Journal of Clinical Tuberculosis and Other Mycobacterial Diseases 35 (2024) 100420

Contents lists available at ScienceDirect

Journal of Clinical Tuberculosis and Other Mycobacterial Diseases

journal homepage: www.elsevier.com/locate/jctube

Assessing the utility of Truenat in extrapulmonary tuberculosis diagnosis – A NRL's experience

Priya Rajendran [a], Lavanya Jayabal [b], Mythili Venkatesan [a], Michel Prem Kumar [a], Radhakrishnan Ramalingam [a], P. Sivaraman [a], Asha Fredrick [c], Sivakumar Shanmugam [a,*]

[a] ICMR -National Institute for Research in Tuberculosis, Chennai, India
[b] District Tuberculosis Office, National Tuberculosis Elimination Programme, Chennai, India
[c] State Tuberculosis Office, National Tuberculosis Elimination Programme, Chennai, India

ARTICLE INFO

Keywords:
Extrapulmonary
Tuberculosis
Truenat
Smear
Culture

ABSTRACT

Background: Diagnosis of extra pulmonary TB (EPTB) remains a big challenge. While data on utility of Xpert testing in EPTB diagnosis is enormous, there is limited data on Truenat MTB testing.
Aim: In this study we aimed to evaluate the usefulness of Truenat in EPTB diagnosis.
Materials and methods: The study included patients suspected and/or treated for EPTB located from Chennai district during the year 2021–2022. All processed EPTB samples were subjected to smear microscopy, culture and Truenat MTB testing.
Results: Of the 195 samples tested, 38 (19.4%) samples were positive for EPTB by any one of the diagnostic methods (smear, culture, microscopy). Out of these 38, 16 (42.1 %) were positive for MTB by Truenat and negative by Culture, 12 (31.5%) were positive by culture but negative by Truenat and 8 (21%) were positive by both Truenat and/or smear and culture. The sensitivity and specificity of the test was calculated with the composite reference standard (Culture (exclusion of colonies as positives), clinical conditions, and smear) and was found to be 60% and 100% respectively.
Conclusion: Truenat MTB test is a cost-effective rapid molecular test that can be used only for the diagnosis of presumptive EPTB and not on follow-up samples.

1 Background

Inspite of being a curable disease, the tuberculosis (TB) notified cases in 2022 by WHO was around 10.1 million out of which 17% were of extrapulmonary TB (EPTB) [1]. While there is significant progress in the development of diagnostics for pulmonary TB (PTB), the diagnostic challenges of extra pulmonary tuberculosis (EPTB) still remain to be addressed. EPTB refers to TB involving organs other than the lungs (e.g., pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, or meninges) and constitutes about 15 to 20% of all cases of TB [2]. Difficulty of EPTB diagnosis could be attributed to various reasons like its paucibacillary status, reduced sensitivity of smear testing, delay in obtaining culture results. Due to these difficulties, when diagnosis and treatment are made on basis of clinical diagnosis, it might result in misdiagnosis and poor treatment outcome. A precise diagnostic test with a rapid turnaround time (TAT) is crucial in cases of EPTB to facilitate earlier detection and treatment. Appropriate point of care (POC) tests if integrated in to the routine TB elimination programme to diagnose EPTB, would in turn contribute towards improving case-detection.

Currently, under programmatic settings, the WHO recommended nucleic acid amplification tests (NAATS) Truenat and Xpert testing are being widely used for EPTB diagnosis. Various studies have demonstrated the utility of the Xpert and Xpert Ultra test in EPTB diagnosis [3–5]. However, there is limited data on the Truenat's utility in diagnosing EPTB especially from India. Studies from Chandigarh documented a sensitivity of 66.6% and 70% in Truenat testing of vitreous fluid and ileocecal biopsy specimens respectively [6,7]. A study from Kerala that tested different extrapulmonary specimens demonstrated 100% sensitivity of Truenat testing for *M.tuberculosis* diagnosis [8]. Since WHO's endorsement of Truenat testing in 2020 [9], the test has been deployed in all the peripheral health centers for patient management under programme which involves EPTB diagnosis as well. However difficult to process samples like biopsies, tissues and excisions are sent to reference labs for further processing before subjecting them to NAAT. In this study we have documented the results of Truenat testing from such samples.

