"ASSOCIATION OF BAL CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST GRADING ON THE BASIS OF CYCLE OF THRESOLD VALUE WITH TIME TO CULTURE POSITIVITY

By

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Dr. Bhagyashri Patil
MD RESPIRATORY MEDICINE
Professor and Head of Department,
Department of Respiratory Medicine,
J.N Medical College
Belagavi-590010

Date: 15/7/24
Place: Belagavi

Dr.(Mrs.)N S Mahantashetti MD (Paed.)
Principal,
J. N. Medical College,
Nehru Nagar,
Belagavi – 590010

PRINCIPAL
JAWAHARLAL NEHRU MEDICAL COLLEGE
BELAGAVI

KAHER
BELAGAVI
J.N. MEDICAL COLLEGE

Date: 15/7/24
Place: Belagavi

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Nehru Nagar, Belagavi- 590 010, Karnataka, INDIA

0831 – 2471350 0831 – 2470759 www.jnmc.edu principal@jnmc.edu

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Dr. (Mrs.) N.S. Mahantashetti.
Chairperson-Antiplagiarism Committee &
Principal,
J. N. Medical College, Belagavi.

To,
Reg. No. BR0121005
Postgraduate Student,
2021-22 Batch,
Department of Pulmonary Medicine
J. N. Medical College, Belagavi.

ETHICAL CLEARANCE CERTIFICATE

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
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Accredited 'A+' Grade by NAAC in (3rd Cycle) Placed in Category 'A' by MHRD (GoI)
JNMC INSTITUTIONAL ETHICS COMMITTEE
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref No.MDC/JNMCIEC/ 31 **Date: 06/10/2023**

To

REG.NO. BR0121005

PG Student in Respiratory Medicine
J. N. Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"ASSOCIATION OF BAL CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST GRADING ON THE BASIS OF CYCLE OF THRESHOLD VALUE WITH TIME TO CULTURE POSITIVITY",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee.

(Dr. Smita Sonoli)
Member Secretary
JNMC Institutional Ethics Committee
J.N.Medical College, Belagavi.

(Dr. Harsha Hegde)
Chairman,
JNMC Institutional Ethics Committee
J.N.Medical College, Belagavi

LIST OF ABBREVIATION

TB	Tuberculosis
WHO	World health organization
NTEP	National tuberculosis elimination programme
FIND	Foundation for Innovative Diagnostics
MDR	Multi-Drug Resistant
CBNAAT	Cartridge Based Nucleic Acid Amplification Test
PCR	Polymerase Chain Reaction
CT	Cycle Threshold
PCR	Polymerase Chain Reaction
BAL	Bronchoalveolar Lavage
AFB	Acid-Fast Bacillus
DST	Drug Susceptibility Testing
rpoB	RNA Polymerase Beta Subunit
HIV	Human Immunodeficiency Virus
HBsAg	Hepatitis B Surface Antigen
LJ	Lowenstein-Jensen
MGIT	Mycobacteria Growth Indicator Tube
IQR	Interquartile Range
SD	Standard Deviation
RNTCP	Revised National Tuberculosis Control Programme
DOTS	Directly Observed Treatment Short-course
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic acid
LPA	Line probe assay
NTM	Non tuberculous mycobacteria
MTB	Mycobacterium tuberculosis
DST	Drug susceptibility testing
NAA	Nucleic acid amplification
EPTB	Extra pulmonary tuberculosis
SMB	Sloppy molecular probe
PTB	Pulmonary tuberculosis

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ABSTRACT

Background and Objectives: Tuberculosis (TB) continues to be a major global health issue, with India bearing a significant burden of the disease. The emergence of drug-resistant TB and the need for rapid and accurate diagnostic methods underscore the importance of this study. This research aims to investigate the association between cycle threshold (CT) values from Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) and the time to culture positivity (TTCP) in patients with pulmonary and extrapulmonary tuberculosis. Specifically, the study focuses on bronchoalveolar lavage (BAL) samples, which have not been extensively researched in this context.

Materials and Methods: This hospital-based prospective observational study was conducted from October 2022 to October 2023 at KLEs Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belagavi. A total of 105 patients suspected of having TB were included in the study. Samples collected included BAL, sputum, pleural fluid, tissue, gastric lavage, cerebrospinal fluid (CSF), and lymph node aspirates. All samples were processed using the Xpert MTB/RIF or Xpert MTB/RIF Ultra assays to determine CT values and subsequently cultured using the BACTEC MGIT 960 system. The correlation between CT values and TTCP was analyzed using statistical methods.

Results: The study population had a mean age of 45 years, with a significant male predominance (71.43%). The analysis revealed a strong inverse correlation between CT values and TTCP, with lower CT values (indicative of higher bacterial loads) associated with earlier culture positivity. Specifically, high CT value categories (<16 cycles) showed the highest culture positivity rates (89.47%), whereas very low CT value categories (>28 cycles) had minimal culture positivity (8.33%). Additionally,

rifampicin resistance was found in 8.57% of the cases, with these cases showing higher culture positivity rates.

Interpretation and Conclusion: The study demonstrates that CT values from CBNAAT are reliable predictors of bacterial load and culture outcomes. Lower CT values are associated with higher bacterial loads and earlier culture positivity, making them valuable indicators for TB management. The findings emphasize the importance of using CT values to guide clinical decisions, particularly in the early initiation of treatment. The study also highlights the diagnostic value of both Xpert MTB/RIF and Xpert MTB/RIF Ultra assays in TB detection, with comparable efficacy in identifying culture positivity. These insights can inform TB control programs and improve patient outcomes by facilitating timely and appropriate treatment interventions.

