



COMPARISON OF INDIGENOUS SPUTUM TRUNAT WITH THE WIDELY IMPLEMENTED GENEXPERT IN DIAGNOSIS OF PULMONARY TB PATIENTS

Respiratory Medicine

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ABSTRACT

Introduction: Eight countries reported the two thirds of the global burden, with India having 26% of the total cases in the world. Case detection remains the crucial factor in control of infection of Tuberculosis. Under RNTCP, three tests are incorporated for the diagnosis of TB (sputum smear microscopy, chest X -ray, and the CB-NAAT test). Both CB-NAAT and TruNAT, not only diagnose tuberculosis accurately, but also identify rifampicin resistance. **Objectives:** To compare sensitivity and specificity of indigenous TruNAT to the widely used CBNAAT test in detection of mycobacterium tuberculosis and presence of rifampicin resistance in suspected PTB patients. **Methodology:** This is a Diagnostic Validation Study done among 54 patients with findings of pulmonary TB, attending OPD of Department of Respiratory Medicine and ENT. Data was obtained on presence of MTB and rifampicin resistance. **Results:** TruNAT positivity testing had a sensitivity, specificity, PPV value, NPV, and diagnostic accuracy of 98.11%, 100%, 100%, 50%, and 98.15% respectively. TruNAT for identification of rifampicin resistance had a sensitivity, specificity, PPV, NPV, and diagnostic accuracy of 98.11%, 100%, 100%, 50%, and 98.15% respectively. **Conclusion:** This study confirms that TruNAT has a good diagnostic accuracy rate with good sensitivity, specificity, positive predictive value, and diagnostic accuracy. With other additional advantages of speedy results and idea on rifampicin resistance (compared to sputum microscopy), lower cost, Portable, battery operated and not requiring temperature ceiling (Compared to CBNAAT) makes TruNAT, a comprehensive choice of diagnostic test and identification of Rifampicin resistance in TB cases.

KEYWORDS

Pulmonary tuberculosis, sputum examination, smear microscopy, CBNAAT, TruNAT.

INTRODUCTION:

Eight countries reported the two thirds of the global burden, with India having 26% of the total TB cases in the world. [1] The five common risk factors (HIV, Diabetes, Smoking, Harmful use of Alcohol and Undernourishment) increases the morbidity and mortality of TB cases. [2-4] This makes India the global leader of Tuberculosis. [5,6]

Case detection remains the crucial factor in control of infection of Tuberculosis. [7] Under RNTCP, three tests are incorporated for the diagnosis of TB (sputum smear microscopy, chest X -ray, and the CB-NAAT test). [8] CB-NAAT, not only diagnose tuberculosis accurately, but also identify rifampicin resistance from the sputum sample in less than 2 hours. [9] In December 2010, WHO suggested the usage of the Xpert MTB/RIF assay. The reduced shelf life of the cartridges, requirement of stable electricity supply annual calibration of the instrument, high cost and temperature ceiling remains a concern in application of CBNAAT test. [10]

On 2 July 2020, World Health Organization approves the usage of TrueNAT Tests for Initial Diagnosis of Tuberculosis and identification of Rifampicin Resistance. [11] The device is portable, battery supported, speedier and has a better sensitivity and specificity compared with the sputum microscopy. It needs a small amount of sputum (0.5ml) compared to the 1ml needed in GeneXpert. Compared to GeneXpert, TrueNAT TB test it is cheaper. [12]

Need for the study / Justification of the study:

This study is chosen aiming to provide more information on TruNAT test's performance in terms of detection rate, feasibility in the regional setup in comparison to the widely established CBNAAT test. TruNAT test is a newer diagnostic test, and the data on accuracy is least available. As there are very few Indian studies performed on feasibility of TruNAT, this study will play a role in providing more information on the sensitivity, specificity, and cost effectiveness to aid in completion of national goal of TB control.

OBJECTIVES:

To compare sensitivity and specificity of indigenous TruNAT to the widely used CBNAAT test in detection of mycobacterium tuberculosis and presence of rifampicin resistance in suspected PTB patients.

