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REVIEW ARTICLE

Line Probe Assay for Rapid Diagnosis of Multidrug Resistant Pulmonary Tuberculosis

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ABSTRACT

Multidrug-resistant tuberculosis (MDR-TB) is a serious public health problem with ultimate concern in the developing countries, including Egypt. In the global TB report issued in 2021 by World Health Organization (WHO), infection caused by the tubercle bacilli Mycobacterium tuberculosis (MTB) represents the 13th chief cause of mortality worldwide. Effective MDR-TB control counts on the accuracy and rapidity of its diagnosis in order to start the proper treatment regimen and as a result limit the transmission of the resistant isolate. Line probe assay (LPA) and GeneXpert MTB/RIF are two WHO approved probe based molecular assays for rapid detection resistant MTB isolates via screening its nucleic acid for any mutations. For the purpose of finding mutations linked to rifampicin resistance using DNA probes, both techniques focus on the same 81 bp Rifampicin Resistance Determining Region. Line probe assay can detect both rifampicin and isoniazid resistance, thus can detect isoniazid mono resistant isolates, which requires special management protocol, in addition to detecting MDR-TB isolates as well with high sensitivity and specificity.

INTRODUCTION

Tuberculosis (TB) is a serious infection, caused by the tubercle bacilli MTB which is the ninth chief cause of mortality worldwide also the leading cause from a single infectious agent and is anticipated to withstand its current position or even ascent to the seventh position[1].

In spite the fact that Egypt is not found within the World Health Organization (WHO) list of the high burden TB countries, Egypt is considered within the highest percentage in Eastern Mediterranean countries where MTB is the third most significant communicable infection after hepatitis C and bilharziasis[2].

Multidrug-resistant tuberculosis (MDR-TB) has emerged as a major health concern around the globe. Alarming reports are arising from several parts all over the world of increasing drug resistance, which potentially threaten disrupting achievements gained in TB control over the last decade[3].

Although mycobacterial culture is considered as the gold standard by WHO for both MTB diagnosis and drug susceptibility testing (DST) nonetheless it is slow and requires high technical expertise. Thus, standardized and optimized MTB culture and DST is difficult as it requires several weeks to months for laboratory results to become available, during which, patients may be prescribed inadequate treatment, hence increasing the development and spread of resistant isolates[4].

Therefore, there is an urgent necessity for developing accurate and prompt DST for MDR-TB to avoid clinical deterioration, improve treatment regimen, and interrupt further transmissions. Since MTB resistance to the treatments is triggered by genetic mutations in the drug targets genes, either in regulatory regions of the target gene or in drug-activating genes, the molecular diagnosis is considered the method of choice for prompt identification of MDR-TB[5].

Multidrug resistant tuberculosis

Multidrug-resistant Tuberculosis is caused by TB strain that are resistant to at least isoniazid and rifampicin, the two most potent TB drugs[6]. MDR-TB has been an area of growing concern to human health worldwide which is hazardous to the TB control[7].

Over the years poor chemotherapy resulted in selective evolution of resistant mutants. The incidence of resistant cases mainly depends on the ratio between native bacilli and the resistant mutants in the lesion. However, inadequate therapy, interrupted treatment, inappropriate drug doses or addition of a single drug to a failing regimen allows selective growth of resistant mutants and acquisition of DR-TB[8].

The history of resistance in MTB is a recent issue, which emerged over the past sixty years accompanied by the anti-tuberculosis drugs development. With the

discovery of rifampicin in 1966 and its extended use between 1970 and 1990, patients who were carriers of isoniazid resistant strains became rifampicin resistant. This marked the beginning of an MDR-TB problem that has since developed into a pandemic in several nations [9].

Also, it was shown that there is a direct correlation between strong National TB Programmes (NTP) practices and low resistance rates. According to recent evidence, NTPs that effectively utilize currently available medications may be able to delay or perhaps decrease the DR-TB prevalence [10]. **Table (1)** lists the commonest reasons for resistance in the general population and how MDR-TB develops during epidemics.

Transmission of MDR-TB depends on both the virulence of the drug-resistant strain and the susceptibility of the population. The prevalence of DR-TB is correlated with 1) previous TB management, 2) current TB management, and 3) MDR-TB transmission. However, there are many factors besides these three that affect the epidemiology of MDR-TB, therefore the anticipated changes in both TB and MDR-TB prevalence varies greatly [11].

