.1128/JCM.01602-13.
18. **Hirano K, Chiyoji A, Takahashi M.** 1999. Mutations in the *rpoB* gene of rifampicin-resistant *Mycobacterium tuberculosis* strains isolated mostly in Asian countries and their rapid detection by line probe assay. J Clin Microbiol **37:**2663–2666.
19. **Ahmad S, Al-Mutairi NM, Mokaddas E.** 2009. Comparison of performance of two DNA line probe assays for rapid detection of multidrug-resistant isolates of *Mycobacterium tuberculosis*. Indian J Exp Biol **47:**454–462.
20. **Bifani P, Mathemu B, Kurepina N, Shashkina E, Bertout J, Blanckis AS, Moghazeh S, Driscol J, Gicquel B, Frothingham R, Kreiswirth BN.** 2008. The evolution of drug resistance in *Mycobacterium tuberculosis*: from a mono-rifampicin resistant cluster to increasingly multidrug-resistant variants in an HIV-seropositive population. J Infect Dis **198:**90–94. http://dx.doi.org/10.1086/588822.
21. **Miotto P, Piana F, Penati V, Canducci F, Migliori GB, Cirillo DM.** 2006. Use of the GenoType MTBDR assay for molecular detection of rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* clinical strains isolated in Italy. J Clin Microbiol **44:**2485–2491. http://dx.doi.org/10.1128/JCM.00083-06.
22. **Yang B, Koga H, Ohno H, Ogawa K, Fukuda M, Hirakata Y, Maesaki S, Tomono K, Tashiro T, Kohno S.** 1998. Relationship between antimycobacterial activities of rifampicin, rifabutin and KRM-1648 and *rpoB* mutations of *Mycobacterium tuberculosis*. J Antimicrob Chemother **42:**621–628. http://dx.doi.org/10.1093/jac/42.5.621.
23. **Cooksey RC, Morlock GP, Glickman S, Crawford JT.** 1997. Evaluation of a line probe assay kit for characterization of *rpoB* mutations in rifampicin-resistant *Mycobacterium tuberculosis* isolates from New York City. J Clin Microbiol **35:**1281–1283.
24. **Kapur V, Lordanescu LLL, Hamrick MR, Wanger A, Kreiswirth BN, Musser JM.** 1994. Characterization by automated DNA sequencing of mutations in the gene (rpoB) encoding the RNA polymerase beta subunit in rifampicin-resistant *Mycobacterium tuberculosis* strains from New York City and Texas. J Clin Microbiol **32:**1095–1098.
25. **Zhang Y, Yew WW.** 2009. Mechanisms of drug resistance in *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis **13:**1320–1330.