* Corresponding author at: Department of Bacteriology, ICMR -National Institute for Research in Tuberculosis, Chetpet, Chennai-31, India.

https://doi.org/10.1016/j.jctube.2024.100420

Available online 20 February 2024

2. Materials and methods

Study population: Patients with suspected EPTB and patients on treatment for EPTB enrolled in the National Tuberculosis elimination programme (NTEP), in and around Chennai district for a one-year period (July 2021 to July 2022) were included in the study. This study was done as part of routine patient management where the samples that require processing are sent to NIRT for Truenat testing.

Processing of the EPTB samples: A variety of extrapulmonary samples such as body fluids, surgically excised tissues, biopsies and fluids from lymph node and other parts of the body, aspirated or draining pus and urine were received for Truenat testing. The samples received are divided into two groups: - Specimens that are free from contaminating normal flora: spinal, pleural, pericardial, synovial, ascitic, blood, pus, bone marrow, tissues (lymph node or tissue biopsies). Specimens known to contain contaminating normal flora: gastric lavage, bronchial washings and urine. Since extra-pulmonary specimens, in general, are paucibacillary in nature, their processing methods require milder decontamination. Fluid specimens other than CSF, Bronchoalveolar lavage and pericardial fluid are centrifuged for 15 min at 3500 rpm. Tissue biopsies are transferred into a sterile tissue grinder tube to which 5 mL of sterile distilled water was added and homogenized with sterile teflon grinding rod. Further, these specimens are inoculated onto multiple media, viz. Lowenstein Jensen's medium, SK medium enriched with sodium pyruvate (SP), and a liquid medium Kirchner's, made selective by incorporating polymyxin B, amphotericin B, carbenicillin, vancomycin and trimethoprim to inhibit growth of other microorganisms (PACT). The processed samples were also subjected to Ziehl-Neelsen staining and examined for acid fast bacilli (100x magnification).

Truenat testing: Truenat MTB test was performed as per manufacturer's instructions [10]. All the samples were treated as per Molbio EPTB sample pre-treatment protocol. After discarding the supernatant of the centrifuged specimens, 0.5 mL of the sediment was transferred to Lysis buffer tube. The homogenized tissue sample and pus aspirate were treated with liquefaction buffer for 5–10 min and then transferred to Lysis buffer tube. The tube was then vortexed and incubated for five minutes. The extraction of Deoxyribonucleic Acid (DNA) from the samples was done using Trueprep AUTO Universal Cartridge Based Sample Prep kit and device. The pre-treated sample was transferred to the sample chamber of the cartridge and was placed in the device. The entire elute was aspirated out from the elute chamber into the Elute Collection Tube (ECT). Truenat MTB Real Time PCR: The 6 μL of purified DNA from ECT was transferred to microtube containing freeze dried PCR reagents. It was then added to the Truenat MTB microchip containing lyophilized master mix and the real-time PCR was done using a pre-programmed profile on True lab Analyser.

3. Results

A total of 195 EPTB samples collected over a period of one year from July 2021 to July 2022 was included in the study. The gender distribution among the study population was found to be 113 male (57.9%) and 82 female (42.1%). Maximum number of enrolled patients were in the age group of 19–39 (43.9%) followed by 40–59 (25.4%). Out of 195 samples received, majority of them were biopsies (n = 144 (73%)) and the remaining were fluid (n = 27 (13.8%)) and tissue (n = 24 (12.3%) samples. Of the biopsies lymph node biopsies were 46.

Of the 195 samples tested, 38 (19.4%) samples were positive for EPTB by any one of the diagnostic methods done (smear, culture, microscopy). Out of these 38 samples, 16 (42.1 %) were positive for MTB by Truenat and negative by Culture (Table 1), 12 (31.5%) were positive by culture but negative by Truenat (Table 2) and 8 (21%) were positive by both Truenat and/or smear and culture (Table 3). Only two samples (pus and bone marrow biopsy of a 49 and 41-year-old male respectively) (5.2%) out of 38 were positive by smear microscopy alone (Table 1). The rifampicin resistance when tested among Truenat positives (n = 24) 6 of them were indeterminate and resistance was not detected in others.