Table 1: Most frequent causes associated with selection of resistance in the community and generation of DR-TB[10].

Non-implementation of DOTS and DOTS expansion strategies	Inadequate supply or poor quality of drugs	Patients: inadequate drug intake	Others
Poorly organized or funded NTPs	History of frequent drug supplies shortages in the country	Inadequate adherence to treatment	Dominant private sector
Inadequate or missing guidelines	Poor quality of anti-TB drugs	Adverse effects and malabsorption	Poor infection control practice
Poor training	Wrong dose or combination	Social barriers	High prevalence of highly virulent DR-TB strain
Lack of treatment monitoring		Financial problems (treatment not for free)	HIV infections
Non-standardized treatment		Substance dependency disorders	

Anti-TB drug resistance screening was considered important and necessary on worldwide. Seventy-one percent of those with bacteriologically proven PTB in 2020 had their rifampicin resistance evaluated, according to an official WHO report in 2021: 61% in 2019 and 50% in 2018. There are ten countries that account for about 70% of the MDR-TB global burden in 2020: China, Democratic Republic of the Congo, India, Indonesia, Nigeria, Pakistan, Philippines, Russian Federation, South Africa and Viet Nam [12].

Molecular diagnosis of MDR-TB

For several ages, patients with un-diagnosed DR-TB who have received the standardized treatment regimens, accelerated the emergence of MDR-TB through drug-specific resistance augmentation. Comprehensive drug susceptibility testing (phenotypic and/or genotypic) is a need to inform physicians about the best treatment regimen tailored individually according to patient susceptibility results [13].

The phenotypic drug susceptibility testing (DST) is considered the gold standard for the diagnosis of DR-TB. Nonetheless, this method is time-consuming (takes up to 3–8 weeks) [14].

Previous studies verified most spontaneous mutations, particularly single nucleotide polymorphisms (SNPs) on the circular chromosome, are the chief cause of treatment resistance in MTB strains. Each anti-tuberculous drug is associated with one or more genetic mutation which is responsible for variable degrees of resistance with variable frequencies [15].

For example, *rpoB* gene mutations, primarily in an eighty-one bp hotspot region, are responsible for 97% of cases of resistance to rifampicin (codon 507 to codon 533), however the highest frequency and highest-level of rifampicin resistance is conferred to mutations at codons 526–531 of *rpoB*. Also, isoniazid resistance is caused by mutations in the *inhA* promoter region, *katG*, *ahpC*, *ndh*, and *furA* genes. The majority of *katG* mutations are found at codon 315, which is responsible for increasing isoniazid resistance by 50% up to 90% [16].

World Health Organization has authorized certain molecular diagnostic tests listed in **Table (2)** for detection of TB and drug-resistant strains to overcome the drawbacks of conventional DST techniques, to allow rapid diagnosis of DR-TB without any delay which is a corner stone step in the control of MDR-TB [17].

Molecular drug susceptibility techniques are more rapid and accurate than the conventional phenotypic tests. Molecular techniques can diagnose both MTB and the associated drug resistance through nucleic acid amplification tests [13].

Table (2): WHO-authorized Molecular Drug-Susceptibility tests [17].

Assay	Drug Resistance Sensitivity, Specificity %	DR-TB Category	Time to Result
GeneXpert assays			
XPERT MTB/RIF (Cepheid) 2010	RIF (96, 98)	RR-TB	2 hours
XPERT MTB/RIF Ultra (Cepheid) 2017	RIF (94, 98)	RR- TB	2 hours
XPERT MTB/XDR (Cepheid) 2021	INH (94.2, 98); ETH (98, 99.7); FQ (93.1, 98.3); AMK (86.1, 98.9); CAP (70, 99.7); KAN (98.1, 97)	RR- TB; MDR- TB; Pre- XDRTB	1.5 hours
Truenat assay			
TRUENAT MTBRIF/Dx (Molbio) 2020	RIF (84, 97)	RR- TB	1 hour
Moderate complexity automated NAATs			
RealTime MTB RIF (Abbott) 2019	RIF (94.8, 100); INH (88.3, 94.3)	RR-TB; MDRTB	10.5hours
BD MAX MDR-TB (Becton Dickson) 2021	RIF (90, 95); INH (82, 100)	RR-TB; MDRTB	4 hours
Cobas MTB-RIF/ INH (Roche) 2021	RIF (97.2, 98.6); INH (96.9, 99.4)	RR-TB; MDRTB	4.5 hours
FluoroType MTBDR (Hain) 2021	RIF (98.9, 100); INH (91.7, 100)	RR-TB; MDRTB	3 hours
Line probe assay			
GenoType MTBDRplus (Hain) 2008	RIF (98.2, 97.8); INH (95.4, 98.8)	RR-TB; MDRTB	5-7 hours
Genoscholar NTM/MDRTB strip (Nipro) 2012	RIF (100, 95); INH (97, 95)	RR-TB; MDRTB	5-7 hours
GenoType MTBDRsl (Hain) 2016	FLQ (100, 98.9); AMK (93.8, 98.5); CAP (86.2, 95.9)	RR-TB; MDRTB	5-7 hours

