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Original Research Article

Optimizing tuberculosis diagnosis: A comparative study of mycobacterial growth indicator tube (MGIT) versus GeneXpert for smear-negative pulmonary and extra-pulmonary cases

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Abstract

Background: Tuberculosis being a major global health issue. Accurate diagnosis of tuberculosis and drug resistance detection are the key components to manage Tuberculosis. Diagnosis in smear-negative pulmonary specimens & Extra-pulmonary specimens constitutes a major challenge. We studied the performance of the micro Mycobacterial Growth indicator tube instrument (MGIT) & GeneXpert the Nucleic Acid Amplification Testing (NAAT) assay for the timely diagnosis of tuberculosis in smear-negative pulmonary and extra-pulmonary specimens from the tuberculosis suspected patients.

Materials and Methods: This study was done at the Department of Microbiology, a tertiary care centre, Stanley medical college and Hospital. Pulmonary specimens (only smear-negative) and extra-pulmonary specimens (both smear-positive and negative) were collected from the tuberculosis suspects and tested for MGIT culture & GeneXpert.

Results: A total of 100 specimens were included among them 49% were pulmonary specimens (smear-negative) and 51% were extrapulmonary specimens (both smear positive and negative). In total Mycobacterial Growth Indicator tube detected 17 % and GeneXpert detected 26%. Out of 49 smear-negative pulmonary specimens MGIT detected 16% (8/49) whereas GeneXpert detected 10/51(20%). Out of 51 extra pulmonary specimens MGIT detected 17.6% (9/51) and GeneXpert detected 31 % (16/51). There was 90% correlation between MGIT & GeneXpert with respect to Mycobacterium tuberculosis complex identification.

Conclusion: This study supports that the concurrent use of MGIT culture with GeneXpert could increase the detection rate of tuberculosis within a shorter Turn around time (TAT), especially in paucibacillary specimens.

Keywords: microMGIT – Mycobacterial growth indicator tube, GeneXpert, NAAT - Nucleic acid amplification technique, TAT - Turn around time.

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1. Introduction

Tuberculosis continues to be a major global health issue. The global Tuberculosis incidence rate in 2023 was 134 / lakh population. Five countries accounted for 56% of the worldwide TB disease, among them India (26%), Indonesia (10%), China (6.8%), the Philippines (6.8%) and Pakistan (6.3%). Since India being the highest in contributing TB cases it is mandatory to act & curtail the TB transmission & focus on TB prevention.[1]

The incidence of Tuberculosis in India for 2022 was 199/lakh population. Among which pulmonary TB constitutes 76% & Extra pulmonary TB cases constitutes 24%. The TB mortality was 23/ lakh population. Among the Tuberculosis suspected patients, only 54.6 % of cases were microbiologically diagnosed and the remaining 45.4 % were clinically diagnosed.[2]

Among the 10.6 million new cases, approximately 5 million were smear-negative. Establishing a diagnosis in smear-negative pulmonary specimens constitutes a major

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problem due to its paucibacillary nature.[3] There are 20% to 40% of cases with extrapulmonary involvement in developed & low-incidence countries, but the prevalence is even more higher in developing countries like India with a higher incidence of Tuberculosis. Diagnostic challenge is the emergence of (MDR-TB) which may occur if patients are infected with resistant strains or owing to inadequate medication or poor compliance for treatment adherence.

TB HIV Co-infection is a major public health concern. HIV infection increases the risk of developing active TB in latent infection. Ruling out TB before starting ART is a vital step in HIV treatment initiation. IRIS, Immune reconstitution Inflammatory syndrome, a paradoxical worsening of TB symptoms can occur in some patients living with HIV (PLHIV) with TB after starting ART. Therefore early & rapid diagnosis in TB HIV Co-infection is very essential for the disease management.[4]

In developing and resource-poor countries like India, acid-fast staining (Ziehl-Neelsen) and culture on conventional Lowenstein-Jensen (LJ) medium are the primary methods used for diagnosing *Mycobacterium tuberculosis*. One main disadvantage of the LJ culture method is the significant delay in detection time. Since the tubercle bacilli are slow-growing organisms, it typically takes more than a month for cultures to show growth, with only a few isolates growing more quickly. On an average, the mean time to detect *Mycobacterium tuberculosis* on an LJ medium is about 25 ± 9 days.

