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EXPEDITED DIAGNOSIS OF MYCOBACTERIUM TUBERCULOSIS USING TRUENAT MTB-RIF ASSAY IN A TERTIARY CARE HOSPITAL OF NORTHERN INDIA

Microbiology

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ABSTRACT

Background: Global tuberculosis control relies heavily on providing reliable and accurate TB diagnosis to patients. Molbio Diagnostics has developed point-of-care tests to detect drug resistance to Mycobacterium tuberculosis (MTB) and Rifampicin. Truenat MTB and Truenat MTB-RIF assay, a nucleic acid amplification test, is a real-time polymerase chain reaction chip-based assay using portable, battery-operated devices. This study aimed to detect TB in suspected patients as earliest. **Methods:** We evaluated the performance of True prep® AUTO/AUTO v2 Universal Cartridge based Sample Prep Device for the automated extraction and purification of DNA, and the True lab® Real Time micro PCR Analyser for performing RT-PCR, resulting in the semi-quantitative detection of MTB in clinical sputum specimens from 350 patients with suspected pulmonary tuberculosis. **Results:** This study analysed 350 samples from suspected TB patients from January 2024 to March 2024. There were 350 samples tested, 74(21.14%) were positive and 276(78.85%) were negative. **Conclusion:** This preliminary study shows that performance of the Truenat tests in primary healthcare centres with very limited infrastructure was feasible. This test allows detection of MTB and rifampicin resistance in approximately two hours to provide quick and accurate diagnosis.

KEYWORDS

Mycobacterium tuberculosis, Nucleic Acid Amplification Test, Real-time polymerase chain reaction, Rifampicin drug resistance.

INTRODUCTION

Tuberculosis (TB) causes the highest number of deaths globally, attributable to a curable infectious agent, despite the availability of potent anti-TB medication [1].As of 2021, India remains the largest TB burden country, with an estimated 2,950,000 cases (2,510,000-3,440,000) and 2.14 million cases notified; this is 18% higher than the incidence in 2020 and also represents 27% of global TB case notifications in 2021[2].Conventional culture and drug susceptibility testing (DST) methods rely on the slow growth of Mycobacterium tuberculosis in solid or liquid media, which can take weeks to months to yield results[3], and can lead to prolonged periods of ineffective therapy and ongoing disease transmission. To enable rapid TB testing, the WHO has recommended low-and moderate-complexity nucleic acid amplification technology (NAAT) for use in the initial diagnostic test. Among the forms of low-complexity NAAT testing available for use in peripheral settings, the WHO has recommended the use of Xpert MTB/RIF, Xpert MTB Ultra, Truenat MTB, MTB plus, MTB-RIF Dx, and TB LAMP assays[4] .All these are robust point-of-care diagnostic tests that are easily implementable at lower levels of the healthcare system.

Molbio Diagnostics Pvt. Ltd. (Goa, India) developed three assays that utilise chip-based real-time micro PCR: two for detection of M. tuberculosis ,the Truenat MTB assay (the ribonucleoside-diphosphate reductase gene, nrdB single copy target and has a limit of detection (LOD) of about 100 colony forming units (CFU)/ml sputum sample) and The Truenat MTB Plus assay (containing both a portion of the nrdZ gene and a portion of the multicopy IS6110 element) has a limit of detection of about 30 CFU/ml. and one for the detection of RIF resistance (the MTB-RIF Dx reflex assay targeting the rpoB gene) [5] . Following the WHO recommendation, India was the first country to implement Truenat MTB-RIF as an upfront molecular test, as early as 2020, under the National TB Elimination Program (NTEP)[6,7].

Truenat is a real-time polymerase chain reaction (RT-PCR) chip-based assay that can detect Mycobacterium tuberculosis (Mtb) and rifampicin (RIF) drug resistance. With the combined advantages of affordability, simplicity in operations, diagnostic sensitivity, and portability, this micro-PCR device represents a strong candidate for wide-scale use in resource-limited settings [8].

Extraction of DNA and detection of MTB takes approximately one hour. If the Truenat MTB or MTB Plus test result is positive, an aliquot of the already extracted DNA may be loaded onto a Truenat™ MTB-RIF Dx chip and analysed in the True lab micro PCR Analyzer. Mutations associated with rifampicin (RIF) resistance are detected by a probe melt analysis of the real-time PCR products.

In adults and children with signs and symptoms of pulmonary TB, WHO recommendation applies to the use of the test with sputum specimens. Some published data are available on the performance of the Truenat TB test with non-sputum or extra pulmonary TB samples, but WHO did not make a recommendation regarding the use of the Truenat TB test for these specimens.

