ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research

Vol. 17, Issue, 02, pp.31872-31876, February, 2025
DOI: https://doi.org/10.24941/ijcr.48622.02.2025

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EVALUATION OF TRUENAT PCR AND MGIT LIQUID CULTURE FOR DIAGNOSING CERVICAL TUBERCULOUS LYMPHADENITIS IN A TERTIARY CARE CENTER IN DEHRADUN

[1]Dr. Arti Negi, [2]Dr. Nidhi Negi, [3]Dr. Shalabh Jauhari, [4]Dr. Neha Arya and [5,*]Dr. Yogita Rawat

[1]Assistant Professor, Department of Microbiology, Govt. Doon Medical College, Patel Nagar, Dehradun, Uttarakhand; [2]Professor, Department of Microbiology, Govt. Doon Medical College, Patel Nagar, Dehradun, Uttarakhand; [3]Professor & Head, Department of Microbiology, Govt. Doon Medical College, Patel Nagar, Dehradun, Uttarakhand; [4]Junior Resident 2, Department of Microbiology, Govt. Doon Medical College, Patel Nagar, Dehradun, Uttarakhand; [5]Assistant Professor, Department of Microbiology, Govt. Doon Medical College, Patel Nagar, Dehradun, Uttarakhand

ARTICLE INFO

Article History:
Received 30th September, 2024
Received in revised form
15th November, 2024
Accepted 26th December, 2024
Published online 27th February, 2025

Key words:
Extrapulmonary TB (EPTB),
Cervical Lymphadenitis,
Truenat PCR, MGIT Liquid Culture, Diagnosis.

***Corresponding author:**
Dr. Yogita Rawat

ABSTRACT

Background: Tuberculosis (TB) remains a major public health concern, with extrapulmonary TB (EPTB) posing significant diagnostic challenges. Cervical tuberculous lymphadenitis is the most common form of EPTB,35% of EPTB and 15-20% of all cases of Tuberculosis (TB).The diagnosis is complicated by the paucibacillary nature of specimen and the limitations of conventional diagnostic methods. This study aimed to evaluate the diagnostic efficacy of Truenat polymerase chain reaction (PCR) compared to Mycobacteria Growth Indicator Tube (MGIT) liquid culture in detecting *Mycobacterium tuberculosis* (MTB) in cervical lymph node aspirate samples. **Methods:** A total of 100 clinically suspected cases of cervical tuberculous lymphadenitis were evaluated in this comparative study, from April 2023 to March 2024. Fine-needle aspiration cytology (FNAC) samples were collected and processed for both Truenat PCR and MGIT liquid culture. Ziehl-Neelsen (ZN) staining was also performed. Culture results were taken as the gold standard for statistical analysis, and diagnostic parameters such as sensitivity and specificity were calculated. **Results:** Out of 100 samples, MTB was detected in 29% (29/100) using Truenat PCR and 22% (22/100) using MGIT liquid culture. Among the 29 PCR-positive samples, 20 (69%) were also culture-positive, while 9 (31%) were culture-negative. Of the 22 MGIT-positive cases, 20 (91%) were also detected by PCR, whereas 2 (9%) were PCR-negative. ZN staining was positive in only 10% of samples. The sensitivity and specificity of Truenat PCR compared to MGIT culture were found to be 90.9% and 88.46%, respectively. **Conclusion:** Truenat PCR demonstrated higher sensitivity than MGIT culture, making it a valuable diagnostic tool for cervical tuberculous lymphadenitis. Given its rapid turnaround time and high accuracy, integrating Truenat into routine diagnostic workflows could improve early case detection, especially in resource-limited settings. This aligns with India's TB elimination goals and the global *End TB* strategy.

