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Original Article

A Comparative Study of Cartridge-based Nucleic Acid Amplification Test and Ziehl–Neelsen Stain with Culture on Lowenstein–Jensen Media as Gold Standard for the Diagnosis of Pulmonary Tuberculosis

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Abstract

Introduction: Tuberculosis (TB) is an airborne disease caused by *Mycobacterium tuberculosis* that usually affects the lungs, leading to fever, cough, and chest pain. Although a declining trend was observed in most developed countries, TB remains a leading cause of morbidity and mortality in many developing countries, including India. **Materials and Methods:** This prospective study was carried out using 150 sputum samples of suspected pulmonary TB patients. All the samples were subjected to Ziehl–Neelsen stain, cartridge-based nucleic acid amplification test (CBNAAT) and culture on Lowenstein–Jensen (LJ) media. They were compared for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) in terms of quantitative results. **Results:** CBNAAT results of the sputum samples showed a sensitivity of 100%, 88.7% specificity, 90.3% PPV, and NPV 94%, whereas culture on LJ media showed a sensitivity, specificity, PPV, and NPV of 68.3%, 100%, 100%, and 73.9%, respectively. **Conclusion:** Whereas culture remains the gold standard for the diagnosis of TB, CBNAAT has taken over the domain of diagnosis owing to its high sensitivity and rapid turn over time.

Keywords: Acid-fast bacilli, cartridge-based nucleic acid amplification test, Lowenstein–Jensen media, *Mycobacterium tuberculosis*

Introduction

Tuberculosis (TB), a major airborne communicable disease, is one of the top 10 causes of death worldwide. The infection is acquired through aerosolization of droplets containing *Mycobacterium tuberculosis*. The bacillus typically affects the lungs, causing pulmonary TB but can also affect other sites of the body leading to extrapulmonary TB.[1]

According to the WHO reports, there was an estimated 10 million cases of TB globally in 2019. People of both gender and almost all age groups were affected, but the highest burden was found in men aged ≥15 years. This accounted for 56% of all the cases in 2019.[1]

Factors contributing to the continued spread of TB include increase in patients coinfected with *M. tuberculosis* and human immunodeficiency virus (HIV) infection, insufficient control procedures, and laboratory delays in the identification and susceptibility testing of *M. tuberculosis* isolates. This emphasizes the need for rapid and cost-effective susceptibility testing to diagnose and treat TB cases at the earliest.[2]

Smear microscopy involving direct examination of sputum smears with Ziehl–Neelsen (ZN) staining for acid-fast bacilli is the most commonly used test for clinically suspected TB patients in resource-limited settings. However, identification of *M. tuberculosis* bacilli by microscopic examination requires at least 10,000 bacilli per mL of sputum.[3,4] Whereas,

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the sensitivity of smear microscopy can be improved with fluorescence, yet, a large number of TB cases still go undiagnosed and extrapulmonary cases are missed.[5]

In early 2011, the WHO endorsed a novel, rapid, and automated cartridge-based nucleic acid amplification test (CBNAAT), that could simultaneously detect *M. tuberculosis* and rifampicin resistance.[6] Its limit of detection is 5 genome copies of purified DNA per reaction (131 colony-forming units per mL of sputum).[7] Since CBNAAT detects both live and dead bacteria, it cannot differentiate between active and cured TB.[8]

While culture on Lowenstein–Jensen (LJ) media is considered time-consuming and necessitates special procedures, it is still cheaper than molecular techniques. Therefore, culture remains the gold standard for diagnosing TB. *M. tuberculosis* grows slowly and takes 4 to 8 weeks to become positive in conventional culture media.[9]

This study has been conducted to evaluate the validity and reliability of sputum smear direct microscopy when compared to CBNAAT and culture on LJ media for the diagnosis of pulmonary TB.

Materials and Methods

A prospective study was carried out using 150 sputum samples. These samples were collected from patients clinically suspected with pulmonary TB and no past history of antitubercular drug intake. Patients presented with fever and cough. Other common presentations were weight loss, anorexia, and occasional chest pain. All 150 samples were subjected to ZN stain, CBNAAT, and culture on LJ media. They were compared for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) in terms of quantitative results.

Ziehl–Neelsen stain

N-Acetyl L-Cysteine–Sodium Hydroxide decontamination method was used for *M. tuberculosis* isolation from sputum samples. Then, the processed sample was smeared for ZN stain, visualized under an oil-immersion microscope (×100 magnification). Each slide was observed for acid-fast bacillus (AFB) for 10 min, corresponding to 300 fields examined [Figure 1].