In the present study, lymph node TB was the predominant type of EPTB (n = 20, 52.6%) among the positives. Maximum number of positive cases were in the age group category 20–40 (n = 22, 57.8%) and 41–60 (n = 8, 21%). Five patients were under the age of 18 out of which two aged 3 and 17 years were positive by Truenat, two aged 17 and 15 years were positive by culture and one 18-year-old patient was positive by both Truenat and culture for EPTB.

The sensitivity and specificity of the test was calculated with the composite reference standard (Culture (exclusion of colonies as positives), clinical conditions, and smear) and was found to be 60% and

Table 2
EPTB samples positive by culture and negative by Truenat and smear.

S. no	Lab.No	Sample Type	LJ Culture	Age	Sex
1	RFEP 5163	Cervical lymphnode biopsy	1 colony	22	Female
2	RFEP 5173	Elbow Septic Arthritis biopsy	7 colonies	40	Female
3	REEP5178	Lateral End of Clavicle	1 colony	15	Male
4	RFEP 5210	Cervical Lymphnode biopsy	1 colony	22	Female
5	RFEP 5215	Cervical Lymphnode biopsy	1+	17	Male
6	RFEP 5222	Endometrial biopsy	3 colonies	26	Female
7	RFEP 5285	Lymphnode biopsy	1+	35	Male
8	RFEP 5346	Bone biopsy	6 colonies	53	Male
9	RFEP 5422	Lymphnode BIOPSY	1+	30	Male
10	RFEP 5463	Cervical lymphnode biopsy	5 colonies	48	Female
11	RFEP 5482	Excision biopsy	1 colony	48	Female
12	RFEP 5497	PUS	1+	26	Female

Table 1
EPTB samples positive by Truenat and negative by culture.

S.no	Lab.No	Sample Type	MTB Status	FM Smear	Cfu/ml	Age	Sex
1	RFEP 5110	Lymphnode Biopsy	Detected	Neg	$4.1x10^{02}$	29	Female
2	RFEP 5156	Breast tissue	Detected	Neg	$8.7x10^{02}$	42	Female
3	RQ 1203	Pott's spine cold abscess	Detected	Neg	$1.2x10^{03}$	17	Female
4	RFEP 5257	Lymphnode biopsy	Detected	Neg	$3.5x10^{03}$	22	Male
5	RFEP 5374	Pus	Detected	Neg	$2.4x10^{03}$	29	Female
6	RFEP 5375	Lymphnode aspirate	Detected	Neg	$1.4x10^{03}$	29	Female
7	RFEP 5377	Lymphnode biopsy	Detected	Neg	$1.4x10^{01}$	29	Female
8	RFEP 5385	Lymphnode aspirate	Detected	Neg	$7.9x10^{02}$	25	Female
9	RFEP 5397	Spot Spine biopsy	Detected	Neg	$1.1x10^{03}$	3	Male
10	RFEP 5398	Hip Joint biopsy	Detected	Neg	$2.1x10^{04}$	58	Male
11	RFEP 5419	Lymphnode biopsy	Detected	Neg	$1.4x10^{03}$	24	Female
12	RFEP 5462	Lymph node Biopsy	Detected	1+	$2.0x10^{04}$	58	Male
13	RFEP 5495	Lymphnode biopsy	Detected	Neg	$4.1x10^{02}$	55	Female
14	RFEP 5532	Pus	Detected	1+	$9.2x10^{05}$	25	Female
15	RFEP 5533	Lymphnode biopsy	Detected	Neg	$9.2x10^{05}$	25	Female
16	RFEP 5552	Lymphnode biopsy	Detected	Neg	$4.3x10^{05}$	39	Female

Table 3
EPTB samples positive by Truenat and/or smear and culture.