Methodology:

This is a Diagnostic Validation Study done for a study period of 18 months, among 54 patients with clinical signs and symptoms of pulmonary TB and radiological findings of pulmonary TB, attending

OPD of Department of Respiratory Medicine and ENT. Patients showing signs of presumptive PTB, Age more than 18 years who were newly diagnosed as sputum smear positive pulmonary tuberculosis patients were included. Patients on Anti TB medication, with Extra pulmonary TB and Patients unwilling to participate in study were excluded from the study.

As per RNTCP protocol, presumptive PTB patients underwent two sample smear microscopy. If they are positive, two more sputum samples were collected for sputum CBNAAT and TruNAT. Data was obtained on presence of MTB and rifampicin resistance.

All presumptive PTB patients have undergone preliminary history taking and physical examination. Two sputum samples – one on the spot and one early morning sputum sample were collected from the patient initially as per RNTCP protocol. For patients who are unable to produce sputum, sputum production was induced by saline nebulisation.

The two samples have undergone smear microscopy (ZN stain) Smear positive patients were identified, and two more sputum samples were collected. Two more sputum samples were collected from these patients and sent for sputum CBNAAT and sputum TruNAT testing respectively. Data obtained on presence of MTB in specimen, rifampicin resistance from Sputum TruNAT were compared to corresponding data obtained from sputum CBNAAT.

RESULTS:

AGE: In the study, The mean age in this study among the subjects was 45.94 ($\pm$ 14.56) years ranging from 22 to 80 years. 14 (25.93%) were present each in 31 - 40 years age group and 41 - 50 years age group and least 8 (14.81%) were in 21 - 30 years age group as represented in the below table,

Table 1. Age distribution of the study population.

Age group	Frequency	Percent
21 - 30 years	8	14.81
31 - 40 years	14	25.93
41 - 50 years	14	25.93
51 - 60 years	8	14.81
> 60 years	10	18.52
Total	54	100.00

Gender: In this study, 39 (72.22%) were males and 15 (27.78%) were females.

Diagnostic Utility of TruNAT positivity in comparison with CBNAAT positivity:

On comparing with the CBNAAT positivity findings as gold standard, the TruNAT positivity testing had a sensitivity of 98.11% (CI - 90.1% - 99.7%), specificity of 100% (CI - 20.6% - 100.1%), positive predictive value of 100% (CI - 93.2% - 100%), negative predictive value of 50% (CI - 9.4% - 90.5%) and diagnostic accuracy of 98.15% (CI - 90.2% - 99.6%) as represented in Table 3.

On comparing with the CBNAAT Rifampicin resistance findings as gold standard, the TruNAT Rifampicin resistance testing had a sensitivity of 100% (CI - 43.9% - 100%), specificity of 100% (CI - 92.7% - 100%), positive predictive value of 100% (43.9% - 100%), negative predictive value of 100% (CI - 70.2% - 100%) and diagnostic accuracy of 100% (CI - 93.2% - 100%) as represented in Table 4.

Table 3. Diagnostic Utility of TruNAT positivity in comparison with CBNAAT positivity

TruNAT positivity	CBNAAT positivity		TOTAL
	Positive	Negative	
Positive	52 True Positive	0 FalsePositive	52
Negative	1 False negative	1 True Negative	2
TOTAL	53	1	54
Sensitivity	98.11% (90.06 - 99.67)		
Specificity	100% (20.65 - 100.01)		
Positive Predictive Value	100% (93.12 - 100)		
Negative Predictive Value	50% (9.45 - 90.55)		
Accuracy	98.15% (90.23 - 99.67)		

Table 4. Diagnostic Utility of TruNAT Rifampicin resistance in comparison with CBNAAT Rifampicin resistance

TruNAT Rifampicin resistance	CBNAAT Rifampicin resistance		TOTAL
	Resistant	Sensitive	
Resistant	3 True Positive	0 False Positive	3
Sensitive	0 False negative	49 True Negative	49
TOTAL	3	49	52
Sensitivity	100% (43.85 - 100)		
Specificity	100% (92.73 - 100)		
Positive Predictive Value	100% (43.85 - 100)		
Negative Predictive Value	100% (70.15 - 100)		
Accuracy	100% (93.12 - 100)		

DISCUSSION:

The main objective of the study is to study the sensitivity and specificity of indigenous TruNAT compared with the widely used CBNAAT test in detection of mycobacterium tuberculosis and presence of rifampicin resistance in suspected PTB patients.