Here, we report results from a multicentre prospective and diagnostic clinical evaluation study of the Truenat MTB, MTB Plus and MTB-RIF Dx assays, in which we assessed performance in the intended settings of use (i.e., microscopy centres like primary healthcare centre), relative to microbiological confirmation (culture and phenotypic DST) as a reference standard.

METHODOLOGY:

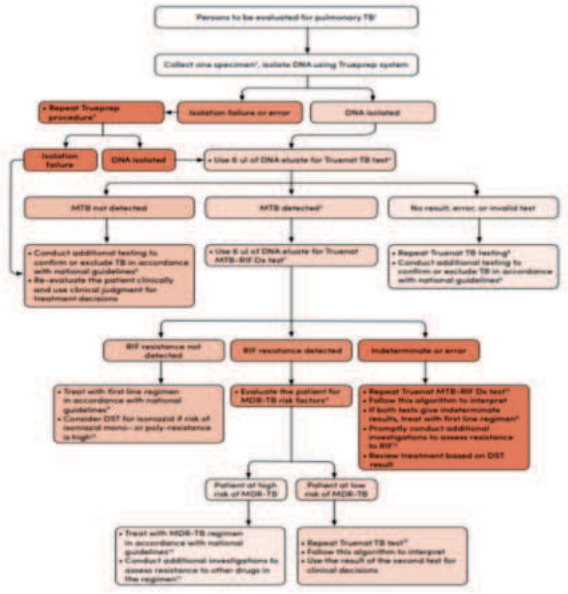
This multicentre prospective and diagnostic accuracy study of the performance of the Truenat TB assays was conducted Molecular (PCR) Lab of Govt. medical college and attached Hospital, Dausa and various clinical sites (with attached microscopy centres). The study population comprised adult men and women presenting to clinical symptoms suggestive of pulmonary TB disease. As part of the consent process, all participants signed and dated their consent forms, either in writing or by thumbprint, if they were illiterate.

Participants enrolled at district hospital, Dausa were asked to provide three sputum specimens for reference laboratory testing and an additional specimen for microscopy centre testing. All three sputum specimens were transported to the centralised reference laboratory for culture, Xpert MTB/RIF or Ultra, Truenat, and smear testing. An additional Spot and morning sputum specimen from each patient were used for Truenat assay testing at Shri Ramkaran Joshi Govt. Hospital, Dausa.

The Truenat™ MTB Plus is based on the principle of Real Time Polymerase Chain Reaction. A sputum specimen is first liquefied and lysed using the Trueprep™ AUTO MTB Sample Pre-treatment Pack. The DNA from the sample is then extracted using the Trueprep™ AUTO v2 Universal Cartridge Based Sample Prep Device and Trueprep™ AUTO v2 Universal Cartridge Based Sample Prep Kit. The extracted DNA is then amplified by the Truelab Real Time micro-PCR analyzer. The Truenat™ MTB Plus chip is placed on the chip tray

of the Truelab™ Real Time micro PCR Analyzer. Six (6) µL of the purified DNA is then dispensed into the reaction well of the Truenat™ MTB Plus chip and the test is started. At the end of the test run, the result screen will display "DETECTED" for Positive result or "NOT DETECTED" for Negative results.

Algorithm for the use of the Truenat TB tests as the Initial Diagnostic Test for pulmonary TB:



If MTB is detected, test the same elute for Rifampicin resistance using Truenat™ MTB-RIF Dx the chip and the test takes extra 55 minutes to complete.

RESULTS AND DISCUSSION:

The present study is a laboratory based prospective observational study. A total of 350 Truenat MTB tests were conducted for the diagnosis of TB among presumptive TB patients from January 2024 to March 2024. There were 350 samples tested ,74(21.14%) were positive and 276(78.85%)were negative.

Table1. Distribution Of Tb Patients In Total Tested Samples

Total sample	Positive	Negative
350(100%)	74(21.14%)	276(78.85%)

Table 2 Gender Distribution Among Detected Tb Patients

Total positive sample	Male	Female
74(100%)	63(85.13%)	11(14.86%)

Table 3. Age Distribution Of The Study Participants

Age group	Total no of sample	Positive	Negative
1-20	31 (8.85%)	04(12.90%)	27(87.09%)
20-40	109(31.14%)	25(22.93%)	84(77.06%)
40-60	118(33.71%)	30(25.42%)	88(74.57%)
60-80	92(26.28%)	15(16.30%)	77(83.69%)
Total	350(100%)	74(21.14%)	276(78.85%)

Table 4. Truenat Rif Test Results Among Detected Tb Patients

Total sample	Rifampicin resistant	Rifampicin sensitive	Indeterminate
74(100%)	00	55(74.32%)	19(25.67%)

DISCUSSION

This multicentre diagnostic accuracy study indicates that the rapid molecular Truenat assays have overall comparable performance characteristics to Xpert and could be considered as initial tests for the diagnosing of TB and detecting of RIF resistance in primary healthcare facilities [9].

