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Major Article

The performance of Xpert MTB/RIF and MTBDRplus within a Programmatic setting at TB Laboratory in Rio de Janeiro, Brazil

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ABSTRACT

Background: Few studies in routine settings have confirmed the high accuracy of the Xpert MTB/RIF assay for detecting rifampicin resistance (RR) and the first-line probe assay (FL-LPA) for detecting both RR and isoniazid resistance (INH^R).

Methods: The performance of Xpert MTB/RIF and MTBDRplus VER 2.0 LPA was evaluated in 180 *Mycobacterium tuberculosis* samples collected from January 2018 to December 2019 in Rio de Janeiro, Brazil. The results were compared with those from BACTEC MGIT 960 culture and drug susceptibility testing (DST). Whole-genome sequencing was performed on the samples with discordant results.

Results: The Xpert MTB/RIF assay showed a sensitivity (Se) of 93.3% and a specificity (Sp) of 97.6%, detecting RR. The performance of FL-LPA to identify RIF and INH resistance was, respectively, (Se) 100% and 83.3% and (Sp) 98.8% and 100%. Among 18 clinical isolates with INH^R detected by FL-LPA, mutations in the *katG* gene were observed in 100% of samples, of which only two (11.1%) had mutations in both *katG* and *inhA* genes. Overall, the discordant results were identified in 9 (5%) samples. Among the four Xpert RIF-resistant and DST-sensitive, two harbored mutations in rpoB Leu430Pro. Among the four FL-LPA-sensitive and DST-resistant, one had a mutation in *inhA* 17G>T. FL-LPA showed high accuracy in detecting RR and INH^R.

Conclusions: The MTBDRplus test demonstrated excellent performance in detecting RR, and INH^R in clinical isolates under routine conditions at a reference laboratory in Rio de Janeiro, Brazil. Incorporating both tests can improve drug-resistant tuberculosis treatment outcomes and monitor the INH^R incidence.

Keywords: Drug-Resistant Tuberculosis. Molecular Diagnostic Techniques. Sensitivity and Specificity. Diagnosis. *Mycobacterium tuberculosis*.

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INTRODUCTION

The 2022 World Health Organization (WHO) Global Report states that in 2021, tuberculosis (TB) will become the second leading cause of death due to infectious diseases, following COVID-19, surpassing HIV/AIDS[1]. While drug-sensitive tuberculosis (DS-TB) is effectively cured within 6 to 9 months with the WHO recommended 1st line multiple anti-TB drug regimen, for TB patients with *Mycobacterium tuberculosis* (MTB) resistant to both isoniazid and rifampicin, defined as multidrug-resistant tuberculosis (MDR-TB), treatment can take up to two years using second-line drugs that are frequently more expensive, toxic, and have low favorable outcome[1]. The burden of drug-resistant

tuberculosis (DR-TB) also increased by 3% between 2020 and 2021, with 450,000 new cases of rifampin-resistant (RR) in 2021[1].

In addition, WHO provided global estimates of the incidence of isoniazid monoresistance (INH^R) for the first time: there were 1.4 million incident cases of INH^R-TB, of which 1.1 million were susceptible to rifampicin[1]. Most of these patients were not diagnosed with DR TB and did not receive appropriate treatment. Furthermore, there are limited data available on TB treatment outcomes among patients with INH^R-TB in high-burden countries[2].

MTB culture-based methods (solid or liquid), the gold standard for TB diagnosis, usually take several weeks and require empirical treatment without TB drug resistance results. To improve the early detection of MDR/RR-TB and reduce the time for initiation of appropriate treatment, the WHO recommended the following molecular tests: Genotype MTBDRplus/First Line – Line Probe Assay (henceforth FL-LPA) (Hain Lifescience, Nehran, Germany) in 2008 and Xpert MTB/RIF (Cepheid, USA) in 2010[3].

The meta-analysis of the Xpert MTB/RIF used for DR-TB diagnosis confirmed that it can be reliably executed directly on a respiratory sample in less than one day, with a sensitivity of 67 to 89% and high specificity, above 95%, and it also allows the direct detection of RR with high accuracy[4].

As the detection of INH^R by molecular tests is not usually possible in low- and middle-income countries, the identification of RR by Xpert MTB/RIF has been used as a predictive marker of MDR-TB, assuming that, in a certain region, there is a low prevalence of INH^R [5].