Keywords: Tuberculosis, CBNAAT, Cycle Threshold (CT) Values, Time to Culture Positivity (TTCP), Xpert MTB/RIF, Xpert MTB/RIF Ultra, Bronchoalveolar Lavage (BAL), Drug-Resistant Tuberculosis, Bacterial Load, Diagnostic Methods.

INTRODUCTION

Tuberculosis is one of the major causative agent leading to infectious disease in over 10.6 million people worldwide with mortality of 1.3 million people.[1] The epidemiology of this infection varies substantially throughout the world with highest rates (300 cases per 100,000 individuals or higher) observed in India, and the islands of Southeast Asia and Micronesia. India alone accounts for major burden of the disease that is almost 60% of new TB cases worldwide.[2] According to the Global Tuberculosis Report 2022, India reported MDR/RR-TB prevalence rate of 316 per 100,000 population and an incidence rate of 8.5 per 1,00,000 population. These statistics highlight the high burden of drug-resistant TB in the country.[3] In a recent survey of 2020 says, an estimate of 38% global TB deaths are among HIV-negative people and for 34% in HIV-negative and HIV-positive patients were there in India alone.[4] The numbers are definitely alarming the healthcare system. The honourable prime minister Shri. Narendra Modi had taken initiative to eliminate tuberculosis by 2025. The programme is strengthened and now called as NTEP. This programme relies still on sputum microscopy for the diagnosis.[2]Liquid culture techniques are used for the early identification of mycobacterial detection and the confirmation of the disease, but it may take almost about 3-4 weeks to obtain the results. The conventional solid culture methods still takes longer time for results that is almost about 6-8 weeks. The current situation warrants an availability of reliable, standardised and user-friendly diagnostic method that enables the direct identification of the MTB complex and drug resistance to important drugs like rifampicin which is necessary for the accurate diagnosis of pulmonary tuberculosis and to detect resistance at the earliest. Cartridge Based Nucleic Acid Amplification test assay, a newer diagnostic method, uses nucleic acid amplification to identify MTB complex

DNA in sputum and rpoB gene alterations linked to RIF resistance. This henceforth serves as a standardized, highly specific, and sensitive diagnostic testing modality for detection of tuberculosis at the earliest with detection of drug resistance. Though CBNAAT is a gold standard for early diagnosis and detection of drug resistant tuberculosis, the mycobacterial load will be known by CT(cycle threshold) value of CBNAAT. The CBNAAT/ Xpert CT values provide a semiquantitative estimate of bacillary load. The bacterial load may be related to infectivity and transmission of the disease. Lesser the CT value should ideally yield earlier culture positivity. The time taken to culture positivity(TTCP) may give a clue about the aggressiveness and virulence of the bacteria. There have been few studies to show the substantial association between CT values and time to positivity for the culture in liquid culture.[5,6,7]

This study used CT values as a potential measure for mycobacterial burden and its association with the ability to predict bacillary load through time to culture positivity, primarily in BAL samples of patients with pulmonary tuberculosis, which has not received much research attention. In addition, sputum, extrapulmonary samples based on CT value and its association with culture positivity have also been studied in this study.

NEED OF THE STUDY

The correlation of the CT value and the time taken to show growth in culture may highlight the behavioural pattern of mycobacteria as an area of research. It may be correlated with the emergence of drug resistant tuberculosis with culture positivity. This study can highlight to consider the factors responsible for the bacillary burden and its relationship with resistance. The CT value and it's time to culture positivity remains unexplained area for the research. In few of the studies, sputum CBNAAT and TTCP is studied. In this study we are aiming to study the relationship of BAL CBNAAT and extrapulmonary sample CT values and its TTCP. This study can highlight the factors responsible for development of drug resistance and its association of CT values, indirect factors responsible for culture positivity, correlation with compliance of the drugs, correlation with past history of tuberculosis, occupational influences on compliance, social stigma, personal habits like alcoholism, smoking, tobacco chewing and its effect on culture positivity, lack of education and awareness of the disease. So this study is novel and maybe considered as an important study for future research purposes.

OBJECTIVE

PRIMARY OBJECTIVE:

- To study the association of cycle of threshold values (CT) of BAL CBNAAT with time to culture positivity values in patients with pulmonary tuberculosis.

SECONDARY OBJECTIVE:

- To study the association of CT values of CBNAAT with TTCP in patients with extrapulmonary tuberculosis and drug resistant tuberculosis.