S.no	Lab.No	Sample Type	MTB Status	Cfu/ml	FM Smear	LJ Culture	Age	Sex
1	RFEP 5127	Cervical lymphnode aspirate	Detected	$1.5x10^{02}$	Neg	1 col	18	Female
2	RFEP 5158	Supra cervical lymphnode	Detected	$1.5x10^{06}$	2+	2+	25	Female
3	RFEP 5202	Lymph node biopsy	Detected	NA	Neg	1+	39	Female
4	RFEP 5208	Lymphnode	Detected	$7.1x10^{02}$	Neg	1+	28	Male
5	RFEP 5209	Axilla node	Detected	$5.6x10^{03}$	1+	1+	28	Male
6	RFEP 5373	Soft tissue	Detected	$5.5x10^{02}$	Neg	8 col	61	Female
7	RFEP 5492	Pus	Detected	$6.1x10^{06}$	2+	1+	25	Female
8	RFEP 5506	Pus	Detected	$5.6x10^{01}$	Neg	1+	60	Female

100% respectively. The positive predictive value and negative predictive value was found to be 100% and 90.6% respectively. When the culture positives with colonies were also included for sensitivity calculation, the value decreased to 47.6% (Table 4).

4. Discussion

Currently, under programmatic settings, the WHO recommended nucleic acid amplification tests (NAATS) Truenat and Xpert testing are being widely used for both pulmonary TB and EPTB diagnosis. In this study, we intended to assess the effectiveness of Truenat in EPTB testing since there is scarcity of documented data from the programmatic settings. The study samples consisted of both presumptive EPTB patients and those under treatment for EPTB (follow-up samples). Most of the samples were from adult population with only 16.4% of distribution from the age group 0–18 and 5 among them being positive for EPTB. The findings are similar to the reports from other parts of the developing world where it has been documented that EPTB affects mostly the adult group. Maximum number of positive cases being in the age group category 20–40 was similar to the finding from another study from South India [8].

While the gender distribution was explored, female had more EPTB disease compared to male (25 out of 38 positives). Although WHO have been reporting higher incidence of the disease in male worldwide [11], the disease incidence is found to be higher in female in India concurring with our study findings [8,12]. Similarly, lymph node TB being the most predominant form of TB is also similar to the reports from other studies even through GeneXpert testing where lymph node TB was most common form of EPTB [3,8]. This could also be attributed to the fact that the lymph node samples are the most common extrapulmonary samples obtained for EPTB testing.

Among 38 EPTB positives, 16 of them were positive for Truenat and negative for culture which could be correlated to paucibacillary status and the processing method. The decontamination procedure used for extrapulmonary specimens might be detrimental to *Mycobacteria* and the retrieval of them culture becomes difficult after NALC NaOH processing [2]. However, in Truenat even if the bacilli is dead, the DNA of the bacilli gets amplified. So the higher number of positives in Truenat can be substantiated as either the presence of bacilli in current disease or the remnants of bacilli from past history of TB. In our study, 7 among the 16 were follow-up patients who were on anti TB treatment indicating the presence of dead bacilli.

On contrary, culture positive and Truenat negative in EPTB samples needs further attention and research. One of the reason could be the limit of detection (LOD) in Truenat where MTB can be detected with a LOD of 100 cfu/ml. Some of the culture positives among Truenat negatives had 1 to 8 colonies when the EPTB samples were inoculated on to LJ medium. However two samples that had 1 colony and 8 colonies in LJ medium and were Truenat positive also needs to be noted. In addition the sample type (biopsy processing needs skilled hands) can also be a contributing factor for this contradictory results and needs further exploration in a larger set of samples.

Two smear positives among 38 EPTB positive samples could be NTM, however this was not further confirmed since culture turned out to be negative for both the samples. Similar scenario was found in another study where one sample was negative by Truenat but positive by smear and culture and was further confirmed as NTM [8].

Table 4
Sensitivity calculation in comparison with reference standard.

Composite reference standard (Smear + LJ (excluding colonies) + Clinical diagnosis)				
Truenat MTB testing		POSITIVE	NEGATIVE	TOTAL
	POSITIVE	24(60.0)	0(1.3)	24(12.0)
	NEGATIVE	16(40.0)	156(98.7)	172(88.0)
	TOTAL	40(100.0)	156(100.0)	196(100.0)

5. Limitations of the study

Since our objective was to assess the ability of MTB detection in extrapulmonary samples by Truenat, we were not specific about the presumptive EPTB patients. Since we had included EPTB patients under anti TB treatment as well, the sensitivity of the Truenat testing could not be calculated accurately. Moreover due to the smaller sample size of 195, the sensitivity was calculated for all the sample types together instead of calculating it for each sample type.