In the study, 14 (25.93%) were present each in 31 - 40 years age group and 41 - 50 years age group and least 8 (14.81%) were in 21 - 30 years age group. The mean and SD of age is 45.94 ($\pm$ 14.56) years ranging from 22 to 80 years. In this study, Totally 2 cases were found negative in TruNAT, one was in >60 years which was true negative and another in 51 - 60 years age group which was false negative. TruNAT can be used in all the ages. While all the diagnostic tests are sputum examination based, the collection of the sample is difficult in children. Age-structure model for the transmission of the disease, vaccination and immune responses are widely studied for TB.

In this study, 39 (72.22%) were males and 15 (27.78%) were females. Males were higher among older age groups whereas females were higher among 31 - 40 years and younger age group. In this study, The two negative cases of TruNAT testing were seen among males and the false positive was also in males. Highest burden of TB occurs among the adult men. Gender is an important factor to be explored because, Gender and socio-cultural determinants of TB related stigma are widely prevalent, which will affect the case detection rate and to an extent the completion of the treatment. [13-15]

In this study, 53 (98.15%) had Positive and 1 (1.85%) had Negative CBNAAT finding. This is slightly higher compared to the findings from the study Meghna Patel et al, in their study, observed that (86.8%) patients had positive results. [22] 50 (92.59%) were sensitive to Rifampicin and 3 (5.56%) were resistant to Rifampicin in CBNAAT testing.

Line probe assay (LPA) is used to identify the multidrug-resistant tuberculosis (MDR-TB) patients for INH resistance. In this study, Among the subjects, 2 (3.7%) were sensitive to INH and 1 (1.85%) were resistant to INH in LPA testing.

On comparing with the CBNAAT positivity findings as gold standard, the TruNAT positivity testing had a sensitivity of 98.11% (CI - 90.1% - 99.7%), specificity of 100% (CI - 20.6% - 100.1%), positive predictive value of 100% (CI - 93.2% - 100%), negative predictive value of 50% (CI - 9.4% - 90.5%) and diagnostic accuracy of 98.15% (CI - 90.2% - 99.6%).

The major strengths of the study include, were firstly, the desired sample size was achieved despite the study period affected by the lock down and other restrictions by induced by the COVID pandemic. Secondly, The CBNAAT equipment were calibrated every year in our institution and hence the measurement bias is very minimal. Thirdly. Line probe assay and INH resistance is also studied and compared.

The major limitations of the study include the sample size was relatively smaller for performing the stratified analysis to study the influence of the factors like age and gender on the diagnostic accuracy of the test. Secondly, This is a Hospital based study in a private setting where a different type of case appear and hence the Berksonian bias is also possible. Eg., MDR TB cases tend to access the Government facility. Lastly, because of the cross-sectional design, and follow up was done, and hence the influence of the diagnosis on the outcomes of the disease could not be explored.

This study confirms that TruNAT has a good diagnostic accuracy rate with good sensitivity, specificity, positive predictive value, and diagnostic accuracy. With other additional advantages of speedy results and idea on rifampicin resistance (compared to sputum microscopy), lower cost, Portable, battery operated and not requiring temperature ceiling (Compared to CBNAAT) makes TruNAT, a comprehensive choice of diagnostic test and identification of Rifampicin resistance in TB cases. Soon the TruNAT test, will replace CBNAAT in the diagnostic algorithm for the diagnosis of TB.

Future studies with increased sample size, exploring the cost benefits, should be performed, in other health care settings such as primary and secondary care, will yield overall diagnostic accuracy of TruNAT. Factors affecting the diagnostic accuracy of TruNAT needs to be explored.

CONCLUSION:

This study confirms that TruNAT has a good diagnostic accuracy rate with good sensitivity, specificity, positive predictive value, and diagnostic accuracy. With other additional advantages of speedy results and idea on rifampicin resistance (compared to sputum microscopy), lower cost, Portable, battery operated and not requiring temperature ceiling (Compared to CBNAAT) makes TruNAT, a comprehensive choice of diagnostic test and identification of Rifampicin resistance in TB cases.