The Mycobacterial Growth Indicator Tube (MGIT) system, is a liquid culture medium that has decreased the mean time of detection by 18 ± 14 days. Implementation of microMGIT reader in any laboratory is easy to adapt, fluorometric technique which is not harmful to the technicians. The MGIT round-bottomed glass tube, manufactured by BD, contains 7ml of modified Middlebrook liquid medium (7H9). The bottom of the tube is embedded with a fluorescent compound. Oxygen (O2) in the liquid culture medium in the uninoculated tube quenches the fluorescence, hence no light visibility. As the bacteria grows it consumes oxygen, unmasks the fluorescence and helps the microMGIT reader to detect the fluorescence under UV light.[5]

The GeneXpert assay is a real-time molecular testing, a Nucleic Acid Amplification technique, cartridge based, processed within a single equipment with cutting edge technology. It can detect both MTb Complex Identification and Rifampicin resistance within two hours and this requires only minimal biohazard preparation.[6] The detection limit of gene X pert is about 131 bacilli/ml of specimen. Compared to culture the sensitivity & specificity of Gene X pert is high.[7,23] Rifampicin resistance mutations is an indicator of multidrug-resistant tuberculosis. However the treatment is different for Rifampicin susceptible and Rifampicin resistant Tuberculosis. Knowing the Rifampicin susceptibility before initiating the treatment is crucial for disease management. Hence WHO endorsed & recommends Gene X pert assay as the initial test for the diagnosis of Smear negative specimens, Extra pulmonary Tuberculosis & pediatric patients. It is very important to diagnose early & rapidly to treat the disease, thereby we can contribute to the overall TB control by reducing the global burden of the disease.[23]

Although RIF-Monoresistance is present in just five percent of RIF-Resistant strains, a significant percentage (95%) of RIF-Resistance is associated with simultaneous resistance for isoniazid. Hence the identification of RIF-Resistance will serve as an indicator for MDR-TB with significant degree of accuracy.[7] This can result in detection of more number of bacteriologically confirmed cases, rapid diagnostics and thereby no delay of treatment so that patients can rapidly receive second-line drugs instead of first-line drugs

The purpose of this study is to test and compare the diagnostic accuracy of two methods the liquid culture medium (MGIT) and GeneXpert, for the detection of Mycobacterium tuberculosis in smear-negative pulmonary specimens and extrapulmonary specimens. Additionally, the study aims to determine the anti-tubercular sensitivity pattern using the Mycobacterial Growth Indicator Tube & GeneXpert.

2. Materials and Methods

This prospective cross-sectional study was conducted in the Department of Microbiology and Respiratory Medicine at Stanley Medical College in Chennai from August 2014 to December 2014. The ethical committee at Stanley Medical College granted ethical and research clearance with number 201214052.

2.1. Specimen collection and processing

100 patients suspected of having tuberculosis infection were included in this study from the Department of TB and Chest Medicine. Among the samples collected, sputum (40), bronchoalveolar lavage fluid (9), pleural fluid (31), ascitic fluid (5), pus/empyema/abscesses (12), and lymph node aspirates (3). These specimens were processed to detect the presence of Mycobacterium tuberculosis using a microMGIT fluorescence reader, (which employs Mycobacterial Growth Indicator tubes) and GeneXpert technology. Around 3 to 5 ml of sputum samples and 5- 10 ml of Extra pulmonary samples were collected in a sterile container in duplicates one for MGIT culture and another for GeneXpert and transported immediately to the laboratory and decontaminated by NaOH – NALC decontamination method.

The procedure was carried out inside Class 2, B2, Biosafety cabinet. AFB staining was performed using Kinyon's method. The sample was also inoculated into an MGIT tube. Following the addition of 0.8 ml of reconstituted (OADC) supplement Oleic Acid, Albumin, Dextrose, and Catalase supplements, as well as an antibiotic mixture consisting of (PANTA) Polymyxin, Amphotericin B, Nalidixic Acid, Trimethoprim, and Azlocillin, The tubes were then incubated at 37°C for a period of minimum of six weeks, and the readings were taken from day two of incubation onwards using a microMGIT instrument **(Figure 1).** Positive tubes were confirmed for Acid-fast bacilli using Kinyon's method (**Figure 2**). Additionally, they were also subcultured onto Blood Agar Plates and Sabouraud Dextrose Agar to rule out bacterial and fungal contamination. For positive control, *Mycobacterium tuberculosis* H37Rv (ATCC 27294) was utilized. Positive tubes were further tested with a Rapid Card TBCID (MPT 64 Antigen) to differentiate *Mycobacterium tuberculosis* complex from MOTT (Mycobacterium other than Tuberculosis complex). The BACTEC MGIT SIRE Kit was used for susceptibility testing of the antibiotics Streptomycin, Isoniazid, Rifampicin, and Ethambutol.