Therefore, FL-LPA is recommended for identifying RR and INH^R, particularly in regions with a high prevalence of mono INH^R. The high accuracy of FL-LPA has been reported by meta-analysis; for RR, it showed a pooled sensitivity and specificity (with 95% confidence intervals) of 96.7% (95.6-97.5%) and 98.8% (98.2-99.2%), respectively; and for INH^R, it demonstrated sensitivity and specificity of 90.2% (88.2-91.9%) and 99.2% (98.7-99.5%), respectively[6]. However, performance studies in the programmatic settings of Xpert MTB/RIF and FL-LPA for detecting RR, INH^R, or MDR-TB are limited, especially in high-TB burden and low-middle-income countries[7-13]. Thus, the main objective of the present study was to evaluate the performance of Xpert MTB/RIF and FL-LPA for the direct detection of RR, INH^R, and MDR-TB under routine diagnostic conditions in a laboratory environment in Rio de Janeiro, Brazil, a high-burden DR-TB setting.

METHODS

• Study Design and Population

This was a retrospective data analysis of the performance of Xpert MTB/RIF (Cepheid, USA) and Genotype MTBDRplus VER 2.0/ FL-LPA (Hain Lifesciences, Nehren, Germany) on 180 MTB isolates obtained between 2018 and 2019. The analysis was conducted at the Molecular Mycobacteriology Laboratory (LMM) of the Clementino Fraga Filho University Hospital (HUCFF) and the Thorax Diseases Institute (IDT) located in the State of Rio de Janeiro, which has the second highest incidence, highest TB mortality rate, and highest number of DR-TB cases in the country[14].

All MTB isolates were obtained from patients with presumed pulmonary TB evaluated at primary health centers in Rio de Janeiro or Duque de Caxias.

• MTB isolation and drug-sensitivity testing (DST)

In this study, MTB isolates obtained from respiratory samples were inoculated into an automated liquid culture. Decontamination, inoculation, and incubation of the samples were performed using a BACTEC MGIT 960 system (Becton Dickinson, USA) according to the manufacturer's instructions.

Samples were decontaminated using the N-acetyl-L-cysteine/ sodium hydroxide (NALC-NaOH, 2% NaOH) method. Part of the sediment was resuspended in 3 ml of phosphate buffer (pH 6.8) and directly inoculated into the liquid culture medium. Another part was enriched by culturing in Löwenstein–Jensen culture medium, cryopreserved using a freezing solution (7H9 + 10% glycerol and 10% OADC in liquid medium), and afterwards, defrosted and inoculated into liquid medium. All cultures were maintained at 37°C in the BACTEC MGIT 960 system.

First-line phenotypic drug sensitivity tests (DST) were also performed using the automated BACTEC MGIT 960 system according to the manufacturer's instructions. Drugs evaluated were streptomycin- SM (1.0 µg/ml), isoniazid- INH (0.1 µg/ml), rifampicin- RIF (1.0 µg/ml), ethambutol- EMB (5.0 µg/ml). DST results for RIF and INH (resistant or susceptible to the critical concentration tested) were considered the gold standard for the evaluation of genotypic results obtained using the Xpert MTB/RIF (Cepheid, USA) and Genotype MTBDRplus/FL-LPA (Hain Lifescience, Nehran, Germany) assays.

• Xpert MTB RIF test

Xpert MTB/RIF was performed with a volume of 1.5 ml taken from the clinical sample. The 1.5 ml volume was placed in a 15 ml conical tube, and the sample reagent was added at a ratio of two reagent volumes to one sample volume (2:1) and shaken vigorously 10-20 times, with or without vortex. The tube was incubated for 10 min at room temperature, vortexed vigorously 10-20 times, and incubated for an additional 5 min at room temperature. Samples that were not fully liquefied were shaken again and left at room temperature for 5-10 minutes. Decontamination and liquefaction steps did not exceed 35 min. For the liquefied samples, 2 ml of the total volume was transferred from inside the conical tube and deposited inside the Xpert MTB/RIF cartridge, and the test was performed automatically in a GeneXpert machine[3].

• GenoType® MTBDRplus Kit PCR reaction.