Cartridge-based nucleic acid amplification test

Sputum liquefaction and inactivation was done by adding a double volume of sample reagent (Sample: Reagent-1:2). Then, the mixture was vigorously shaken for 10–20 times (or vortexed for at least 10 s). After that, the mixture was incubated for 10 min at room temperature and again shaken vigorously for 10–20 times (or vortexed for at least 10 s). Again, the sample was incubated at room temperature for an additional 5 min. Using a fresh transfer pipette, 2 ml of the processed sample was transferred to the cartridge. The cartridge was loaded into the instrument as per the manufacturer's instructions.[10]

Culture

For culture, the sediment from the processed sample was inoculated in the LJ media and incubated aerobically at 36°C for 4–6 weeks. *M. tuberculosis* grew as a buff-colored, dry colony, which is very distinctive [Figure 2].[11]

Results

A total of 150 sputum samples were subjected to ZN stain, CBNAAT, and culture on LJ media.

Correlation between Ziehl–Neelsen stain and culture

In the present study, among the 150 samples, 54 (36%) were acid-fast stain positive, all of which showed growth on culture. Among the 96 acid-fast negative samples, 25 (16.6%) showed growth on culture and 71 (47.3%) were negative [Table 1].

Correlation between acid-fast bacillus smear and cartridge-based nucleic acid amplification test

Among the 150 samples, 54 were AFB positive, all of which were also CBNAAT positive. Among the 96 AFB-negative samples, 29 (19.3%) were CBNAAT positive [Table 2].

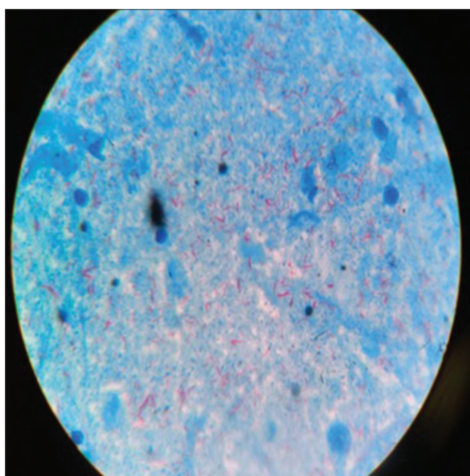

Figure 1: Ziehl–Neelsen stain of sputum sample showing the presence of acid-fast bacilli

Figure 2: Growth of mycobacterium tuberculosis on Lowenstein–Jensen medium

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Correlation between cartridge-based nucleic acid amplification test and culture

Out of the 83 CBNAAT-positive samples, 8 (5.3%) were culture negative. Among the 67 CBNAAT-negative sputum samples, 4 (2.6%) were positive on culture [Table 3].

Sensitivity, specificity, positive predictive value, and negative predictive value values of Ziehl–Neelsen stain and cartridge-based nucleic acid amplification test with culture as gold standard

CBNAAT has a very high sensitivity (100%) in comparison to ZN staining in the diagnosis of *M. tuberculosis*. On the other hand, ZN stain is highly specific (100%) for the detection of acid-fast bacilli in the sputum samples [Table 4].

Discussion

The purpose of this study was to evaluate the diagnostic yield of CBNAAT to detect *M. tuberculosis* in sputum samples and compare it with ZN staining and AFB culture.

Table 1: Comparison of results from acid-fast bacilli smear and culture

ZN stain smear for AFB	Culture positive	Culture negative
Positive (*n*=54)	54	0
Negative (*n*=96)	25	71

AFB: Acid-fast bacilli, ZN: Ziehl–Neelsen

Table 2: Comparison of results from acid-fast bacilli smear and cartridge-based nucleic acid amplification test

ZN stain smear for AFB	CBNAAT positive	CBNAAT negative
Positive (*n*=54)	54	0
Negative (*n*=96)	29	67

AFB: Acid-fast bacilli, ZN: Ziehl–Neelsen, CBNAAT: Cartridge-based nucleic acid amplification test

Table 3: Comparison of results from cartridge-based nucleic acid amplification test and culture

CBNAAT	Culture positive	Culture negative
Positive (*n*=83)	75	8
Negative (*n*=67)	4	63

CBNAAT: Cartridge-based nucleic acid amplification test

Table 4: Sensitivity, specificity, positive predictive value, and negative predictive values of Ziehl–Neelsen stain and cartridge-based nucleic acid amplification test with culture as gold standard

Method	Sensitivity	Specificity	PPV	NPV
ZN stain (%)	68.3	100	100	73.9
CBNAAT (%)	100	88.7	90.3	94

ZN: Ziehl-Neelsen, CBNAAT: Cartridge-based nucleic acid amplification test, PPV: Positive predictive value, NPV: Negative predictive value

CBNAAT showed a sensitivity of 100%, specificity of 88.7%, PPV of 90.3%, and NPV of 94%. A study by Agrawal *et al*.[12] showed similar results with a sensitivity of 100%, specificity of 90%, PPV of 91.6%, and NPV of 100%. Similar results were also seen in the study by Sharma *et al*.[13] Out of 67 CBNAAT-negative cases, 4 samples were culture negative. The reason for this is mostly because CBNAAT can detect only MTB. Another possible reason might be the bacterial load in the sample which was too low for CBNAAT to detect the DNA. Thus, it shows that a patient with a negative CBNAAT may still have TB.