REVIEW OF LITERATURE

Tuberculosis (TB) is an infectious disease which can be prevented and also be completely cured taking necessary precautions and adequate treatment. In spite newer treatment regimens and prompt diagnostic methods that strengthened NTEP & WHO initiatives,

In 2022, 7.5 million people were newly diagnosed with tuberculosis, and it remained the world's second biggest cause of mortality from a single infectious agent after COVID 19, accounting for twice as many deaths as HIV/AIDS. The worldwide number of people newly diagnosed with tuberculosis was 7.5 million in 2022, the highest figure since WHO began global TB tracking in 1995. More than 60% of these cases attributed to India, Indonesia and Philippines. Globally, an estimated 10.6 million people have been affected with TB in 2022, when compared to 10.3 million in 2021 and 10.0 million in 2020. The estimated TB incidence rate was 133 in 2022. In 2022, 30 high-TB burden nations accounted for 87% of global TB cases, with India accounting for the most (27%), followed by Indonesia (10%), China (7.1%), the Philippines (7.0%), Pakistan (5.7%), Nigeria (4.5%), and Bangladesh (3.6%). The impacted group consisted of 55% men and 33% women. Children accounted for 12% of global cases in 2022. In 2022, 4.1 million individuals got multidrug-resistant or rifampicin resistant tuberculosis (MDR/RR-TB), but only 1,75,650 were recognised and treated, accounting for roughly two in every five people. Approximately 1.6 million people with HIV died from tuberculosis, which is formally categorised as HIV/AIDS.[1]

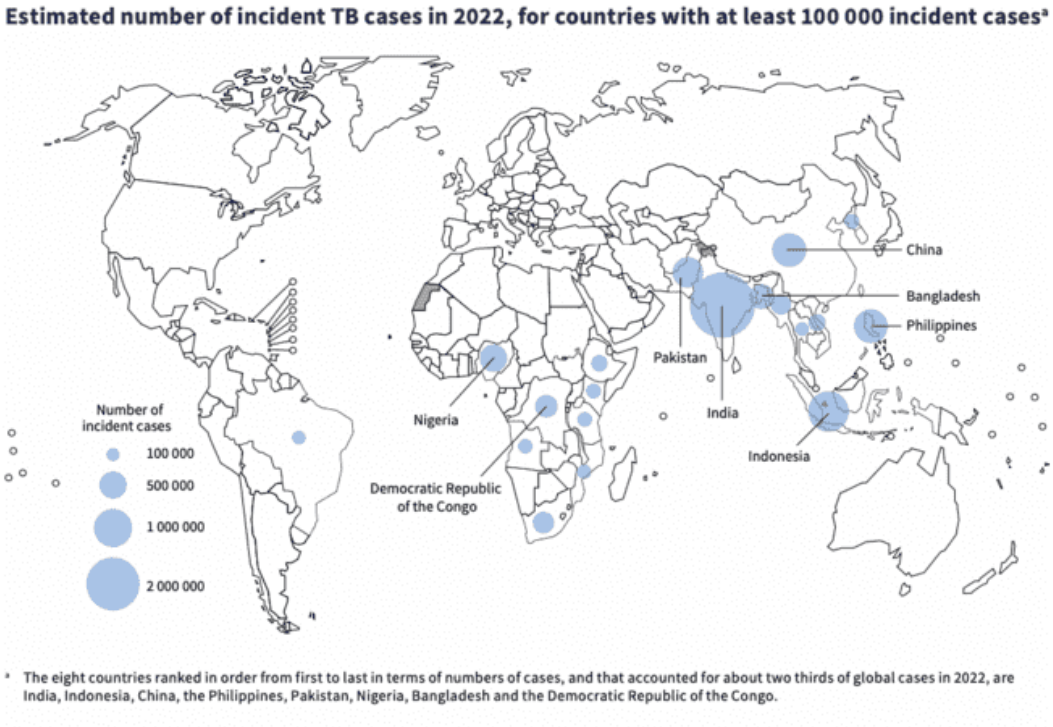


Figure 1 : Global incidence of TB cases in 2022.

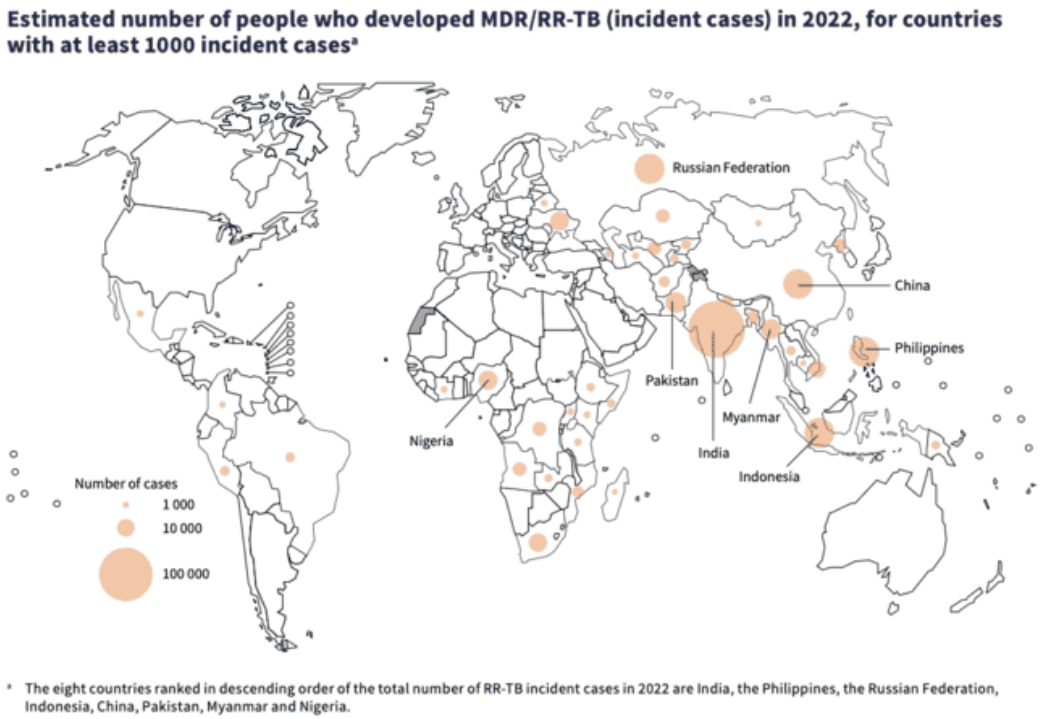


Figure 2 : Global incidence of MDR/RR-TB cases in 2022.