Abbreviations: RR-TB, rifampicin-resistant tuberculosis; MDR-TB, multidrug-resistant tuberculosis; N/A, not applicable; RIF, rifampicin; INH, isoniazid; FLQ, fluoroquinolone; AMK, amikacin; KAN, kanamycin; ETH, ethionamide; CAP, capreomycin

Line probe assays (LPAs):

Line probe assays are DNA hybridization assays that can detect different mutations at the same time by using multiple probes. After DNA extraction and target amplification, amplicons are hybridized to specific oligonucleotide probes that are complementary to the target sequences and are immobilized on the surface of a strip. After several post-hybridization washes to remove non-specific binding, the amplicon-probe hybrids are visualized by eye as colored bands on the strip. The turnaround time of the whole assay is 5–7 h [18].

Line probe assays are rapid molecular tests that can diagnose MTB and drug resistance, even though LPAs are designed for reference or regional laboratory settings, because they are more technically complex and take longer time to perform, however LPAs have an advantage over the Xpert MTB/RIF assay, they are able to detect both isoniazid and rifampicin resistance, in addition to second line injectables and fluoroquinolone[19].

Principle of Line probe assays:

Line probe assays are a group of strip-based DNA tests which detect drug resistance profile of an MTB isolate through the binding pattern of amplicons to probes targeting the commonest mutations associated with first and second-line agents resistance as well as to probes targeting the wildtype (WT) DNA sequence [19]. Mutation are detected either though binding of biotin-labeled amplicons to the target mutations, or the absence of the corresponding wild type probes **(Fig. 1).** On the test strip, colorful bands appear because of the post-hybridization (probe binding) reaction [20-21].

First Line- Line Probe Assay:

The WHO[6] endorsed LPAs for MTB and rifampicin resistance detection in smear-positive TB cases in 2008**,** at the beginning Genotype MTBDR assay (Hain Lifescience, Nehren, Germany) and INNO-LiPA Rif.TB assay (Innogenetics, Ghent, Belgium) were initially used, later it was replaced by more recent versions of the LPA technology [22].

Further researches have been issued and authorized by the WHO toward guiding an updated policy for using molecular-based diagnostic assays, where the performance of three LPAs were assessed: GenoType MTBDR plus version 1 and 2, and Nipro NTM+MDRTB Detection Kit 2, for screening isoniazid and rifampicin resistant MTBisolates [18].

Nipro NTM+MDR-TB distinguishes four significant *Mycobacterium* species (*M. tuberculosis*, *M. avium*, *M. intracellulare*, and *M. kansasii*) that cause the human

disease in addition to detecting MDR-TB cases by targeting *rpoB*, *katG*, and *inhA*. Although the sensitivity of the Nipro strips varies between studies (from 50 to 95%), they have a good specificity for isoniazid and rifampicin resistance screening between 97 and 100% in both clinical samples and cultured isolates [23]. On the other hand, GenoType MTBDR plus version 2 sensitivity varies between 83.3 and 96.4%, and specificity between 98.6 and 100% [24].

Second Line LPA:

In May 2016, WHO approved this rapid diagnostic test called MTB DRsl (SL-LPA) a DNA-based test that identifies mutations within MDR/Rifampicin resistant-TB strains, responsible for fluoroquinolone and injectable second-line TB drugs resistance. It is the initial and only WHO-recommended assay for identifying new resistance in MDR and XDR-TB without delay. It provides results within 1-2 days, which is a chief advantage over the 3 months or longer results obtained by phenotypic DST [25].