All samples that presented a valid Xpert MTB/RIF test detection were subsequently evaluated using GenoType® MTBDRplus VER 2.0 Kit (FL-LPA). FL-LPA was performed on all sampled MTB isolates, one per patient, using the MGIT DST results. Cultures were subjected to DNA extraction one day before entering the MGIT 960 system instrument for DST. DNA was extracted from the liquid cultures using a Genolyse kit (version 1.0; Hain). The reactions detected on the strips were visually interpreted using a cardboard template. In the case of invalid results, such as no signal with a conjugate or any of the other control probes, and doubtful reactions as weak signals with the gene bands, the test was repeated using a new DNA extraction[6].

• Whole genome sequencing (WGS)

WGS was performed only for samples that showed discordant results between the genotypic and phenotypic assays. DNA from

the four samples was subjected to Next-Generation Sequencing (NGS) to obtain the whole-genome sequence. Paired-end sequencing (2 × 150 bp) was performed on an Illumina NextSeq machine using either a 300 cycle v2 mid-output or high-output kit (Illumina, Code FC-404-2003 or Code FC- 404-2004) under standard Illumina® procedure as previously described[15].

• Statistical analysis

Kappa concordance index (K) statistics were calculated based on (a) the proportion of RIF and/or INH results reported as resistant versus (b) the proportion of RIF and INH results reported as susceptible, using each of the genotypic molecular methods, Xpert MTB/RIF and FL-LPA, compared to the respective DST results obtained by the gold standard, the BACTEC MGIT 960 phenotypic method. The sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and accuracy (A) of the Xpert MTB/RIF and FL-LPA tests were calculated from the ratio of genotypic results to phenotypic gold-standard results.

WGS bioinformatics analysis of the raw reads was conducted as previously described by Salvato et al[15]. SPSS software, version 21.0, was used for the kappa index analyses. The remaining statistical analyses were performed using the online software MedCalc Software - Diagnostic Test Evaluation Calculator (version 20.027), available at https://www.medcalc.org/calc/diagnostic_test.php.

• Data availability

Mycobacterium tuberculosis WGS data are available in the NCBI BioProject ID PRJNA719107.

RESULTS

• Resistance detected by First Line – Line Probe Assay

The resistance profiles of 180 clinical samples were analyzed using Xpert MTB/RIF, FL-LPA, and drug susceptibility testing after isolation in a liquid medium (MGIT 960).

The performance of Xpert MTB/RIF in detecting RR showed a sensitivity (Se) of 93.3% and a specificity (Sp) of 97.6% (**Table 1**). The performance of FL-LPA in identifying RR and INH^R was 100% and 83.3% for sensitivity and 98.8% and 100% for specificity, respectively. The performance of FL-LPA in detecting MDR-TB was high, with a PPV of 93.3%.

Compared to MGIT 960 phenotypic DST, a high agreement with FL-LPA and Xpert MTB/RIF for RR was found – 0.93 and 0.83, respectively, and for INH^R with FL-LPA, 0.88. The agreement between Xpert MTB/RIF and FL-LPA for RR detection was 0.91. Monoresistance to SM, INH, and RIF was found respectively in 10%, 10%, and 0.6% of the samples. Ethambutol resistance was detected in one MDR-TB case.

Among the 11 samples with RR, the most prevalent mutations in the *rpoB* gene were in Wt 8 (codons 530-533) and Wt 7 (codons 526-529), representing 60% and 10%, respectively.

Among the 18 samples with INH^R detected by FL-LPA, a mutation in the *katG* gene was observed in 100% of the samples, with only two (10%) having mutations in both *katG* and *inhA* genes. None of the samples harbored mutations in the *inhA* gene alone. In addition, only one INH^R sample showed both WT1 (wild type) and mutant bands, suggesting heteroresistance or mixed infection.

• Discordant results analysis

Overall, discordance between genotypic and phenotypic results was identified in nine (5.0%) samples (**Table 2**): discordance between Xpert MTB/RIF and MGIT-960 DST in five cases, four cases which Xpert MTB/RIF indicated resistance and MGIT 960 DST showed susceptible and one case where Xpert MTB/RIF indicated rifampicin susceptibility, but the MGIT 960 DST showed resistance. For the four Xpert MTB/RIF resistant discordant cases, two had a Leu430Pro at *rpoB*, which is a borderline rifampicin resistance mutation that accounts for highly discordant results in phenotypic DST[16]. FL-LPA for RIF resistance was discordant with MGIT 960 DST in two cases, and no WGS results were available. Four samples with FL-LPA susceptible to INH and MGIT 960 DST resistant to INH showed one mutation in hA 17G>T, which is an INH^R canonical mutation in the inhA promoter region[17].