Citation: Dr. Arti Negi, Dr. Nidhi Negi, Dr. Shalabh Jauhari, Dr. Neha Arya and Dr. Yogita Rawat. 2025. *"*Evaluation of Truenat PCR and MGIT Liquid Culture for Diagnosing Cervical Tuberculous Lymphadenitis in a Tertiary Care Center in Dehradun.*". International Journal of Current Research, 17, (02), 31872-31876.*

INTRODUCTION

Tuberculosis (TB) is a chronic, infectious disease that causes a significant morbidity and mortality if not diagnosed timely and treatment initiated early (https://www.cdc.gov/globalhealth /newsroom/topics/tb/index.html). Despite being a curable disease, the tuberculosis (TB) notified cases in 2022 by WHO was around 10.1 million out of which 17% were of extra pulmonary TB (EPTB) (World Health Organization, 2022). An estimated global total of 10.8 million people fell ill with TB in 2023, equivalent to 134 incident cases per 100 000 population. Among all the incident TB cases, 6.1% were among people living with HIV (World Health Organization, 2024). While there is significant progress in the development of diagnostics for pulmonary TB (PTB), the diagnostic challenges of extra pulmonary tuberculosis (EPTB) still persists and need 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 it constitutes about 15 to 20% of all cases of TB (Purohit, 2025). Cervical lymph nodes are the most common site of tuberculous

lymphadenopathy in 60%–90% of cases, and its diagnosis remains a challenge, especially in developing countries (Hegde, 2014). In comparison to developing countries, NTM is the most common cause of lymphadenopathy in developed nations. Difficulty of EPTB diagnosis could be attributed to various reasons like its paucibacillary nature, reduced sensitivity of smear testing, delay in obtaining culture results (4-6 weeks) (Prakash, 1985). When diagnosis and treatment are based solely on clinical presentation, there is a high risk of misdiagnosis, leading to suboptimal treatment outcomes. In resource poor settings, cytology and conventional smear (AFB) microscopy are routinely used as the initial diagnostic tools for Lymph node TB (LNTB). Fine needle aspiration cytology (FNAC) is a simple and rapid diagnostic technique but cytomorphological features of LNTB can overlap with few other lesions other than TB and causes delay in diagnosis, it lacks sensitivity due to the paucibacillary nature of fine needle aspirates (FNAC). Moreover the outcome also depends on the quality and quantity of samples, it also have inter observer variability (Corbett, 2003).

The gold standard recommended by the WHO for the diagnosis of TB is the use of culture method. In 2007, the WHO endorsed the use of liquid culture media as a gold standard for TB diagnosis based on the recommendations of international experts (Valsan, 2022). But it has major limitation of turnaround time of 2–4 weeks leading to a delay in the commencement of anti-tuberculosis treatment (ATT). It has low sensitivity in case of paucibacillary TB. Also in a developing country like ours, where TB burden is high, there is likely high chances of drug resistance both in pulmonary and extrapulmonary TB cases. Early detection of drug resistance in LNTB will help in early detection of resistance cases and timely initiation of treatment. Considering all these limitations, more rapid and reliable methods are the need of hour (Tadesse, 2025). A precise diagnostic test with a good diagnostic yield and 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, in programmatic settings, WHO-recommended nucleic acid amplification tests (NAATs), such as Truenat and Xpert, are widely utilized for the diagnosis of extrapulmonary tuberculosis (EPTB) (Ninan, 2022). Apart from routine TB diagnosis, TrueNat also gives Rifampicin sensitivity by melt curve analysis. Furthermore, Truenat has been shown to have high concordance with Xpert MTB/RIF, another molecular diagnostic test, while also offering the benefit of detecting rifampicin resistance when used in combination with Truenat MTB-RIF Dx . This is particularly beneficial in high-burden settings, where drug-resistant tuberculosis poses a major challenge (Kumar, 2023). But it still remains a dilemma to choose between PCR and culture as the most dependent and reliable diagnostic test for diagnosis of TB. We planned a study to evaluate the diagnostic efficacy of Truenat polymerase chain reaction and MGIT Liquid-culture in Extrapulmonary tuberculosis (Cervical Tuberculous lymphadenitis) samples in our hospital.