However, adoption of SL-LPA does not eliminate the importance of conventional culture and DST. Despite good specificity of SL-LPA in detecting resistance to fluoroquinolone and the second-line injectable drugs, culture and phenotypic DST are required to completely exclude resistance to these drug classes along with other second-line drugs [26].

In conclusion, LPAs are simple and easy assay to perform and straightforward to interpret which can be performed either directly from respiratory samples or indirectly from culture material with short turnaround time to detect isolates with isoniazid and rifampicin resistance, allowing early appropriate treatment, which reduces transmission and spread of MDR-TB [19].

However, some mutations that confer resistance are outside the regions covered by the test therefore resistance cannot be fully excluded even if all WT probes are present. Also, LPA is less efficient than conventional DST in detecting resistance in samples comprising hetero-resistance strains [27]. More precisely, with LPA, it is possible to detect resistant bacteria with mutations detected by the MUT probes if resistant bacteria represent at least 5% from entire population. Still, resistant bacteria with mutations inferred by the absence of WT probes would be missed if the resistant population is less than 95% from the entire population[28].

Potential clinical and epidemiologic relevance of LPA use in practice

The main pillar of integrated patient centered care and prevention within the Strategy by End TB committee has stated the need for the early TB detection including anti-tuberculous sensitivity testing. This emphasizes how crucial LPAs are for the quick detection of MTB and its resistance pattern [29]. As well, the International Standards for TB Control recommends LPA for MDR-TB diagnosis, especially in environments where the risk of isoniazid mono-resistance is high [30].

Currently available commercial LPA detect mutations in *rpoB* codons 516, 526 and 531. There is a high-level agreement between genotypic and phenotypic DST. This is because the mutations associated with rifampicin resistance are located within 81 bp core region of *rpoB*, while mutations outside this region are unusual. Molecular based techniques for screening rifampicin resistance could be considered a standard for the diagnostic evaluation of presumptive MDR-TB cases [31].

Although, majority of rifampicin resistant strains are also resistant to isoniazid, molecular testing for isoniazid drug resistance is important as it offers the possibility of adding isoniazid to a second-line drug regimen, in addition the management of rifampicin resistant TB is different if accompanied by isoniazid resistance [32].

As for isoniazid resistance LPA detect mutations in *inhA* positions 16, 15 and 8, and *katG* codon 315. High-level isoniazid resistance is associated with S315T mutation without affecting ethionamide susceptibility. The limited data on the direct association between *katG* S315T mutation and clinical outcome suggest increased risk of first-line treatment failure, death and relapse [33]. In the case of *katG* S315T mutation, isoniazid should therefore be excluded from treatment. Compared to *katG* S315T, *inhA* promoter mutations confer low-level isoniazid resistance, but significantly affect ethionamide susceptibility [34].

Evidence which supports continuing of using LPAs was provided based on data collected from a variety of laboratory settings in various countries, based on their diagnostic accuracy when used directly on sputum smear-positive specimens or indirectly on culture isolates, as an initial test or in conjunction with culture-based DST for the detection of MTB and MDR [35]. In countries with middle or low incidence, LPAs may also be a vital tool in the efforts exerted to eradicate TB [36].

Based on recent MDR-TB treatment guidelines, isoniazid resistance does not prevent its use, as the usefulness of high-dose isoniazid in patients infected with strains harboring mutations either within *inhA* promotor or region *katG* is uncertain, taking in consideration that *inhA* mutant strains are naturally resistant to ethionamide and prothionamide [37].

Hence, identifying strains with isoniazid resistance by applying first line LPAs will be valuable to clinicians, if it provides the results quickly and does not cause any delay the start of therapy [38].