REFERENCES:

- Chakaya J, Khan M, Ntoui F, Akillu E, Fatima R, Mwaba P, Kapata N, Mfinanga S, Hasnain SE, Katoto PD, Bulabula AN. Global Tuberculosis Report 2020-Reflections on the Global TB burden, treatment and prevention efforts. International journal of infectious diseases. 2021 Dec 1;113:S7-12.
- Silva DR, Muñoz-Torrico M, Duarte R, Galvão T, Bonini EH, Arbex FF, Arbex MA, Augusto VM, Rabahi MF, Mello FC. Risk factors for tuberculosis: diabetes, smoking, alcohol use, and the use of other drugs. Jornal Brasileiro de Pneumologia. 2018 Mar;44:145-52.
- Duarte R, Lönnroth K, Carvalho C, Lima F, Carvalho AC, Muñoz-Torrico M, Centis R. Tuberculosis, social determinants and co-morbidities (including HIV). Pulmonology. 2018 Mar 1;24(2):115-9.
- Gupta S, Shenoy VP, Mukhopadhyay C, Baiy I, Muralidharan S. Role of risk factors and socio-economic status in pulmonary tuberculosis: a search for the root cause in patients in a tertiary care hospital, South India. Tropical Medicine & International Health. 2011 Jan;16(1):74-8.
- Harries AD, Satyanarayana S, Kumar AM, Nagaraja SB, Isaakidis P, Malhotra S, Achanta S, Naik B, Wilson N, Zachariah R, Lönnroth K. Epidemiology and interaction of diabetes mellitus and tuberculosis and challenges for care: a review. Public health action. 2013 Nov 4;3(1):3-9.
- Satyanarayana S, Thekkur P, Kumar AM, Lin Y, Dlodlo RA, Khogali M, Zachariah R, Harries AD. An opportunity to end TB: Using the sustainable development goals for action on socio-economic determinants of TB in high burden countries in WHO South-East Asia and the Western Pacific Regions. Tropical medicine and infectious disease. 2020 Jun 18;5(2):101.
- TBfacts - TB Statistics, India [Internet]. [cited 2021 Oct 19];Available from: <https://tbfacts.org/tb-statistics-india/>
- Guidelines:: Central TB Division [Internet]. [cited 2021 Oct 19];Available from: <https://tbcindia.gov.in/index1.php?lang=1&level=1&sublinkid=4571&lid=3176>
- Sachdeva K, Shrivastava T. CBNAAT: a boon for early diagnosis of tuberculosis-head

- and neck. Indian Journal of Otolaryngology and Head & Neck Surgery. 2018 Dec;70:572-7.
10. Genexpert Cepheid test - Test for drug resistant TB - TBFacts [Internet]. [cited 2021 Oct 19]; Available from: <https://tbfacts.org/genexpert/>
 11. FIND, MolBio, ICMR. World Health Organization Endorses Truenat Tests for Initial Diagnosis of Tuberculosis and Detection of Rifampicin Resistance. Find [Internet] 2020;1(June):2019–21. Available from: https://www.finddx.org/wp-content/uploads/2020/07/PR_TrueNat-WHO-endorsement_2July2020_FINAL.pdf
 12. TRUENAT- rapid molecular testing for TB [Internet]. [cited 2021 Oct 19]; Available from: http://www.stoptb.org/news/stories/2021/ns21_013.html
 13. Gosoni GD, Ganapathy S, Kemp J, Auer C, Somma D, Karim F, et al. Gender and socio-cultural determinants of delay to diagnosis of TB in Bangladesh, India and Malawi [Special section on gender and TB]. Int J Tuberc Lung Dis 2008;12(7):848–55.
 14. Allotey P, Gyapong M. Gender in tuberculosis research [Special section on gender and TB]. Int J Tuberc Lung Dis 2008;12(7):831–6.
 15. Somma D, Thomas BE, Karim F, Kemp J, Arias N, Auer C, et al. Gender and socio-cultural determinants of TB-related stigma in Bangladesh, India, Malawi and Colombia [Special section on gender and TB]. Int J Tuberc Lung Dis 2008;12(7):856–66.