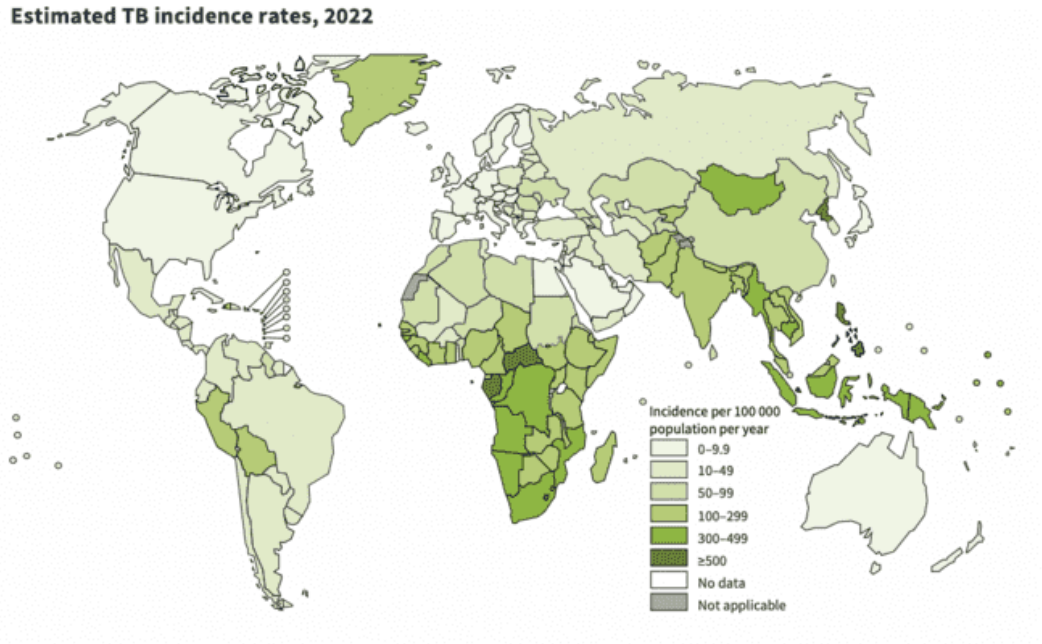


Figure 3: Global incidence rates of TB in 2022.

With the aim of improving TB in the country, Revised National Tuberculosis programme was launched in India in the year 1997 was later expanded to cover the entire country in 2006. Since then, RNTCP screened about 20 million symptomatic individuals annually for TB using microscopy and treated around 1.5 million people in one year. Rapid molecular diagnostics, including line probe assay and CBNAAT were introduced in 2009 and were implemented nationwide. LPA's are used for detecting drug resistance to first- and second-line anti-TB agents, including pyrazinamide. They are used to test culture isolates by indirect testing and AFB smear microscopy-positive specimens (first-line LPA) along with smear-positive and smear-negative sputum specimens (second-line LPA) by direct testing. LPA's detect mutations by binding of amplicons to probes which target the most common mutations (MUT probes) with first and second line LPA's or by the lack of hybridization in the corresponding WT probes which is defined as "inferred resistance". Development of coloured bands on the strip at the site of probe binding is seen post hybridization.[8,9]There are three types of line probe assay available.

The INNO-LiPA MYCOBACTERIA v2 is a line probe assay which simultaneously detects and identifies the genus mycobacterium and 16 different mycobacterial species.[10,11]The LiPA rpoB PCR detects MTB and rifampicin resistance which can performed on pulmonary and extra pulmonary samples.[11,12]The mycobacterium genus is identified by the genotype MTBDR +, which also detects potentially multidrug-resistant and extensively multidrug-resistant tuberculosis. Through mutations in the rpoB and katG/inh A (high/low isoniazid resistance) genes, it aids in the detection of MTB complex. Although national policy for diagnosis under RNTCP recommends direct sputum smear microscopy by Ziehl-Neelsen acid-fast staining/ Fluorescence Microscopy as a tool for primary case detection and for treatment monitoring in drug sensitive TB cases, it requires special laboratory tests to detect the resistance patterns and also to test drug sensitivity patterns which led to the use of molecular or culture based techniques. Molecular techniques like CBNAAT can provide results within 2 hrs which can prevent the delay in the diagnosis of MDR TB cases when compared to the conventional DST which take 4-6 weeks for the results. The RNTCP strengthening was achieved by establishing CBNAAT facility at every district level, which led to recent policy of sputum AFB with CBNAAT became the first and foremost approach for early detection of disease and resistance also.