Specimens were transported under strict aseptic conditions with triple-layer packing according to standard guidelines to the National Institute of Research in Tuberculosis, Chetpet, Chennai. GeneXpert which is a single-step semi-nested multiplex real-time PCR was done in NIRT. Clinical specimens were treated with lysis buffer and then approximately 2 ml of the treated specimen was added to GeneXpert cartridges. The barcoded Catridges were loaded into the instrument. The detection of MTBcomplex was identified using $IS6_{110}$ Primers and rifampicin (RIF) resistance was analyzed using the highly conserved sequence of rpoB gene.

3. Results

Among tuberculosis suspected patients 74% were males, and 26% were females**. (Table 1).**The commonest age group affected was between 41 to 60 years. **(**Error! Reference source not found.). A total of 100 specimens were studied among which 49 were smear-negative pulmonary specimens and 51 were extrapulmonary specimens which included both (smear-positive and negative**). (Table 3)** Specimens such as Sputum, BAL & Extra pulmonary specimens such as Pleural fluid, Ascitic fluid, Pus and Lymph node aspirates were included**. (Table 4)**

The overall positivity rate of Gene X pert was 26/100(26%) & MGIT was 17/100(17%). (**Table 5**) Among the 49smear negative pulmonary specimens MGIT detected 8/49(16.3%) and Gene X pert detected 10/49(20%) **(Table 6).** Among the 51 extrapulmonary samples MGIT detected 9/51(18.5%) and Gene X pert detected 16/51(31%) **(Table 7)**

Out of 17 MGIT-positive samples, 58% (10/17) were found to be MTB complex whereas 42% (7/17) were found to be Mycobacterium other than tuberculosis (MOTT)**. (Table 8)**

Out of the 10 *M. tuberculosis* complex (MTBC) detected, all the isolates were susceptible to Streptomycin, Isoniazid, Rifampicin, and Ethambutol. Among the 7 Mycobacterium Other Than Tuberculosis (MOTT) isolates all were resistant to Streptomycin, Isoniazid, Ethambutol & Rifampicin except one isolate which was found to be susceptible to Rifampicin alone.

GeneXpert detected only one Rifampicin resistance, the rest of 25/26 isolates in which Rifampicin resistance was not detected.

Figure 1: Bactec Micro MGIT Reader.

Figure 2: Showing suspended mycobacterial colonies in the MGIT tube.

Table 1: Gender distribution in pulmonary and extrapulmonary specimens

	Male	Female	Total
Pulmonary	36	13	49
Extra pulmonary	38	13	51

Table 2: Age distribution

Age	Pulmonary symptoms	Extra pulmonary	Total
Upto 20 yrs	4	5	9
21 to 40 yrs	9	22	31
41 to 60 yrs	21	18	39
Above 60 yrs	15	6	21
Total	49	51	100

Table 3: Category

Total Samples	Pulmonary specimens 49 (Smear negative)	Extra Pulmonary specimens (51)	
		AFB Smear Negative	AFB smear positive
100	49 %	46%	5%

Table 4: Specimen type

Category	Specimen Type	Number	Percentage %
Pulmonary (49)	Sputum	40	40
	BAL	9	9
Extra Pulmonary (51)	Pleural Fluid	31	31
	Ascitic Fluid	5	5
	Pus	12	12
	Lymph Node Aspirate	3	3
	Total	100	100

Table 5: Overall MGIT & Gene X pert positivity

Specimens	MGIT	Gene X pert
Pulmonary (49)	8	10
Extrapulmonary(51)	9	16
Total (100)	17/100(17%)	26/100(26%)

Table 6: MGIT & Gene X pert positivity in pulmonary specimens

S.No	Pulmonary samples (AFB Smear negative)	MGIT Positive	GeneXpert Positive
1.	Sputum 40 (82%)	6 (12%)	10 (20%)
2.	BAL 9 (18%)	2(4%)	0
Total	49	8/49(16.3%)	10/49 (20%)

Table 7: MGIT & Gene X pert positivity in extra pulmonary specimens

S.No	Extrapulmonary samples (AFB Smear positive & negative)	MGIT Positive	Gene Xpert Positive
1.	Pleural Fluid 31 (61%)	2 (4%)	4 (8%)
2.	Ascitic Fluid 5 (10%)	0	0
3.	Pus 12 (24%)	6 (12%)	9 (18%)
4.	Lymph Node Aspirate 3 (6%)	1 (2%)	3(6%)
Total	51	9/51 (18.5%)	16/51(31%)