Comparing Xpert MTB RIF and FL-LPA results, among the three samples with discordant RIF resistance results, MGIT SIRE confirmed the FL-LPA results in one RIF-resistant sample and two RIF-sensitive samples.

TABLE 1: Performance of Xpert MTB RIF and MTBDRplus assays for tuberculosis and for rifampicin and isoniazid resistance detection at reference laboratory (n=180).

	True positive	False Positive	False Negative	True Negative	Sensitivity (95% CI)	Specificity (95% CI)	Positive Predicitve Value (95% CI)	Negative Predictive Value (95% CI)	Kappa value (95% CI)
RIF resistance									
Xpert MTB RIF	14	4	1	161	93.3%	97.6%	77.8%	99.4%	0.833
					(68.1-99.8)	(93.9-99.3)	(56.8-90.3)	(96.0-99.9)	(0.759-0.908)
MTBDRplus	15	2	0	163	100%	98.8%	88.24%	100%	0.931
					(78.2-100)	(95.7-99.9)	(65.4-96.8)	(97.8-100)	(0.857-1.000)
INH resistance									
MTBDRplus	20	0	4	156	83.3%	100%	100%	97.5%	0.887
					(62.6-95.3)	(97.7- 100)	(83.2-100)	(94.1-98.7)	(0.818-0.956)
MDR-TB resistance									
MDTDRplus	14	1	0	165	100%	99.4%	93.3%	100%	0.963
					(76.8-100)	(96.7-99.9)	(66.5-99.9)	(97.7-100)	(0.889-1000)

RIF: rifampicin; **INH:** isoniazide; **MDR:** multidrug-resistant; **CI:** Confidence interval.

TABLE 2: Evaluation of discordant results by whole genome sequencing and clinical history.

Nº MTB Isolate	RIF (Xpert)	RIF MTBDR plus	RIF (MGIT)	INH MTBDR plus	INH (MGIT)	WGS (rpoB)	WGS (katG/ inhA)
2640/18	S	R	R	R	R	Ser450Leu	-
747/18	R	S	S	R	R	Leu430Pro	-
17/19	R	S	S	R	R	Leu430Pro	-
2112/19	R	R	S	R	R	-	-
485/18	R	R	S	S	S	-	-
1191/18	S	S	S	S	R	-	-
2427/18	S	S	S	S	R	-	-
475/18	S	S	S	S	R	-	-
451/18	S	S	S	S	R	-	inhA-17G>T

N: Number; **RIF:** Rifampicin; **INH:** Isoniazid; **WGS:** whole genome sequencing; **S:** Susceptible; **R:** Resistant.

DISCUSSION

The Xpert MTB/RIF test for clinical samples and the FL-LPA molecular test for MTB clinical isolates showed high accuracy in the early diagnosis of RR/INH^R and MDR-TB when used under routine diagnostic conditions in Rio de Janeiro, Brazil. Although the predictive values may vary according to the prevalence of the disease in different settings[18], the sensitivity and specificity of the FL-LPA molecular assay obtained in this study are in accordance with the literature for high-burden settings in low- and middle-income countries under field conditions[7-13,19]. The performance of Xpert MTB/RIF to detect RR was also high, showing a sensitivity (Se) of 93.3% and a specificity (Sp) of 97.6%, similar to those described elsewhere[3]. In addition, compared to MGIT 960 phenotypic DST, a high agreement with FL-LPA and Xpert MTB/RIF for RR was found (0.93 and 0.83, respectively), and 0.88 for INH^R with FL-LPA.

FL-LPA was superior to Xpert MTB/RIF in the detection of RR, which is consistent with previously published data[20]. This is likely because FL-LPA detection is performed on DNA isolated from cultures, which yields high quantities of the template and high sensitivity (100% and 92.9%, respectively)[21].

Furthermore, FL-LPA can detect RR and INH^R directly in clinical samples, irrespective of the smear status, without the need for MTB culture growth, as highlighted in other series[7,8,10,12,22]. It is important to accelerate decision-making by the clinical team in defining the appropriate treatment course for RIF resistance, INH resistance, and MDR-TB. Thus, FL-LPA with Xpert MTB/RIF may be helpful in regions with a high prevalence of INH resistance that is not detected by Xpert[3]. The discordant results among genotypic and phenotypic tests were identified in only nine (5.0%) samples. In addition, the discordant results between Xpert MTB/RIF and FL-LPA for RIF resistance were identified in only three samples, confirmed by MGIT SIRE the FL-LPA results in 1 RIF resistant and 2 RIF sensitive samples.