A MTB culture can detect organisms even at very low concentrations. However, Turnaround times for liquid and solid culture systems range from a few days to several weeks. In addition, a liquid culture system requires sophisticated instruments, a dedicated laboratory, and specially trained technicians. This set-up is not feasible for use in remote areas**. Nikam, C. et al**[10] studied that, the overall performance of the test with MGIT culture as gold standard, sensitivity of smear was 61.5% (CI:53.3–69.3%), while TrueNAT and Genexpert was, 94.7% (CI:89.8–97.6%) & 96.0% (CI: 91.5–98.5%), respectively.

There is an urgent need for affordable, easy to use, point-of-care molecular diagnostic assays that augment the efforts to treat disease before its spread and irreversible damage to the individual's health. WHO endorsed the Truenat assays as initial tests to identify TB and detect rifampicin resistance rather than smear microscopy, culture and phenotypic DST following the FIND study. These assays were recommended at the point-of-care in low resource primary healthcare settings[11]. For MTB/RIF Dx, Truenat costs about the same as GeneXpert (1000 rupees) per sample in **Lee, D. J. et al** [12].This assay when utilized as a point-of-care for TB diagnosis in India, demonstrated improvement in linkage-to-care, increase in life expectancy and cost-effectiveness when compared with AFB smear or GeneXpert MTB/RIF.

In this study, A total of 350 specimen were screened, 74(21.14%) were positive and 276(78.85%) were negative for tuberculosis by Truenat assay testing. Similar studies done by **Nikam C et al.** out of 247 samples, 107 smear positive and 93 smear negative were detected by TrueNAT system[10].

In our study, out of 74 detected TB samples , 55(74.32%) samples are rifampicin sensitive and 19(25.67%) are indeterminate results while no samples are rifampicin resistant. In addition to some delays in performing the Rif test, prolonged storage of extracted DNA may result in DNA degradation as well as low bacterial load in samples leading to indeterminate results **(Penn-Nicholson, A. et al)**[13]. Similar studies have reported that indeterminate results (n=14) for rifampicin detection may be attributed to the low bacterial load in samples (**Singh UB et al)** [14]. A recent study reported after a median of 10 tests, the percentage of invalid results has declined after short training and assay operations[15]. In a study (**Gopalaswamy R et al.)**[16] the rate of invalid MTB results was 5.2% [95% confidence interval (CI): 5.11–5.26; n = 16,998] and the rate of errors was 2.5% (95% CI: 2.46–2.57; n = 8,240) in Truenat MTB chip testing.

Out of 74(100%) ,63(85.13%)samples were detected in male while 11(14.86%) were female patients. This was similar to study conducted by **Nhamoyebonde S et al.** [17] Men tend to have a higher number of social contacts, work in high-risk occupations such as mining, and engage in high-risk behaviour's such as smoking. The age group most commonly affected was 40-60 years (33.71%) followed by 20-40 years (22.93%) and 60-80 years (16.30%), 1-20 years (16.30%). Some of the associated risk factors are diabetes, smoking, alcohol use and use of other drugs, all of which can also contribute to poor tuberculosis treatment results.[18]

CONCLUSION

Based on this prospective clinical study, the Truenat assays provide accurate diagnosis of TB and RIF resistance in intended settings. These results indicate that the Truenat MTB, MTB Plus and MTB-RIF Dx assays can be performed at the primary healthcare centre level, although data were limited for the MTB-RIF Dx assay. Findings from the Truenat assays have been reviewed by the WHO, and meet the minimal criteria for recommendation for use as an initial test for detection of TB and RIF resistance instead of smear microscopy, culture and phenotypic DST.