Table 8: MTB complex & MOTT in MGIT positive isolates

	MTB Complex (TBCID positive)	MOTT (TBCID negative)	MGIT total positive
Smear Negative Pulmonary (49)	3	5	8
Extra Pulmonary (Smear Positive & Negative) (51)	7	2	9
Total (100)	10/17 (58%)	7/17(42%)	17

4. Discussion

In developing countries like India, smear microscopy is the primary method used to diagnose tuberculosis. According to INDIA TB report 2024, Only 21% of the patients suspected to have Tuberculosis were tested by Nucleic Acid Amplification Testing methods either by GeneXpert or TruNAAT.[2] This study focuses on diagnosing tuberculosis in smear-negative pulmonary specimens and extrapulmonary specimens using rapid diagnostic methods such as MGIT and GeneXpert. Generally, smear-negative pulmonary specimens and extrapulmonary specimens have a lower bacillary load.

According to **Table 1**, among the patients suspected of having tuberculosis, 74% were males and 26% were females. This finding is consistent with the research conducted by Kumari P)[9] and Jagadevi,[6] which reported that 68.8% and 57.7% of their subjects were males, while 31.2% and 42.3% were females, respectively. In the Indian context, males in the productive age group are typically the primary earners in their families and are more likely to go out for work, which increases their chances of getting infected.

According to **Table 2,** the most commonly affected age group in this study was between 41 and 60 years. This finding aligns with the results of Jalal D[10] and Jagadevi,[6] who reported mean age groups of 51 to 60 years and 30 to 49 years, respectively.

In this study, pulmonary specimens, including sputum and bronchoalveolar lavage (BAL), as well as extrapulmonary specimens such as pleural fluid, ascitic fluid, pus, and lymph node aspirates, were included **(Table 4).** The distribution of samples was as follows sputum constituted 40%, pleural fluid accounted for 31%, pus represented 12%, BAL made up 9%, ascitic fluid comprised 5%, and lymph node aspirates comprised 3%.

According to **Table 5,** Among the 100 tuberculosis-suspected patients, MGIT detected 17/100 out of 100 cases (17%). This detection rate is concordant with the study done by Gopi A,[11] who found a detection rate of 12/100 cases (12%) & also this finding correlates with the work of Sumit Goyal[5] who reported a detection rate of 18/101 cases (17.9%). In comparison, Hilleman[7] reported a much lower detection rate of 448/9558 cases (4.6%) & Jalal D[10] reported higher detection rate of 24/85(28%).

In this study, the Mycobacterium tuberculosis complex was identified in 10/17 positive cases (58%), while Mycobacteria other than tuberculosis (MOTT) accounted for 7/17 cases (42%). The percentage of MOTT detected by MGIT in this study is 42%, which is consistent with Hillemann[12] who reported 223/ 446 cases (50%).

The detection rates of MGIT in pulmonary specimens were 8/49 (16.3%) , However the positivity rate for MGIT in pulmonary specimens was lower compared to Shah R,[14] who reported 29/51 (56%) & Mulengwa DL[15] reported 310/1328(23%) respectively. In this study the positivity in extrapulmonary specimens were 9/51 (18.5%), according to the study of Uppe A[13] the reported detection rate of 42/150 (28%) for extrapulmonary specimens was slightly on higher side. This may be because of inclusion of more number of sterile fluids in this study.

The mean time of detection of MGIT was 9.6 days in this study. This correlates with Kumari P[9] in which mean time to detect Mtb complex is 9 days. However, in different studies, the time to detect Mycobacteria by MGIT falls in the range of 14 days according to Pfyffer GE.[16] The mean time of detection for MOTT (*Atypical Mycobacteria*) by MGIT is just 5.8 days, this is lesser than Pfyffer[16] in which it is 11.9 days, and also in the same study for *Mycobacterium avium complex* it is 7.2 days. The mean time to detect mycobacteria depends on the bacterial load in the specimen. Lower median TTD in present study might be due to less contamination, precise sample collection and inclusion of more clinically relevant cases. Lower mean TTD for sputum smear positive samples than smear negative samples were also reported in Kumari P.[9]

9% of cultures were contaminated with Gram-positive organisms, this correlates with Pfyffer GE[16] 6-8 %, & Hillemann[7] 7.4%.

Out of 17 MGIT positives, all 10/10(100%) of MTBC isolates were sensitive to Streptomycin, Isoniazid, Rifampicin and Ethambutol which correlates with Gopi A.[11] Among the 7 MOTT isolates, 6/7 were resistant to Rifampicin, streptomycin, Isoniazid and Ethambutol, only one isolate was susceptible to Rifampicin. This is in concordance with the study conducted by Gomathi NS,[11] in which atypical mycobacterial isolates were resistant to routine anti-tubercular drugs.