Mycobacterium tuberculosis is a member of a MTB complex which consists of closely related mycobacteria. The MTB complex consists of the species M. tuberculosis, M. bovis, M. bovis Bacille-Calmette Guérin (BCG), M. africanum, M. caprae, and M. microti, all of which belong to the genus Mycobacterium along with few unvalidated species including M. orygis, M. mungi, M. canettii, and M. surricatae. In addition to the M. tuberculosis complex, the genus Mycobacterium, includes more than 200 other species, referred to collectively as non-tuberculous

mycobacteria or nontuberculous mycobacterium (NTM)[13].Tuberculosis is caused by rod shaped, weakly gram positive, non sporing , obligate intracellular aerobic bacteria called Mycobacterium tuberculosis which belongs to the family mycobacteriaceace.[14]The rods are 0.2–0.5 μm in diameter and 2–4 μm long. *These are obligate aerobic* bacteria which cannot survive without oxygen, explaining why they are found in the most aerated part of the lung i.e, upper lobes. It is an internal facultative parasitic bacterium that infects macrophages and it contains lipids such as mycolic acid in its cell membranes which is responsible for the virulence of the pathogen. It is a contagious pathogen which spreads through droplet by air borne transmission causing pulmonary and extra pulmonary tuberculosis which can cause serious forms of the disease.[15] Cell wall remains the distinguishing feature of mycobacterium tuberculosis which account for the unique 'acid-fast' staining of this bacteria. It refers to the ability of the cell wall components to form stable complexes with the primary stain and resist decolorization with harsh acid-alcohol or mineral acid.[16] Because of this, all mycobacterium species—not just M. tuberculosis—are referred to be acid-fast bacilli, and a sputum sample must contain 10^4–10^5 Bacilli/ml in order to be considered positive[17]

The various staining methods are as follows:

Ziehl-Neelsen staining:

The Ziehl-Neelsen staining procedure uses the principle of application of heat during the staining process which facilitates the uptake of the dye through the hydrophobic cell wall and envelope. It uses a carbolfuchsin stain, acid alcohol decolourizer, and methylene blue counterstain. Acid-fast organisms stain pink, while the background of debris stains blue. The ZN stain confirms the acid-fast property of

mycobacteria. This makes it more mycobacterial-specific as they reveal the 'acid-fast' characteristics of the cell wall. However, application of heat can also generate infectious aerosols for which it warrants a bio safety level 3 laboratory. Since not all laboratories have such capabilities, the Kinyoun procedure (which does not require the application of heat), is often used as a 'cold' acid-fast staining alternative.[16]However, the use of light microscopy for detection of AFB has its own set of limitations i.e., specimens must contain at least 10^4 colony forming units (CFU)/mL, the lower limit of detection for most light microscopes, to yield a positive smear.[16]

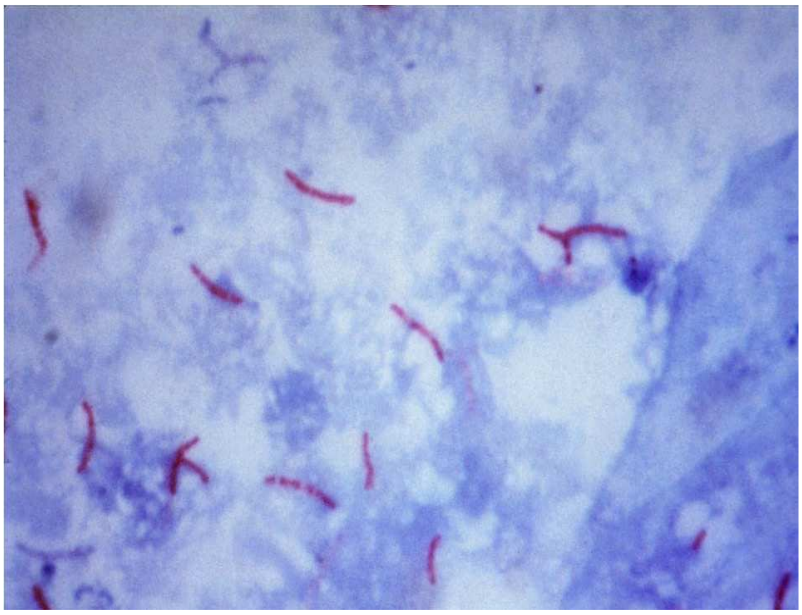


Figure 4: Zn stain showing MTB bacilli.

Fluorochrome (auramine or auramine-rhodamine) staining:

Fluorescence microscopy uses fluorochrome dyes (such as auramine-O or auramine-rhodamine) which permits more rapid assessment of a greater number of microscopic fields using a lower power objective lens there by improving sensitivity over the Ziehl-Neelsen by 10 fold to 10^3 colony forming units (CFU)/mL.[16]

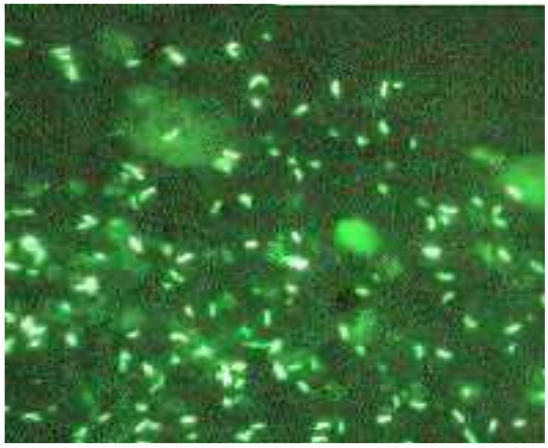


Figure 5: Fluorochrome showing MTB bacilli.