MATERIAL AND METHODS

This comparative study was conducted in Department of Microbiology, Government Doon Medical College & Hospital, Dehradun from April 2023 to March 2024, over a period of one year. A total of 100 clinically suspected cases of cervical tuberculous lymphadenitis samples were included in the study . Patients with cervical lymphadentis having FNAC suggestive of granulomatous inflammation with or without necrosis or AFB positive were all included in the study. Samples were received in the laboratory for both TrueNat PCR and AFB MGIT culture. Patients with Age >12 years, with palpable or radiologically detected cervical lymphadenopathy, FNAC suggestive of granulomatous inflammation were included in the study group. Samples with inadequate volume for both the testing, patients on ATT and immunocompromised individuals were excluded from the study. The study was approved by the Institutional Ethics Committee. The samples were collected from the enlarged superficial lymph nodes using 22–23 gauge needle and 10 ml plastic syringe with a detachable syringe holder. In each case, one part of the aspirated material was put in a sterile container with normal saline for Truenat and other part of the aspirated material was also put in a sterile container with normal saline for MGIT liquid culture. For Mycobacterium growth indicator tube culture a samples were processed in Biosafety cabinet level II. Samples were decontaminated to inhibit other bacterial growth and concentrated by N-acetyl L-cysteine-sodium hydroxide method (Global Laboratory Initiative, 2014). After decontamination, smears were made from all specimens and stained by Ziehl-Neelsen(ZN) staining for acid fast bacilli (AFB). MGIT tubes were inoculated and incubated at 37° for six weeks. The tubes were read every day using MGIT reader (BD). Cultures were considered positive if the green light of the MGIT reader moves towards the red part (between 14-20) and granular growth appears at the bottom of the liquid medium in the tube. AFB smear was prepared from the granular growth in the tube and if acid fast bacilli are seen, the growth is confirmed by TBc ID card (BD Bactec) which detects the MPT64 antigen of MTB complex (Mangayarkarasi, 2019).

TrueNat PCR was done as per the SOP provided by the manufactures. Samples were processed by MTB Plus PCR. In a positive sample, both the target and the internal positive control curves will take a steep, exponential path when the fluorescence crosses the threshold value. At the end of the cycle, the MTB PCR result screen displays “Detected” with Ct value and the colony-forming units per millilitre (CFU/ml). In MTB PLUS PCR, the result screen would display the MTB load as “High”, “Medium”, “Low” or “Very low” for positive specimens (World Health Organization, 2021). The findings of PCR were compared with other diagnostic tecniques, mainly liquid culture.Culture was taken as a gold standard for the statistical analysis. The results were analyzed with SPSS version 21(SPSS Inc., Chicago, Illinois, United States of America). p-values < 0.01 were considered to significant.

RESULTS

During the study period, a total of 100 cervical lymph node aspirates samples were received in the laboratory. Out of 100 samples MTB was detected in 29 samples (29%) in PCR and 22 samples (22%) were positive in MGIT liquid culture system. Out of 29 PCR positive samples 20 (69 %) were MGIT liquid culture positive and 9 samples (31 %) were MGIT liquid culture negative. So 20 samples were positive for both culture and PCR. Out of total 22 MGIT liquid culture positive samples, 20 samples (91 %) were positive in PCR

reaction and 2 (9%) culture positive samples were not detected by the PCR i.e. they were negative in PCR reaction. ZN staining was also performed in 100 cervical lymph node aspirates samples, out of which 10 samples (10 %)) were positive by acid fast staining. Overall, 29 samples were positive by either method. In 29 positive samples, 15 were found to be females (52%) and 14 (48%) were males Fig 1. We also determined the sensitivity and specificity of Truant PCR when compared with MGIT liquid culture medium was found to be 90.9% and 88.46%, respectively Figure 1

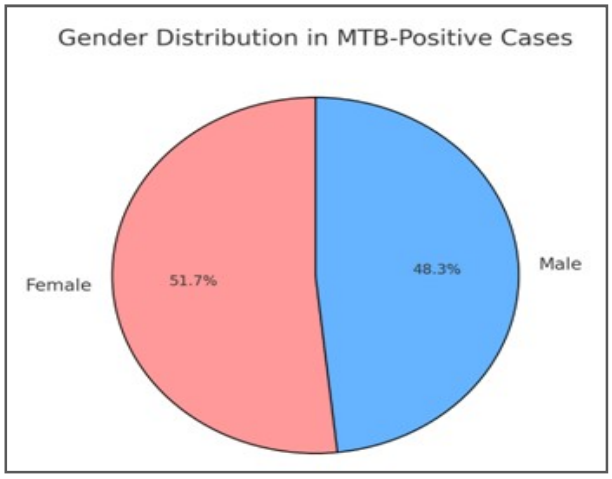

Figure 1. Gender Distribution in MTB Positive cases (n=29)