Out of the 83 CBNAAT-positive samples, 8 were culture negative. This is mainly because PCR amplifies DNA of both live and dead bacilli. The only way to eliminate this error is to take a clear history of treatment with antitubercular drugs.

In comparison with culture on LJ media used as a gold standard, the sensitivity, specificity, PPV, and NPV values for smear microscopy for sputum sample were 68.3%, 100%, 100%, and 73.9%, respectively. Similar results were found in the study by Afsar *et al*.,[14] where the sensitivity and specificity of AFB were found to be 53% and 100%, respectively. A study in Thailand also showed similar sensitivity of 48% and specificity of 84%.[15]

Conclusion

Culture remains the gold standard for diagnosing TB, although growth can take up to 6–8 weeks. Conventional direct smear microscopy is rapid and inexpensive but far from being sensitive for the diagnosis of TB. Besides, it gives no information about the viability of the organism. Molecular method (CBNAAT) is a rapid diagnostic tool for both smear negative or positive patients clinically suspected with TB. In addition, it also gives information about rifampicin resistance. This can be beneficial in treating patients with multidrug-resistant tuberculosis TB and TB-HIV coinfection. The only major disadvantage besides its cost-effectiveness is that it cannot differentiate between active and cured TB. In conclusion, CBNAAT is a very helpful tool for the early diagnosis of TB with reliable results.

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Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization, WHO Global Tuberculosis Report 2020, Executive Summary. Vol 1. Geneva: World Health Organization; 2020.
2. Jacobs RF. Multiple-drug-resistant tuberculosis. Clin Infect Dis 1994;19:1-8.
3. Tuberculosis Coalition for Technical Assistance. International Standards for Tuberculosis Care (ISTC). 2nd ed. The Hague: Tuberculosis Coalition

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for Technical Assistance; 2009.
4. Toman K. How many bacilli are present in a sputum specimen found positive by smear microscopy? In: Frieden T, editor. Toman's Tuberculosis: Case Detection, Treatment, and Monitoring – Questions and Answers. WHO/HTM/TB/2004. 2nd ed. Geneva: World Health Organization; 2004. p. 11-3.
5. Steingart KR, Henry M, Ng V, Hopewell PC, Ramsay A, Cunningham J, *et al.* Fluorescence versus conventional sputum smear microscopy for tuberculosis: A systematic review. Lancet Infect Dis 2006;6:570-81.
6. World Health Organization. Policy Statement: Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System. WHO/HTM/TB/2011. Geneva: World Health Organization; 2011.
7. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, *et al.* Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 2010;48:229-37.
8. Miotto P, Bigoni S, Migliori GB, Matteelli A, Cirillo DM. Early tuberculosis treatment monitoring by Xpert® MTB/RIF. Eur Respir J 2012;39:1269-71.
9. World Health Organization. Policy Framework for Implementing New Tuberculosis Diagnostics. Geneva: World Health Organization; 2011.
10. Xpert MTB/RIF Manual, Cepheid. GENE XPERT MTB RIF USER MANUAL Pdf; 2013. Available from: https://Manualslib.com. [Last accessed on 2021 May 03].
11. Stinson KW, Eisenach K, Kayes S, Matsumoto M, Siddiqi S, Nakashima S, *et al*. Mycobacteriology Laboratory Manual. Publication of Global Laboratory Initiative a Working Group of the Stop TB Partnership. 1st ed. April;2014.
12. Agrawal M, Bajaj A, Bhatia V, Dutt S. Comparative study of GeneXpert with ZN stain and culture in samples of suspected pulmonary tuberculosis. J Clin Diagn Res 2016;10:C09-12.
13. Sharma SK, Kohli M, Yadav RN, Chaubey J, Bhasin D, Sreenivas V, *et al.* Evaluating the diagnostic accuracy of Xpert MTB/RIF assay in pulmonary tuberculosis. PLoS One 2015;10:e0141011.
14. Afsar I, Gunes M, Er H, Gamze Sener A. Comparison of culture, microscopic smear and molecular methods in diagnosis of tuberculosis. Rev Esp Quimioter 2018;31:435-8.
15. Reechaipichitkul W, Suleesathira T, Chaimanee P. Comparıson of genexpert MTB/RIF assay with conventional AFB smear for dıagnosıs of pulmonary tuberculosıs ın northeastern Thailand. Southeast Asian J Trop Med Public Health 2017;48:313-21.