6. Conclusion

To conclude, use of Truenat MTB testing proves to be a useful molecular test for detection of MTB in extrapulmonary samples. The findings indicates that Truenat can be used as an add on diagnostic test for EPTB diagnosis but with the limitation of detecting non-viable bacilli. Hence, its utility can be confirmed only if they are used for detection of EPTB from presumptive patients and not on the follow-up samples of these patients under treatment adhering to WHO guidelines on restricting the molecular tests in follow-up samples.

Ethical statement

This manuscript used only the Truenat testing data of the patient samples obtained as part of programme management and waiver of ethical approval was obtained.

CRediT authorship contribution statement

Priya Rajendran: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **Lavanya Jayabal:** Formal analysis, Writing – review & editing. **Mythili Venkatesan:** Formal analysis, Validation, Writing – original draft. **Michel Prem Kumar:** Data curation, Formal analysis, Investigation. **Radhakrishnan Ramalingam:** Formal analysis, Investigation, Methodology, Writing – review & editing. **P. Sivaraman:** . **Asha Fredrick:** Conceptualization, Investigation, Writing – review & editing. **Sivakumar Shanmugam:** Conceptualization, Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Siva Kumar Shanmugam reports article publishing charges, equipment, drugs, or supplies, and statistical analysis were provided by National Institute for Research in Tuberculosis. Siva Kumar Shanmugam reports a relationship with National Institute for Research in Tuberculosis that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jctube.2024.100420.

References

[1] WHO, Global TB report. 2022.
[2] Purohit M, Mustafa T. Laboratory diagnosis of Extra-pulmonary Tuberculosis (EPTB) in resource-constrained setting: state of the art, challenges and the need. J Clin Diagn Res 2015;9(4). p. EE01-6.
[3] Denkinger CM, Schumacher SG, Boehme CC, Dendukuri N, Pai M, Steingart KR. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. Eur Respir J 2014;44(2):435–46.
[4] Perez-Risco D, Rodriguez-Temporal D, Valledor-Sanchez I, Alcaide F, Land GA. Evaluation of the Xpert MTB/RIF ultra assay for direct detection of mycobacterium tuberculosis complex in smear-negative extrapulmonary samples. J Clin Microbiol 2018;56(9).
[5] Ninan MM, Rupali P, Varghese GM, Shalini EB, Venkatesh K, Jesudason MR, et al. Xpert Ultra in diagnosing extrapulmonary TB: accuracy and trace calls. Int J Tuberc Lung Dis 2022;26(5):441–5.
[6] Sharma K, et al. Evaluating Truenat assay for the diagnosis of ocular tuberculosis and detection of drug resistance. Ocul Immunol Inflamm 2023:1–7.
[7] Sharma K, Sharma M, Sharma V, Sharma M, Samanta J, Sharma A, et al. Evaluating diagnostic performance of Truenat MTB Plus for gastrointestinal tuberculosis. J Gastroenterol Hepatol 2022;37(8):1571–8.
[8] Jose RA, Gopal K, Samuel Johnson AK, Johnson Samuel JA, Abraham SS, Goswami T, Thomas M, Mathew R. Evaluation of Truenat MTB/RIF test in comparison with microscopy and culture for diagnosis of extrapulmonary tuberculosis in a tertiary care centre. J Clin Diagn Res 2021;15.
[9] Molecular assays intended as initial tests for the diagnosis of pulmonary and extrapulmonary TB and rifampicin resistance in adults and children: rapid communication Policy update, 2020. Geneva: World Health Organization.
[10] Nikam C, Jagannath M, Narayanan MM, Ramanabhiraman V, Kazi M, Shetty A, et al. Rapid diagnosis of Mycobacterium tuberculosis with Truenat MTB: a near-care approach. PLoS One 2013;8(1):e51121.
[11] Global tuberculosis report. 2022, World Health Organization: Geneva.
[12] Gaur PS, Suryakant S, Bhaskar R, Singh S, Saxena P, Agnihotri S. Incidence and clinical profiles of pulmonary and extra-pulmonary tuberculosis patients in north Indian population: a hospital based retrospective study. Int J Res Dev Pharm L Sci 2017;6(5):2773–8.