With staining methods having their own limitations like inability to detect MDR TB and also with the emergence of new strains, it warranted a simple, rapid and accurate diagnostic method for the earliest detection of drug resistant tuberculosis. Mycobacteria, unlike other bacteria have a chromosomal drug resistance and are often caused by mutations which are limited to the region of the genome. This resistance is capable of transmission to subsequent generations of bacteria and disrupt TB control and treatment programs.[18,19] Though staining is a backbone of the diagnosis, culture remains the gold standard for detection and confirmation of the disease. Unlike staining method, culture method requires as few as 10^1 to 10^2 bacilli/mL to be detected making it the most reliable tool for detection of TB. The sensitivity and specificity in sputum samples of roughly 80% and 98%, respectively. It is also utilized for species identification and drug susceptibility testing. Culture remains the most effective method for detection of *MTB*, as it can detect as few as 10^1 to 10^2 bacilli/ml[20–22]. These culture media are mainly categorised in to two groups i.e., solid media (egg- and agar-based) and liquid media. One benefit of solid media over liquid media is the ability to examine colonies that contain contaminants and mixed

cultures, while liquid media encourages mycobacteria to grow more quickly. Mycobacterial cultures cannot be cultured using standard bacteriology procedures because MTB develops slowly, taking 18–24 hours to generate a single bacterium. Furthermore, MTB development needs are such that it cannot thrive on straightforward, chemically specified substrate in primary isolation. Egg-enriched media supplemented with asparagine, glycerol and agar or liquid media enhanced with serum or bovine albumin are the only media that permit copious growth.[23,24] The type of specimen is the primary determinant of the medium selection. It is advised to use selective medium with contaminated (or potentially contaminated) specimens (sputum, abscess contents, bronchial washings, gastric lavage fluid, urine) since it contains antimicrobial compounds that stop growth by contaminating bacteria and fungi. While samples from typically sterile locations (bone marrow, tissue biopsy samples, cerebrospinal fluid and other bodily fluids, etc.) should be used with non-selective medium, according to studies.[23,24]

Egg-based media, including Ogawa and Lowenstein-Jensen (LJ) mediums, Agar-based media, like Middlebrook 7H10 and 7H11, and liquid media, like Middlebrook 7H9 broth, are the most widely used non-selective media. Agar-based media like Selective 7H11 (Mitchison's medium), which contains trimethoprim, amphotericin B, polymyxin B, and carbenicillin as a selective agent, and egg-based media like Gruft modification of LJ and Mycobactosel LJ are the most widely used selective media. Modified Middlebrook 7H9 broth and a combination of antimicrobial agents such as MB/BacT, ESP culture System II, and BACTEC MGIT 960 system are included in the liquid medium. One of the most popular automated technologies for quickly identifying mycobacteria in liquid media is the BACTEC MGIT 960 system.

Deland and Wagner devised a technique for semi-automated detection of bacterial metabolism in 1969 by detecting the 14CO2 released during growth and decarboxylation of 14C-labeled substrate in the growth medium. This radiometric approach was often employed for blood culture at the time, with the BACTEC 460 apparatus. This technology was first commercialised in 1980 for mycobacterial recovery from clinical specimens and medication susceptibility testing. The BACTEC 960 TB System was shown to yield 15-20% greater culture positivity of clinical specimens when compared to standard solid media such as LJ medium, with an average time-to-detection of positive growth from 8 to 14 days as opposed to 3 to 5 weeks on solid media.[25]The most reliable method for identifying drug-susceptible and drug-resistant tuberculosis is still drug susceptibility testing (DST), a traditional (phenotypic) culture-based drug susceptibility test. This approach determines the presence or absence of drug resistance by comparing growth on a drug-containing medium to growth on a control medium[22]. Four methods of drug susceptibility testing have been standardized. They include absolute concentration method, resistance ratio method, proportion method and BACTEC-460 radiometric method. [26]

The approach can be performed with either liquid or solid medium (the agar proportion method being the reference standard). But for liquid media, culture-based drug susceptibility testing takes minimum of 7 days, and for solid media, it takes a month.[22,27] *To combat the raising burden of drug resistant TB cases and to minimise the time to detect MTB species, WHO has initiated rapid genotypic diagnostic methods called nucleic acid amplification tests like* Xpert MTB/RIF assay and Xpert MTB/RIF Ultra assay which yield results within 2 hrs. Nucleic acid amplification (NAA) testing is based on the amplification of a specific target RNA or DNA sequence, which can be discovered using a nucleic acid probe.[28,29] The sensitivity &

specificity of NAA are 95 and 98 % in AFB smear-positive respiratory specimens, respectively, and 75 to 88 percent and 95 percent, respectively, in smear-negative specimens.[21,22,23]When clinical and epidemiologic conditions are met, a positive NAA test lends support to the diagnosis of tuberculosis (TB); smear positivity in addition to a positive NAA is regarded as adequate for TB diagnosis.[33–35] This test employs single-use plastic cartridges with numerous chambers preloaded with liquid buffers and lyophilized reagent beads required for sample processing, DNA extraction, and heminested rt-PCR.