Table 1. Table Showing Comparison of Truenat PCR and MGIT Liquid Culture results in Cervical Tuberculous Lymphadenitis samples

Test Result	MGIT Liquid Culture Positive	MGIT Liquid Culture Negative	Total
PCR Positive	20	9	29
PCR Negative	2	69	71
Total	22	78	100

PCR: Polymerase Chain Reaction; MGIT: Mycobacterium growth indicator tube

DISCUSSION

India is nearing the completion of the 100 Days Intensified TB Campaign, a nationwide initiative aimed at reducing tuberculosis (TB)-related mortality and preventing new infections. As one of the highest TB-burden countries, India faces significant challenges in controlling the disease, making such intensified efforts crucial (World Health Organization, 2021). A key aspect of TB control is the early detection of extrapulmonary TB (EPTB), particularly cervical tuberculous lymphadenitis, commonly called Scrofula, is the most common form of EPTB. It constitutes 35–40% of EPTB (Sharma, 2021). Since EPTB often presents with nonspecific symptoms and is more challenging to diagnose than pulmonary TB, increased awareness and access to rapid molecular diagnostics is the need of the hour Tuberculous lymphadenitis (TBL), In this study, we evaluated the presence *of Mycobacterium tuberculosis* (MTB) in100 cervical lymph node aspirate samples using two diagnostic modalities: polymerase chain reaction (PCR), Truenat PCR and the Mycobacteria Growth Indicator Tube (MGIT) liquid culture system. Our findings primarily highlight the key differences in the performance parameters of liquid culture and Truenat PCR, emphasizing on the challenges associated with the early diagnosis of cervical tuberculous lymphadenitis. Among the 100 patients included in this study, the distribution of cases by gender wise revealed a higher prevalence in females as compared to males Figure 1 Cervical tuberculous lymphadenitis affects both genders, with a slight predominance in females. This observation of ours is consistent with previous studies conducted by Sharma *et al.* indicating a higher prevalence of tuberculous lymphadenitis among females, this may be attributed to variations in immune response, sociocultural factors, or differences in healthcare-seeking behavior, stigma attached to it especially in developing countries like ours (Sharma, 2021; Narang, 2019). Understanding this demographic pattern is crucial for targeted screening and early diagnosis, especially in India and other high-TB burden countries. Among the 100 cervical lymph node aspirate samples analyzed, MTB was detected in 29% of cases using PCR, whereas the MGIT liquid culture system identified 22% as positive. The higher positivity rate observed in PCR highlights its superior sensitivity, particularly in paucibacillary conditions where bacterial load is low. This aligns with previous studies performed by Chakravorty *et al*., Denkinger *et al*., demonstrating that nucleic acid amplification tests (NAATs), such as Truenat and Xpert, can detect MTB even in samples with low bacterial burden or prior antibiotic exposure, where culture methods may yield false-negative results (Chakravorty, 2017; Denkinger, 2014). Of the 29 PCR-positive samples, 20 (69%) were also positive by MGIT liquid culture, whereas 9 (31%) were culture-negative. The data was in concordance with the study conducted by Denkinger *et al.* In their study for lymph node tissues and aspirates, which are pertinent to tuberculous lymphadenitis, the pooled sensitivity was 83.1% and specificity was 94.6%.