As per **(Table 5)**, Overall GeneXpert detected 26/100(26%) in this study, this is lesser than Iram S[18] in which 111/245 (45.3 %) & higher than Terzi Ha[19] in which it is 203/2082(9.7%). Among the 49 pulmonary specimens, Gene Xpert detected 10/49 (19.7%) which is lesser than Ioannidis P[20] in which it is 31/80 (38%) but higher than Terzi Ha[19] 175/1526 (11.4%), and in extrapulmonary specimens, GeneXpert detected 16/51(31.3%) as per (**Table 6)** which is higher than the study conducted by Jagadevi[6] & Terzi Ha[19] in which it is 23/241(9.54 %) & 28/556(5%) respectively for extrapulmonary specimens., The detection rate was higher for Pus 9/12(75%) and lymph node aspirates 3/3 (100%). All the lymph node aspirates were detected by GeneXpert in this study.

According to (**Table 6)** In this study the positivity of Xpert was much less among sterile fluids, Ascitic & Pleural fluids 4/36(11%), this correlates with Jagadevi[6] in which it is 8/144(5%), but it is lesser than Friedrich SO[21] in which the detection rate is 5/25 (25%). In Pleural & Ascitic fluid

samples, the positivity rate is much less, because of its paucibacillary nature.

All the 5 smear-positive Extra pulmonary specimens were detected by both GeneXpert & MGIT culture and all the isolates were MTB Complex. Among the 10 Mycobacterium tuberculosis complex isolates, Gene X pert detected 9/10 (90%). Both MGIT & GeneXpert commonly detected 9/10 (90%) of isolates among the MTB complex isolates. Gene X pert did not pick one isolate, Because Gene X pert is designed to detect the MTB complex using primer $IS6_{110}$. There are few studies in which not all the isolates can be picked by the primer $IS6_{110}$. In this case, it can be detected by the primer TRC4 according to S. Narayanan.[22] The sensitivity of Gene X pert can be increased by using both the primers of TRC4 and $IS6_{110}$.

Out of 100 isolates, 17 were detected by Gene X pert alone, and MGIT culture was negative, Gene X pert is a Nucleic Acid Amplification technique that is more sensitive than culture because very few DNA copies can get amplified.

8/100 (8%) of isolates were grown only in MGIT culture, but GeneXpert were negative. Among them 7 were Mycobacterium other than Tuberculosis complex (MOTT) and one isolate was MTB complex. Gene X pert is designed to detect only MTB complex, it is not designed to detect MOTT, because this does not have the primer to detect MOTT.

Among the commonly detected MTB complex isolates (9/100), Rifampicin susceptibility by MGIT culture and GeneXpert were 100 % concordant in this study.

Adapting microMGIT instrument is very well possible even in medium sized laboratory with proper Biosafety precautions, thereby we can increase the yield of mycobacterium including MOTT within shorter turn around time. And also we can determine the susceptibility testing of Antitubercular drugs which will help the clinicians to choose the right drug for the treatment. Emergence and the spread of MDR organisms can be prevented.[23]

Implementation of GeneXpert offers several advantages such as enhanced sensitivity in detecting Mycobacterium tuberculosis, faster results in less than 2 hours, comprehensive diagnosis by giving Rifampicin resistance result in a single test and early detection by a simplified process especially in a country like India.[24] By providing timely diagnosis to the immunocompromised patients like HIV will guide the clinicians to choose Anti tuberculosis treatment / Tuberculosis preventive therapy appropriately according to NACO guidelines. The disadvantage of the instrument is more expensive & it will amplify even the dead bacilli, so this cannot be used for the prognosis monitoring.

Gene Xpert has improved the efficacy of TB diagnostic process. The accuracy of Xpert may vary according to different diagnostic specimens and TB infection sites. Therefore, the selection of adequate and appropriate specimens is critical when using GeneXpert in identifying TB.[25]

5. Conclusion

Although the cost of the equipment, cost per sample & maintenance charges are higher for Gene Xpert it detects MTBC DNA & Rifampicin Resistance very rapidly in less than 2 hrs directly from the clinical specimens compared to all other methods. According to this study concurrent use of GeneXpert assay and Liquid culture by MGIT could increase the yield of Mycobacterium tuberculosis, particularly in smear-negative pulmonary & extrapulmonary specimens such as pus & lymph node aspirates. Atypical Mycobacteria constitutes 7/17(41%) among the positive cultures, it remains the eye opener for the clinicians to consider the atypical mycobacteria by MGIT culture for accurate diagnosis and appropriate treatment.

6. Source of Funding

None.

7. Conflict of Interest

None.

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