In our study, false rifampicin resistance was detected by Xpert in four samples and by FL-LPA in two samples. These results may be associated with mutations in the *rpoB* gene, including what others have referred to as 'disputed mutations' or silent mutations[23-25]. Notably, among the four Xpert RR and DST sensitive cases, two showed mutations in rpoB Leu430Pro similar to those described by Brandao et al. in São Paulo, Brazil[24], and different from those described by Abanda et al., in Cameroon[23] and by Miotto et al[25] in Italy. Being part of the "RIF^R disputed" mutations (L430P, D435Y, H445C/L/N/S, and L452P), MTB isolates carrying this genetic profile are known to have a slow growth delta, taking about 30 days, in liquid culture medium with the drug rifampicin. Therefore, resistance was not detected in phenotypic testing, which was completed within 12 days according to the MGIT 960 base protocol[23-25].

Using Xpert MTB RIF results, as recommended by WHO, as an indicator of MDR[3], a low rate of discrepancy between genotypic and phenotypic results is expected, as the clinical staff could start RR/MDR-TB treatment. However, in São Paulo State, a high false RR resistant results rate (55%) was described by Brandao et al[24] and was associated with unusual clusters of rpoB mutations largely associated with low resistance levels. Unfortunately, in countries with a high TB burden, data on the clinical and economic impact of Xpert and/or MTBDRplus under field conditions[26-28] is scarce. In a nationwide study, Villalva-Serra K et al[27] described that the Xpert implementation in Brazil resulted in a 9.7% increase in TB notification and substantial improvements in DR-TB (63.6%) detection compared to expected notifications if it had not been implemented[26] showed in a pragmatic clinical trial, compared to the MGIT group, physicians received the genotypic DST result earlier using Xpert MTB RIF than those using MGIT (median 7.0 vs. 55.5 days; $p<0.01$), and using MTBDR plus in MTB isolate (30.0 vs. 40.2 days, $p<0.01$). Culture conversion after six months was higher for Xpert (90.9% vs. 79.3%, $p=0.39$) and not for LPA (80.0% vs. 83.0%, $p=0.81$). Soares et al[28], comparing the activity-based costs (ABC) using phenotypic and genotypic DST in a reference laboratory showed that ABC were higher for MGIT SIRE (US$ 136.80) and lower for Genotype® MTBDRplus (US$ 48.38) and for Xpert® MTB/RIF (US$ 9.89). Therefore, interactions between public managers, clinicians, and laboratory technicians are urgently needed to provide a more rapid and precise diagnostic algorithm at the local level and appropriate TB treatment initiation.

In the analysis of discordant results for INH^R, four samples showed sensitivity in FL-LPA and resistance in the phenotypic test; among these samples, one was confirmed to have the mutation

inhA-17G>T. Such false-negative results can be explained by the analysis profile of the FL-LPA test, which features only two detection genes for INH^R. Similar results have been described previously and attributed to the amplification of DNA released from non-viable bacilli in cases of heteroresistance[10]. Furthermore, it is well known that about 10% to 25% of INH^R strains are thought to have mutations outside *katG* and *inhA* loci, which, regrettably, we were not able to detect[29]. The molecular mechanisms underlying INH^R involve several genes in multiple networks and biosynthetic pathways. The recent association between efflux pumps, other genes, and INH^R has also gained considerable attention[30]. Substitutions were observed in fabG1, fabD, nat, accD6, and fbpC as reported by Unissa, et al[31]. Understanding the mechanisms associated with INH^R would allow better detection of INH^R. This information will aid in the design of new drug strategies.

Overall, *rpo*B Ser531Leu and *kat*G Ser315Thr mutations were predominant in our study, whereas inhA mutations were found in a small number of cases, similar to other settings[19,22,24,32-34]. These genetic markers show high accuracy, as we found in our study, where 93.3% of the RR samples detected by Xpert were characterized as MDR-TB by phenotypic DST. We also observed a low rate (0.5%) of probable heteroresistance (the clinical relevance of which is unknown), as described by Kumar et al[35] and Figueiredo et al[36]. Among the weaknesses of this study, we cite the limitation of WGS coverage on all discordant samples complemented by clinical data and the absence of MIC determination, as it is not part of the routine procedure, which determines the levels of resistance, especially for borderline mutations.