It works based on a molecular beacon technology[36,37] to detect DNA sequences amplified in a hemi-nested RT-PCR assay. Five different nucleic acid hybridization probes are used in the same multiplex reaction.[36] Each probe is complementary to a different target sequence within the rpoB gene of rifampicin-susceptible M. tuberculosis and is labelled with a differently coloured fluorophore. Together, these overlapping probes span the entire 81 base pair core region of the rpoB gene[36] and rpoB sequences. A mutation in these regions inhibits hybridization, allowing the probe's conformational integrity to be preserved in the non-fluorescing state. Thus, a mutation anywhere in the core region of the rpoB gene causes either delayed onset (partial inhibition) or full suppression of fluorescence of the associated molecular beacon. This prototype technique detects rifampicin resistance with great sensitivity and specificity, using genomic DNA or culture lysates from many clinical strains of M. tuberculosis.[36] This test approach can be utilised immediately on clinical specimens, either fresh sputum samples or sputum pellets, obtained after decontamination and concentration of the sputum.[38]The test material is then treated with a sample reagent containing sodium hydroxide and isopropanol, mixed by hand or vortex, and incubated for 15 minutes at room temperature. After incubation, 2 ml

of the treated sample is added to the cartridge, and the run begins. The test platform uses a sonic horn that inserts into the cartridge base, causing ultrasonic lysis of the bacilli and liberation of the genetic material.[36] The assay then amplifies a 192 bp segment of the rpoB gene using a hemi-nested rt-PCR reaction. Mycobacterium tuberculosis is detected by the five overlapping molecular probes (probes A–E) that collectively are complementary to the entire 81 bp rpoB core region. If a beacon fails to attach to the matching sequence or binding is delayed, the sample may be resistant to rifampicin.[36,38]The number of positive beacons and the timing of their detection, as well as the outcomes of sample processing controls, CBNAAT gives result as → No TB, TB detected & rifampicin resistance detected, TB detected & no rifampicin resistance detected, TB detected & rifampicin resistance indeterminate and an invalid result.[38]

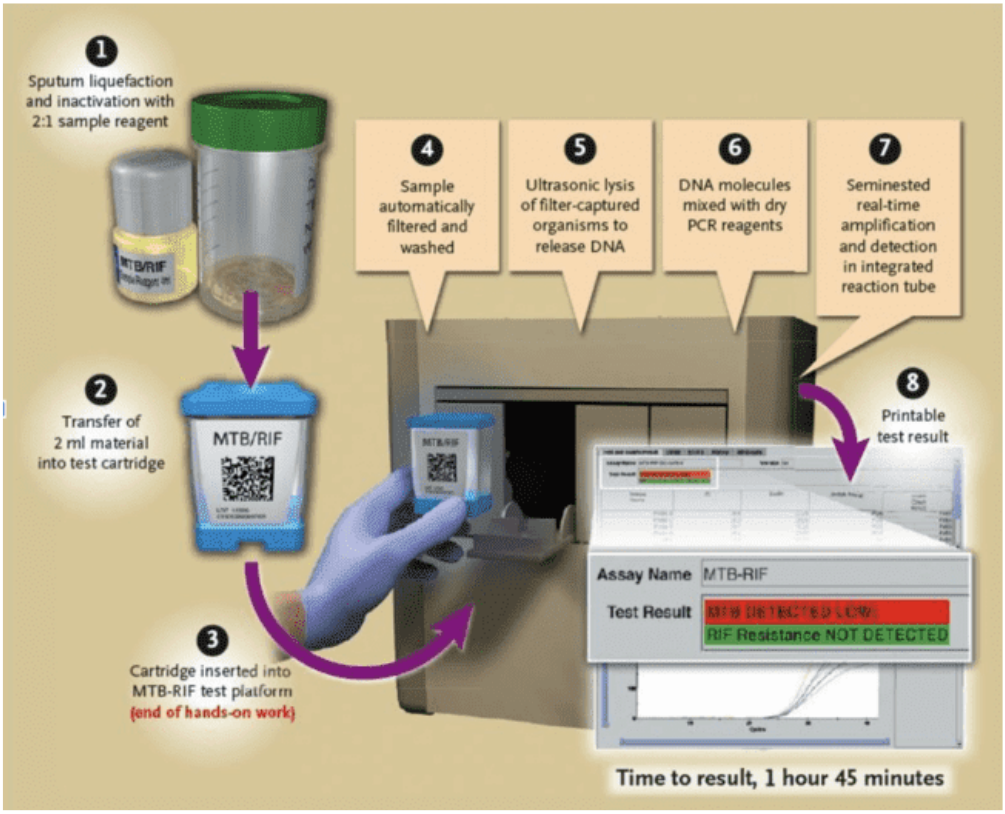


Figure 6: Xpert MTB/RIF Assay procedure.

There were various studies which tested GeneXpert on different types of samples. Based on the review of studies published in Xpert MTB/RIF implementation manual 2014, when Xpert MTB RIF assay was used as an initial diagnostic test replacing smear microscopy on sputum sample, it achieved a pooled sensitivity of 88%[38] and a pooled specificity of 99% which included 22 studies. When used as an add-on test following a negative smear microscopy result, Xpert MTB/ RIF yielded a pooled sensitivity of 68% and pooled specificity of 99% which included 23 studies. In people living with HIV, the pooled sensitivity of Xpert MTB/RIF assay was 79%.[38] When used in diagnosing EPTB, taking culture as the reference standard, pooled sensitivity of MTB/RIF assay in lymph node tissues or aspirates was 84.9% in 14 studies. The pooled sensitivity in gastric fluid was 83.8% and in other tissue specimens it was 81.2% [38].In csf, the pooled sensitivity of MTB/RIF assay compared with culture as a reference standard was 79.5%, whereas in pleural fluid, the pooled sensitivity of Xpert MTB/RIF as compared against culture was 43.70%[38]. Irrespective of the assay used, Pleural fluid remained to be a suboptimal sample for the bacterial confirmation of pleural TB & pleural biopsy is the preferred sample. The sensitivity of Xpert MTB/RIF in testing samples of pleural fluid is very low. Xpert MTB/RIF for diagnosing EPTB is still comparatively weak.