REFERENCES

1. World Health Organization (2004) World Health Report 2004: Changing history. Geneva: WHO
2. WHO. Global TB report (2022). Available at: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022.
3. World Health Organization. Global tuberculosis report 2020. 2020. www.who.int/publications/i/item/ 9789240013131 Date last accessed: 11 April 2021.
4. WHO consolidated guidelines on tuberculosis Module 3: Diagnosis – Rapid diagnostics for tuberculosis detection. (2021). Available at: https://www.who.int/ publications/i/item/who-consolidated-guidelines-on-tuberculosis-module-3-diagnosis---rapid-diagnostics-for-tuberculosis-detection.
5. WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Web Annex 4. Evidence synthesis and analysis. Geneva: World Health Organization; 2020.
6. WHO consolidated guidelines on tuberculosis Module 3: Diagnosis – Rapid diagnostics for tuberculosis detection. (2020). Available at: https://apps.who.int/iris/handle/10665/332862.
7. Central TB Division. India TB Report (2021). Available at: https://tbcindia.gov.in/showfile. php?lid=3587.
8. Stop TB Partnership. Practical Guide to Implementation of Truenat Tests for the Detection of TB and Rifampicin Resistance. (2021).
9. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 3: diagnosis rapid diagnostics for tuberculosis detection.2020.www.who. int/publications

/i/item/who-consolidated-guidelines on-tuberculosis-module-3-diagnosis—rapid-diagnostics-for-tuberculosis-detection Date last accessed: 11 April 2021.
10. Nikam C, Kazi M, Nair C, Jaggannath M, M M, R V, Shetty A, Rodrigues C. Evaluation of the Indian TrueNAT micro RT-PCR device with GeneXpert for case detection of pulmonary tuberculosis. Int J Mycobacteriol. 2014 Sep;3(3):205-10. doi: 10.1016/j.ijmyco.2014.04.003. Epub 2014 Jun 2. PMID: 26786489.
11. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 3: diagnosis—rapid diagnostics for tuberculosis detection 2021 update. https://www.who.int/publications/i/item/9789240029415 (2021). Date Last Accessed 14 Jan 2022.
12. Lee DJ, Kumarasamy N, Resch SC, Sivaramakrishnan GN, Mayer KH, Tripathy S, Paltiel AD, Friedberg KA, Reddy KP. Rapid, point-of-care diagnosis of tuberculosis with novel Truenat assay: Cost-effectiveness analysis for India's public sector. PLoS One. 2019 Jul 2;14(7):e0218890. doi: 10.1371/journal.pone.0218890. PMID: 31265470; PMCID: PMC6605662.
13. Penn-Nicholson A, Gomathi SN, Ugarte-Gil C, Meaza A, Lavu E, Patel P, Choudhury B, Rodrigues C, Chadha S, Kazi M, Macé A, Nabeta P, Boehme C, Gangakhedkar RR, Sarin S, Tesfaye E, Gotuzzo E, du Cros P, Tripathy S, Ruhwald M, Singh M, Denkinger CM, Schumacher SG; Truenat Trial Consortium; Members of the Truenat Trial Consortium:. A prospective multicentre diagnostic accuracy study for the Truenat tuberculosis assays. Eur Respir J. 2021 Nov 4;58(5):2100526.
14. Singh UB, Singh M, Sharma S, Mahajan N, Bala K, Srivastav A, Singh KJ, Rao MVV, Lodha R, Kabra SK. Expedited diagnosis of podiatric tuberculosis using Truenat MTB-Rif Dx and GeneXpert MTB/RIF. Sci Rep. 2023 Apr 28;13(1):6976.
15. Jeyashree, K., Shanmugasundaram, D., Rade, K., Gangakhedkar, R. R. & Murhekar, M. V. Impact and operational feasibility of TrueNat™ MTB/Rif under India's RNTCP. Public Health Action. 10(3), 87–91. https://doi.org/10.5588/pha.20.0004 (2020).
16. Gopalaswamy R, Kumar N, Vashistha H, Rajendran P, Kayesth J, Peravali CJ, Kashyap S, Ghosh S, Yumo H, Moore M, Anand S, Ramachandran R, Alavadi U, Saini S and Shanmugam S (2023) Comprehensive assessment of invalid and indeterminate results in Truenat MTB-RIF testing across sites under the national TB elimination program of India. Front. Public Health 11:1255756. doi: 10.3389/fpubh.2023.1255756
17. Nhamoyebonde S, Leslie A. Biological differences between the sexes and susceptibility to tuberculosis. *J Infect Dis* 2014; 209:S100–6.
18. Silva DR, Muñoz-Torrico M, Duarte R, Galvão T, Bonini EH, Arbex FF, Arbex MA, Augusto VM, Rabahi MF, Mello FCQ. Risk factors for tuberculosis: diabetes, smoking, alcohol use, and the use of other drugs. J Bras Pneumol. 2018 Apr;44(2):145-152.