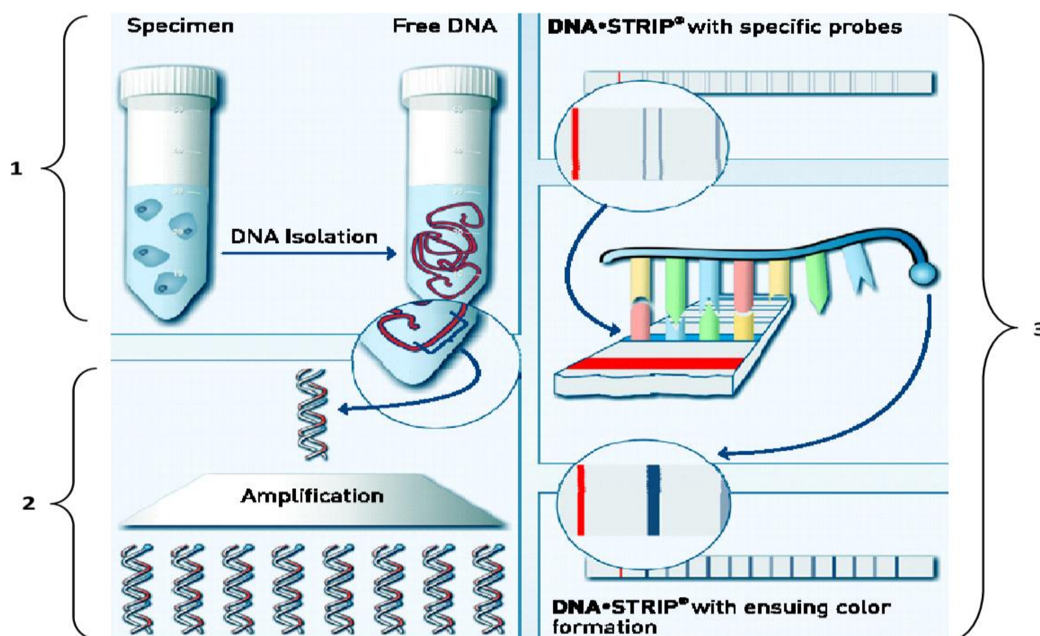

Fig. 1: Schematic representation of the three LPA procedures[38]

CONCLUSION

Line Probe assay application in MDR-TB early diagnosis of will provide rapid and more accurate identification of resistant TB strains, which support its alternative use over conventional DST in daily clinical practice. So, applying LPA would in turn enhance the proper MDR anti-tuberculous management with the proper drug regimen, increasing cure rate, decreasing rate of transmission, avoid redundant use of medication, thus providing good control of MDR-TB.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

REFERENCES

1. Al-sheikh NM, El-Hefnway SM, El-Shetahy AA. Cholesterol 7-alpha hydroxylase gene (CYP7A1) promoter polymorphism rs3808607 as a risk factor for pulmonary tuberculosis in Egypt. Meta Gene. 2018 Jun 1;16:66-71.
2. Ibrahim E, Baess AI, Al Messery MA. Pattern of prevalence, risk factors and treatment outcomes among Egyptian patients with multidrug resistant tuberculosis. Egyptian Journal of Chest Diseases and Tuberculosis. 2017 Jul 1;66(3):405-11.
3. Sharma SK, Kohli M, Yadav RN, Chaubey J, Bhasin D, Sreenivas V, Sharma R, Singh BK. Evaluating the diagnostic accuracy of Xpert MTB/RIF assay in pulmonary tuberculosis. PloS one. 2015 Oct 23;10(10):e0141011.
4. Asante-Poku A, Otchere ID, Danso E, Mensah DD, Bonsu F, Gagneux S, Yeboah-Manu D. Evaluation of GenoType® MTBDRplus for the rapid detection of drug-resistant tuberculosis in Ghana. The international journal of tuberculosis and lung disease. 2015 Aug 1;19(8):954-9.
5. Bai Y, Wang Y, Shao C, Hao Y, Jin Y. GenoType MTBDR plus Assay for rapid detection of multidrug resistance in Mycobacterium tuberculosis: a meta-analysis. PloS one. 2016 Mar 2;11(3):e0150321.
6. World Health Organization. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Policy statement. 2008 Jun;27.
7. Loddenkemper R, Sagebiel D, Brendel A. Strategies against multidrug-resistant tuberculosis. European respiratory journal. 2002 Jul 1;20(36 suppl):66s-77s.
8. Joshi JM. Tuberculosis chemotherapy in the 21st century: Back to the basics. Lung India. 2011 Jul 1;28(3):193-200.
9. Pym AS, Cole ST. 13 Mechanism of Drug Resistance in Mycobacterium tuberculosis.

Bacterial Resistance to Antimicrobials. 2007 Dec 5:313.