In conclusion, the MTBDRplus showed excellent performance as a rapid molecular test for the detection of RR-, INH^R, and MDR-resistant TB in clinical isolates under routine use in a reference laboratory in Rio de Janeiro, Brazil. Considering the low proportion of discordant results in the detection of RIF resistance between Xpert MTB RIF and MTBDRplus, such tests should be incorporated into the routine diagnosis of drug-resistant TB in regions with high DR-TB burden, as they expedite and support staff in choosing the appropriate clinical therapeutic approach for patients with TB, thus promoting a lower proportion of unfavorable TB treatment outcomes. Of special importance is the ability to routinely detect mono INH^R by FL-LPA and the need to follow up on these cases as their outcomes and progression towards MDR-TB are barely known, and their incidence is increasing dramatically in Brazil and worldwide[37].

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REFERENCES

1. World Health Organization (WHO). Global Tuberculosis Report 2022 [Internet]. Geneva: WHO. 2022. [cited 2023 Nov 5]. Available from: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022
2. Araújo-Pereira M, Arriaga MB, Carvalho ACC, Spener-Gomes R, Schmaltz CAS, Nogueira BMF, et al. Isoniazid Monoresistance and Antituberculosis Treatment Outcome in Persons With Pulmonary Tuberculosis in Brazil. Open Forum Infect Dis. 2024;11(1):ofad691. Available from: https://doi.org/10.1093/ofid/ofad691
3. World Health Organization (WHO). WHO consolidated guidelines on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection, 2021 update. Geneva: WHO, 2021. [cited 2023 Nov 11]. Available from: https://www.who.int/publications/i/item/9789240029415
4. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2014;2014(1):CD009593. Available from: https://doi.org/10.1002/14651858.cd009593.pub3
5. World Health Organization (WHO). Xpert MTB/RIF implementation manual: technical and operational 'how-to'; practical considerations. Geneva: WHO, 2014. 59p.
6. Nathavitharana RR, Cudahy PG, Schumacher SG, Steingart KR, Pai M, Denkinger CM. Accuracy of line probe assays for the diagnosis of pulmonary and multidrug-resistant tuberculosis: a systematic review and meta-analysis. Eur Respir J. 2017;49(1):1601075. Available from: https://doi.org/10.1183/13993003.01075-2016
7. Albert H, Bwanga F, Mukkada S, Nyesiga B, Ademun JP, Lukyamuzi G, et al. Rapid screening of MDR-TB using molecular Line Probe Assay is feasible in Uganda. BMC Infect Dis. 2010;10:41. Available from: https://doi.org/10.1186/1471-2334-10-41
8. Chen C, Kong W, Zhu L, Zhou Y, Peng H, Shao Y, et al. Evaluation of the GenoType(®) MTBDRplus line probe assay on sputum-positive samples in routine settings in China. Int J Tuberc Lung Dis. 2014;18(9):1034-9. Available from: https://doi.org/10.5588/ijtld.13.0857
9. Mironova S, Pimina E, Kontsevava I, Niklaveskyy V, Balabanova Y, Skenders G, et al. Performance of the GenoType® MTBDRPlus assay in routine settings: a multicenter study. Eur J Clin Microbiol Infect Dis. 2012;31(7):1381-7. Available from: https://doi.org/10.1007/s10096-011-1453-1
10. Barnard M, van Pitius NCG, van Helden PD, Bosman M, Coetzee G, Warren RM. The diagnostic performance of the GenoType MTBDRplus version 2 line probe assay is equivalent to that of the Xpert MTB/RIF assay. J Clin Microbiol. 2012;50(11):3712-6. Available from: https://doi.org/10.1128/jcm.01958-12
11. Bablishvili N, Turkvadze N, Avalian Z, Blumberg HM, Kempker RR. A comparison of the Xpert® MTB/RIF and GenoType® MTBDRplus assays in Georgia. Int J Tuberc Lung Dis. 2015;19(6):676-8. Available from: https://doi.org/10.5588/ijtld.14.0867
12. Singh BK, Sharma SK, Sharma R, Sreenivas V, Myneedu VP, Kohli M, et al. Diagnostic utility of a line probe assay for multidrug resistant-TB in smear-negative pulmonary tuberculosis. PLoS One. 2017;12(8):e0182988. Available from: https://doi.org/10.1371/journal.pone.0182988
13. Brandao AP, Pinhata JMW, Oliveira RS, Galesi VMN, Caifaffa-Filho HH, Ferrazoli L. Speeding up the diagnosis of multidrug-resistant tuberculosis in a high-burden region with the use of a commercial line probe assay. J Bras Pneumol. 2019;45(2):e20180128. Available from: https://doi.org/10.1590/1806-3713/e20180128
14. Ministério da Saúde (MS). Secretaria de Vigilância em Saúde. Departamento de Doenças de Condições Crônicas e Infecções Sexualmente Transmissíveis. Boletim Epidemiológico da Tuberculose. Boletim Epidemiológico Especial, Mar. 2022. 51 p.
15. Salvato RS, Costa ERD, Reis AJ, Schiefelbein SH, Halon ML, Barcellos RB, et al. First insights into circulating XDR and pre-XDR *Mycobacterium tuberculosis* in Southern Brazil. Infect Genet Evol. 2020;78:104127. Available from: https://doi.org/10.1016/j.meegid.2019.104127
16. Xia H, Song Y, Zheng Y, Wang S, Zhao B, He W, et al. Detection of *Mycobacterium tuberculosis* Rifampicin Resistance Conferred by Borderline rpoB Mutations: Xpert MTB/RIF is Superior to Phenotypic Drug Susceptibility Testing. Infect Drug Resist. 2022;15:1345-52. Available from: https://doi.org/10.2147/idr.s358301