This discrepancy could be attributed to the fact that tuberculous lymphadenitis often presents as a paucibacillary disease, where bacterial load is too low for successful culture. Conversely, among the 22 MGIT culture-positive samples, 20 (91%) were also detected by PCR, while 2 (9%) remained undetected by PCR. Possible explanations for the PCR-negative but culture-positive cases include the presence of PCR inhibitors in clinical samples or genetic mutations affecting primer-binding regions (Marlowe, 2011). In their study, Marlowe *et al.* evaluated the performance of the Cepheid Xpert MTB/RIF assay for the direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. They reported a sensitivity of 97.6% and a specificity of 95.1% for the detection of MTBC which is high as compared with extrapulmonary samples. In our study 31% PCR positive samples were not detected in culture, PCR, being a highly sensitive molecular method, can amplify MTB DNA even in such low-burden samples as PCR amplifies bacterial DNA regardless of viability, while MGIT culture requires live bacilli for growth. Similar findings have been reported in previous studies by Hillemann *et al*, underscoring that while PCR enhances early case detection, it cannot differentiate between viable and non-viable bacilli, posing a limitation in assessing active infection (Torres-Sangiao, 2016). ZN staining was performed on all 100 samples, with only 10% testing positive for acid-fast bacilli. This highlights the low sensitivity of smear microscopy, especially in extrapulmonary TB cases, which often present with a paucibacillary load. Consistent with previous reports, our findings reinforce the need to integrate molecular diagnostic tools into routine diagnostic workflows to improve early case detection and reduce TB transmission (Denkinger, 2014). We also determined the sensitivity and specificity of Truenat PCR in comparison with MGIT liquid culture, which were found to be 90.9% and 88.46%,

respectively. According to a Cochrane systematic review, conducted by Wang *et al*. the assay exhibited a pooled sensitivity of 81% and specificity of 98% across various EPTB specimens (Wang, 2025). These values indicate that Truenat PCR is a highly sensitive and specific diagnostic tool for detecting *Mycobacterium tuberculosis* in cervical lymphadenitis. Similar findings have been reported by Boehme *et al*. Denkinger *et al*., Boehme *et al*. reported a sensitivity of 98.2% and a specificity of 99.2% for detecting MTB in smear-positive, culture-positive cases. In smear-negative, culture-positive cases, the sensitivity was 72.5%. Denkinger *et al*. conducted a meta-analysis and found a pooled sensitivity of 88% and specificity of 98% for detecting MTB across various specimen types, including extrapulmonary samples **(**Boehme, 2011). Thus suggesting that Truenat is a reliable alternative to conventional culture-based methods, particularly in resource-limited settings where rapid and accurate TB diagnosis is critical. Sharma *et al* study reported a sensitivity of 80.49% and a specificity of 77.78% for the Truenat assay when evaluated against fine-needle aspiration cytology (FNAC) with necrosis as the reference standard (Sharma, 2024). Similarly, Mandal *et al*. observed that the Truenat MTB assay exhibited a sensitivity of 100% and a specificity of 95.1% in diagnosing extrapulmonary tuberculosis cases (Mandal, 2023). These findings highlight the diagnostic utility of Truenat in cervical tuberculous lymphadenitis while emphasizing that its sensitivity and specificity may vary based on the reference standard and study population.

CONCLUSION

In our study, PCR demonstrated superior sensitivity by identifying additional cases of tuberculous lymphadenitis that were undetected by microscopy and culture. Given its rapid turnaround time (~2 hours), high sensitivity, and operational feasibility, Truenat represents a valuable alternative for the diagnosis of tuberculous lymphadenitis. Integrating Truenat into diagnostic workflows, particularly in resource-limited settings with constrained laboratory infrastructure, has the potential to enhance early case detection and improve patient outcomes.Detecting and treating EPTB early can prevent complications, reduce transmission risk, and contribute significantly to India's broader goal of eliminating TB by 2025, aligning with global End TB targets.

Conflict of Interest: The authors declare that there was no conflict of interest.

Source of funding: None

REFERENCES

Centers for Disease Control and Prevention. Tuberculosis (TB) (Internet). Atlanta: CDC; 2024 (cited 2025 Mar 8). Available from: https://www.cdc.gov/globalhealth/ new sroom/ topics/tb/index.html

World Health Organization. Global tuberculosis report 2022 (Internet). Geneva: WHO; 2022 (cited 2025 Mar 8). Available from: https://www.who.int/teams/global-tuberculosis- programme/tb-reports/global-tuberculosis-report-2022

World Health Organization. Global tuberculosis report 2024. Geneva: WHO; 2024.

Purohit M, Mustafa T. Laboratory diagnosis of extrapulmonary tuberculosis (EPTB) in resource-constrained settings: state of the art, challenges, and the need. J Clin Diagn Res. 2015;9(4):EE01–6.