10. Caminero JA. Multidrug-resistant tuberculosis: epidemiology, risk factors and case finding [State of the art series. Drug-resistant tuberculosis. Edited by CY. Chiang. Number 4 in the series]. The International Journal of Tuberculosis and Lung Disease. 2010 Apr 1;14(4):382-90.
11. Marais BJ. The global tuberculosis situation and the inexorable rise of drug-resistant disease. Advanced drug delivery reviews. 2016 Jul 1;102:3-9.
12. World Health Organization. Global tuberculosis report 2021: supplementary material.
13. Nguyen TN, Anton-Le Berre V, Bañuls AL, Nguyen TV. Molecular diagnosis of drug-resistant tuberculosis; a literature review. Frontiers in microbiology. 2019 Apr 16;10:794.
14. McGrath M, Gey van Pittius NC, Van Helden PD, Warren RM, Warner DF. Mutation rate and the emergence of drug resistance in Mycobacterium tuberculosis. Journal of Antimicrobial Chemotherapy. 2014 Feb 1;69(2):292-302.
15. Laurenzo D, Mousa SA. Mechanisms of drug resistance in Mycobacterium tuberculosis and current status of rapid molecular diagnostic testing. Acta tropica. 2011 Jul 1;119(1):5-10.
16. Nandlal L, Perumal R, Naidoo K. Rapid Molecular Assays for the Diagnosis of Drug-Resistant Tuberculosis. Infection and Drug Resistance. 2022 Jan 1:4971-84.
17. Mitarai S, Kato S, Ogata H, Aono A, Chikamatsu K, Mizuno K, Toyota E, Sejimo A, Suzuki K, Yoshida S, Saito T. Comprehensive multicenter evaluation of a new line probe assay kit for identification of Mycobacterium species and detection of drug-resistant Mycobacterium tuberculosis. Journal of clinical microbiology. 2012 Mar;50(3):884-90.
18. Kazemian H, Kardan-Yamchi J, Bahador A, Khonsari S, Nasehi M, Hamzehloo G, Vaziri F, Salehi MR, Feizabadi MM. Efficacy of line probe assay in detection of drug-resistant pulmonary tuberculosis in comparison with GeneXpert and phenotypic methods in Iran and genetic analysis of isolates by MIRU-VNTR. Infection and Drug Resistance. 2019;12:3585.
19. Domínguez J, Boettger EC, Cirillo D, Cobelens F, Eisenach KD, Gagneux S, Hillemann D, Horsburgh R, Molina-Moya B, Niemann S, Tortoli E. Clinical implications of molecular drug resistance testing for Mycobacterium tuberculosis: a TBNET/RESIST-TB consensus statement. The international journal of tuberculosis and lung disease. 2016 Jan 1;20(1):24-42.
20. Ling DI, Zwerling AA, Pai M. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. European Respiratory Journal. 2008 Nov 1;32(5):1165-74.
21. Desikan P, Panwalkar N, Mirza SB, Chaturvedi A, Ansari K, Varathe R, Chourey M, Kumar P, Pandey M. Line probe assay for detection of *Mycobacterium tuberculosis* complex: An experience from Central India. Indian J Med Res. 2017 Jan;145(1):70-73. doi: 10.4103/ijmr.IJMR_831_14. PMID: 28574017; PMCID: PMC5460577.
22. Nathavitharana RR, Hillemann D, Schumacher SG, Schlueter B, Ismail N, Omar SV, Sikhondze W, Havumaki J, Valli E, Boehme C, Denkinger CM. Multicenter noninferiority evaluation of Hain GenoType MTBDR plus version 2 and Nipro NTM+ MDRTB line probe assays for detection of rifampin and isoniazid resistance. Journal of clinical microbiology. 2016 Jun;54(6):1624-30.
23. Dantas NG, Suffys PN, Carvalho WD, Gomes HM, Almeida IN, Figueiredo LJ, Gonçalves AD, Gomgnimbou MK, Refregier G, Sola C, Miranda SS. Correlation between the BACTEC MGIT 960 culture system with Genotype MTBDRplus and TB-SPRINT in multidrug resistant Mycobacterium tuberculosis clinical isolates from Brazil. Memorias do Instituto Oswaldo Cruz. 2017;112:769-74.
24. World Health Organization. The use of molecular line-probe assays for the detection of resistance to second-line antituberculosis drugs. WHO/HTM/TB/2016.07 Geneva, Switzerland: WHO, 2016. h ttp://www.who.in t/ tb/ WHOPolicyStatementSLLPA.pdf Accessed January 2019.
25. Choerunisa TF, Lismayanti L, Rostini T, Bayusantika R, Parwati I. Comparison of Line Probe Assay (LPA) and Mycobacterium Growth Indicator Tubes (MGIT) Assay for Second-line TB Drug Susceptibility Testing. The Indonesian Biomedical Journal. 2021 Sep 9;13(3):256-60.
26. Cox V, Cox H, Pai M, Stillo J, Citro B, Brigden G. Health care gaps in the global burden of drug-resistant tuberculosis. The International Journal of Tuberculosis and Lung Disease. 2019 Feb 1;23(2):125-35.
27. 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. European Respiratory Journal. 2017 Jan 1;49(1).
28. Jian J, Yang X, Yang J, Chen L. Evaluation of the GenoType MTBDRplus and MTBDRsl for the detection of drug-resistant Mycobacterium