17. Torres JN, Paul LV, Rodwell TC, Victor TC, Amallraja AM, Elghraoui A, et al. Novel katG mutations causing isoniazid resistance in clinical M. tuberculosis isolates. Emerg Microbes Infect. 2015;4(7):e42. Available from: https://doi.org/10.1038/emi.2015.42

18. Altman DG, Bland JM. Diagnostic tests 2: Predictive values. BMJ. 1994;309(6947):102. Available from: https://doi.org/10.1136/bmj.309.6947.102

19. Feliciano CS, Nascimento MMP, Anselmo LMP, Pocente RHC, Bellissimo-Rodrigues RF, Bollela VR. Role of a GenoType MTBDRplus line probe assay in early detection of multidrug-resistant tuberculosis at a Brazilian reference center. Braz J Med Biol Res. 2015;48(8):759-64. Available from: https://doi.org/10.1590/1414-431x20154458

20. Yadav RN, Kumar Singh B, Sharma R, Chaubey J, Sinha S, Jorwal P. Comparative Performance of Line Probe Assay (Version 2) and Xpert MTB/RIF Assay for Early Diagnosis of Rifampicin-Resistant Pulmonary Tuberculosis. Tuberc Respir Dis (Seoul). 2021;84(3):237-44. Available from: https://doi.org/10.4046/trd.2020.0171

21. Rufai SB, Kumar P, Singh A, Prajapati S, Balooni V, Singh S. Comparison of Xpert MTB/RIF with line probe assay for detection of rifampin-monoresistant *Mycobacterium tuberculosis*. J Clin Microbiol. 2014;52(6):1846-52. Available from: https://doi.org/10.1128/jcm.03005-13

22. Crudu V, Stratan E, Romancenco E, Allerheiligen V, Hillemann A, Moraru N. First evaluation of an improved assay for molecular genetic detection of tuberculosis as well as rifampin and isoniazid resistances. J Clin Microbiol. 2012;50(4):1264-9. Available from: https://doi.org/10.1128/jcm.05903-11

23. Abanda NN, Diieugoué Y, Khadka VS, Perfura-Yone EW, Mbacham WF, Vernet G, et al. Absence of hybridization with the wild-type and mutant rpoB probes in the Genotype MTBDRplus assay detects 'disputed' rifampicin mutations. Clin Microbiol Infect. 2018;24(7):781.e1-e3. Available from: https://doi.org/10.1016/j.cmi.2017.11.021

24. Brandao AP, Pinhata JMW, Simonsen V, Oliveira SO, Ghisi KT, Rabello MCS, et al. Transmission of *Mycobacterium tuberculosis* presenting unusually high discordance between genotypic and phenotypic resistance to rifampicin in an endemic tuberculosis setting. Tuberculosis (Edinb). 2020;125:102004. Available from: https://doi.org/10.1016/j.tube.2020.102004