Hegde S, Rithesh KB, Baroudi K, Umar D. Tuberculous lymphadenitis: early diagnosis and intervention. J Int Oral Health. 2014;6:96–8.

Prakash UB, Reiman HM. Comparison of needle biopsy with cytologic analysis for the evaluation of pleural effusion: analysis of 414 cases. Mayo Clin Proc. 1985;60:158–64.

Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, *et al*. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. Arch Intern Med. 2003;163:1009–21.

Valsan PM, Sudarasana J. Comparison of TrueNat polymerase chain reaction and mycobacterium growth indicator tube culture in the diagnosis of pulmonary and extrapulmonary tuberculosis. J Acad Clin Microbiol. 2022;24(1):21–5.

Tadesse M, Abebe G, Abdissa K, Bekele A, Yamuah L, Apers L, *et al*. GeneXpert MTB/RIF assay for the diagnosis of tuberculous lymphadenitis on concentrated fine needle aspirates in high tuberculosis burden settings. PLoS One. 2015;10:e0137471.

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.

Kumar M, Singh R, Garg P, Kumar A, Sharma S, Gupta R, *et al*. Comparative evaluation of Truenat and Xpert MTB/RIF assays for pulmonary and extrapulmonary tuberculosis diagnosis. Infect Disord Drug Targets. 2023;23(1):54–62.

Global Laboratory Initiative. Mycobacteriology laboratory manual. 1st ed. Geneva: Stop TB Partnership; 2014.

Mangayarkarasi V, Sneka P, Sujith R, Jayaprakash S. Ergonomic diagnostic tool based on chip mini RT PCR for diagnosis of pulmonary and extrapulmonary tuberculosis. J Pure Appl Microbiol. 2019;13:1185–90.

World Health Organization. Global tuberculosis report 2021 (Internet). Geneva: WHO; 2021 (cited 2025 Mar 8). Available from: https://www.who.int/publications/i/item/ 9789240037021

Sharma SK, Mohan A. Extrapulmonary tuberculosis: an overview. Indian J Med Res. 2021;154(2):219–46.

Narang P, Mendiratta DK, Tyagi NK, Malhotra A, Gupta S, Srivastava K, *et al*. Diagnostic efficacy of Xpert MTB/RIF in tubercular lymphadenitis: a comparative study from India. J Lab Physicians. 2019;11(4):315–9.

Chakravorty S, Sen MK, Tyagi JS. Diagnosis of extrapulmonary tuberculosis by PCR. FEMS Immunol Med Microbiol. 2017;66(1):20–36.

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.

Marlowe EM, Novak-Weekley SM, Cumpio J, Sharp SE, Momeny MA, Babst A, *et al*. Evaluation of the Cepheid Xpert MTB/RIF assay for direct detection of Mycobacterium tuberculosis complex in respiratory specimens. J Clin Microbiol. 2011;49(4):1621–3.

Torres-Sangiao E, Holban AM, Gestal MC. Advanced DNA-based methods for tuberculosis detection: promises and challenges. Front Microbiol. 2016;7:648.

Wang MQ, Zheng YF, Hu YQ, Liu XD, Chen HY, Li Q, *et al*. Diagnostic accuracy of Xpert MTB/RIF Ultra for detecting

pulmonary tuberculosis and rifampicin resistance: a systematic review and meta-analysis. Eur J Clin Microbiol Infect Dis. 2025;44:681–702.

Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, *et al*. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med***.** 2011;363(11):1005-15.

Sharma R, Sharma R, Sai KP. Analysis of the diagnostic yield of nucleic acid amplification tests in immunocompetent patients with tuberculous cervical lymphadenitis. Int J Sci Res. 2024;13(8):965–9.

Mandal S, Rai Y, Gogoi S, Sharma A, Kaur R, Jais MB. Evaluation of Truenat MTB test in comparison with Ziehl-Neelsen staining and MGIT automated liquid culture for diagnosis of suspected pulmonary and extrapulmonary tuberculosis in pediatric patients in a tertiary care hospital. Int J Pharm Clin Res. 2023;15(11):1564–8.

****