tuberculosis on isolates from Beijing, China. Infection and Drug Resistance. 2018;11:1627.

29. Al-Mutairi NM, Ahmad S, Mokaddas E, Eldeen HS, Joseph S. Occurrence of disputed rpoB mutations among Mycobacterium tuberculosis isolates phenotypically susceptible to rifampicin in a country with a low incidence of multidrug-resistant tuberculosis. BMC infectious diseases. 2019 Dec;19(1):1-9.
30. Prasad R, Naorem R, Saxena H, Singh J, Gupta N. Rapid Diagnosis of Drug Resistant Tuberculosis Using Line Probe Assay. 1. Obstacles in the Diagnosis and Treatment of Head & Neck Carcinoma: An Institutional Based Study. 2020 Apr;14:36.
31. Welekidan LN, Skjerve E, Dejene TA, Gebremichael MW, Brynildsrud O, Agdestein A, Tessema GT, Tønjum T, Yimer SA. Characteristics of pulmonary multidrug-resistant tuberculosis patients in Tigray Region, Ethiopia: A cross-sectional study. PloS one. 2020 Aug 14;15(8):e0236362.
32. Lönnroth K, Migliori GB, Abubakar I, D'Ambrosio L, De Vries G, Diel R, Douglas P, Falzon D, Gaudreau MA, Goletti D, Ochoa ER. Towards tuberculosis elimination: an action framework for low-incidence countries. European Respiratory Journal. 2015 Apr 1;45(4):928-52.
33. Valafar SJ. Systematic Review of Mutations Associated with Isoniazid Resistance Points to Continuing Evolution and Subsequent Evasion of Molecular Detection, and Potential for Emergence of Multidrug Resistance in Clinical Strains of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2021 Feb 17;65(3):e02091-20. doi: 10.1128/AAC.02091-20. PMID: 33361298; PMCID: PMC8092511.
34. Pitso L, Potgieter S, Van der Spoel van Dijk A. Prevalence of isoniazid resistance-conferring mutations associated with multidrug-resistant tuberculosis in Free State Province, South Africa. South African medical journal. 2019 Sep 1;109(9):659-64.
35. Parsons LM, Somoskövi A, Gutierrez C, Lee E, Paramasivan CN, Abimiku A, Spector S, Roscigno G, Nkengasong J. Laboratory diagnosis of tuberculosis in resource-poor countries: challenges and opportunities. Clin Microbiol Rev. 2011 Apr;24(2):314-50. doi: 10.1128/CMR.00059-10. PMID: 21482728; PMCID: PMC3122496.
36. Harries AD, Lin Y, Kumar AMV, Satyanarayana S, Takarinda KC, Dlodlo RA, Zachariah R, Olliaro P. What can National TB Control Programmes in low- and middle-income countries do to end tuberculosis by 2030? F1000Res. 2018 Jul 5;7:F1000 Faculty Rev-1011. doi: 10.12688/f1000research.14821.1. PMID: 30026917; PMCID: PMC6039935.
37. Sotgiu G, Tiberi S, D'Ambrosio L, Centis R, Zumla A, Migliori GB. WHO recommendations for multidrug-resistant tuberculosis–Authors' reply. The Lancet. 2016 Nov 5;388(10057):2234-5.
38. Barnard M, Gey van Pittius NC, Van Helden PD, Bosman M, Coetzee G, Warren RM. The diagnostic performance of the GenoType MTBDR plus version 2 line probe assay is equivalent to that of the Xpert MTB/RIF assay. Journal of clinical microbiology. 2012 Nov;50(11):3712-6.