25. Miotto P, Cabibbe AM, Borroni E, Degano M, Cirillo DM. Role of Disputed Mutations in the rpoB Gene in Interpretation of Automated Liquid MGIT Culture Results for Rifampin Susceptibility Testing of *Mycobacterium tuberculosis*. J Clin Microbiol. 2018;56(5):e01599-17. Available from: https://doi.org/10.1128/jcm.01599-17

26. Kritski A, Oliveira MM, Almeida IN, Ramalho D, Andrade MKN, Carvalho M, et al. Clinical Impact of the Line Probe Assay and Xpert® MTB/RIF Assay in the Presumptive Diagnosis of Drug-Resistant Tuberculosis in Brazil: A Pragmatic Clinical Trial. Rev Soc Bras Med Trop. 2022;55:e0191. Available from: https://doi.org/10.1590/0037-8682-0191-2021

27. Villalva-Serra K, Barreto-Duarte B, Miguez-Pinto JP, Queiroz ATL, Rodrigues MM, Rebeiro PF, et al. Impact of Xpert MTB/RIF implementation in tuberculosis case detection and control in Brazil: a nationwide intervention time-series analysis (2011-2022). Lancet Reg Health Am. 2024;36:100804. Available from: https://doi.org/10.1016/j.lana.2024.100804

28. Soares VM, Almeida IN, Vater MC, Alves S, Figueredo LJA, Scherer L, et al. Genotype® MTBDRplus and Xpert® MTB/RIF in the diagnosis of tuberculosis and resistant tuberculosis: cost analysis in a tertiary referral hospital. Rev Soc Bras Med Trop. 2020;53:e20190175. Available from: https://doi.org/10.1590/0037-8682-0175-2019

29. Zhang Y, Yew WW. Mechanisms of drug resistance in *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis. 2009;13(11):1320–30.

30. Machado D, Coelho TS, Perdigao J, Pereira C, Couto I, Portugal I, et al. Interplay between Mutations and Efflux in Drug Resistant Clinical Isolates of *Mycobacterium tuberculosis*. Front Microbiol. 2017;8:711. Available from: https://doi.org/10.3389/fmicb.2017.00711

31. Unissa AN, Subbian S, Hanna LE, Selvakumar N. Overview on mechanisms of isoniazid action and resistance in *Mycobacterium tuberculosis*. Infect Genet Evol. 2016;45:474-92. Available from: https://doi.org/10.1016/j.meegid.2016.09.004

32. Tilahun M, Shimelis E, Wogavehu T, Assefa G, Wondimagem G, Mekonnen A, et al. Molecular detection of multidrug resistance pattern and associated gene mutations in M. tuberculosis isolates from newly diagnosed pulmonary tuberculosis patients in Addis Ababa, Ethiopia. PLoS One. 2020;15(8):e0236054. Available from: https://doi.org/10.1371/journal.pone.0236054

33. Kabir S, Junaid K, Rehman A. Variations in rifampicin and isoniazid resistance associated genetic mutations among drug naïve and recurrence cases of pulmonary tuberculosis. Int J Infect Dis. 2021;103:56-61. Available from: https://doi.org/10.1016/j.ijid.2020.11.007

34. Abanda NN, Dieugoué JY, Lim E, Perfura-Yone EW, Mbacham WF, Vernet G, et al. Diagnostic accuracy and usefulness of the Genotype MTBDRplus assay in diagnosing multidrug-resistant tuberculosis in Cameroon? a cross-sectional study. BMC Infect Dis. 2017;17(1):379. Available from: https://doi.org/10.1186/s12879-017-2489-3

35. Kumar P, Balooni V, Sharma BK, Kapil V, Sachdeva KS, Singh S. High degree of multi-drug resistance and hetero-resistance in pulmonary TB patients from Punjab state of India Tuberculosis (Edinb). 2014;94(1):73-80. Available from: https://doi.org/10.1016/j.tube.2013.10.001

36. Figueiredo LJA, Almeida IN, Augusto CJ, Soares VM, Suffys PN, Carvalho WS, et al. Characterization of *Mycobacterium tuberculosis* heteroresistance by genotyping. Int J Mycobacteriol. 2020;9(4):368-72. Available from: https://doi.org/10.4103/ijmy.ijmy_132_20

37. Kritski AL, Viveiros M, Carvalho ACC. Rapid molecular diagnostics to detect resistance to second-line anti-TB drugs. Int J Tuberc Lung Dis. 2022;26(5):385-87. Available from: https://doi.org/10.5588/ijtld.22.0121