In a study conducted by Scott et al, it showed that when culture was used as the reference, Xpert MTB/RIF's overall sensitivity was 59% and specificity was 92% for pus, 80% for lymph node aspirates, and 51% for fluids (ascitic, 59% and pleural, 47%).[39]

In the current study we used CBNAAT in HIV positive pulmonary tuberculosis to determine the bacillary burden, identify resistance and its association with culture based on a study conducted by Sumangala et al[40] where it showed that

GeneXpert helped in increased early case detection in lesser time to diagnose pulmonary TB in people living with HIV as compared to fluorescent microscopy and also to detect rifampicin resistance with high specificity and also can be used for screening for MDR-TB for starting anti-tubercular treatment early.

In a study done by Bernard et al, the bronchoalveolar lavage and aspirates were be subjected to Xpert assay. Study revealed that bronchoalveolar lavage fluid showed sensitivity of 92.3% and specificity of 87.7% for detecting MTB.[41] Apart from providing information about MTB detection and rifampicin resistance, the GeneXpert assays also provide Xpert cycle threshold (CT) findings which provide a semi-quantitative estimate of bacillary load.

Understanding CT Values

The findings of the observed fluorescence signals and embedded calculation algorithms are produced by the software known as the Cartridge Based Nucleic Acid Amplification Test (CBNAAT). The programme offers semi-quantitative data as well as qualitative results, giving details on the bacterial load in the specimens that were examined. The interpretation of semi-quantitative results is predicated on the Cycle threshold (CT) values of targets unique to Mycobacterium tuberculosis (MTB) as shown in **figure 7**.The fluorescence released is feeble and appears as background noise when the cycle number is low. A line can be utilised to separate the relevant signal from the background level after the fluorescence growth is linear. We refer to this as the threshold line. The point at which a response achieves a fluorescence intensity above background levels, or the detection threshold, is shown by the threshold line as shown in **figure 8**. The number of PCR cycles in which the amplification curve crosses the threshold line is known as the cycle threshold (CT).

A detectable CT value in a PCR reaction indicates the presence of target DNA. There will be no CT value if the target DNA is absent since there won't be any rise in fluorescence as shown in figure **9&10**. The CBNAAT programme identifies four levels of MTB detection by analysing load base using CT values which are high, medium, low, and very low. The MTB detection data are also presented in a semiquantitative way. The mean CT value of each MTB-specific probe in the sample is used to calculate this quantitation. The result is shown as MTB high because an early CT value of less than 16 will suggest that the specimen contains more target molecules. An MTB identified medium has a mean CT value between 16 and 22. CT values between 22 and 28 are considered low MTB detection. When the mean CT value is higher than 28, MTB detection occurs very low as shown in **figure 11.**[42]

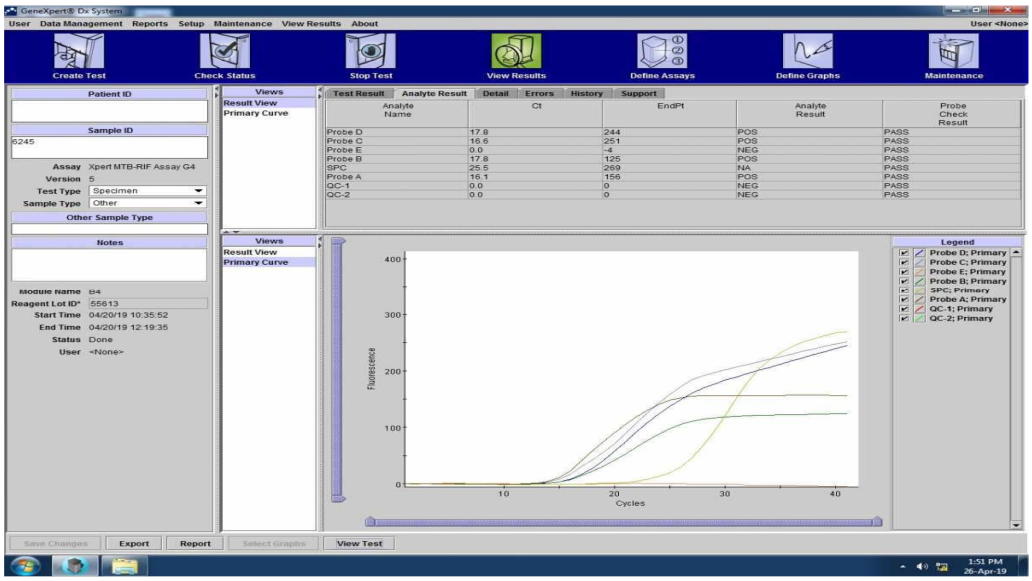


Figure 7: CT values from CBNAAT results can be seen in the second column

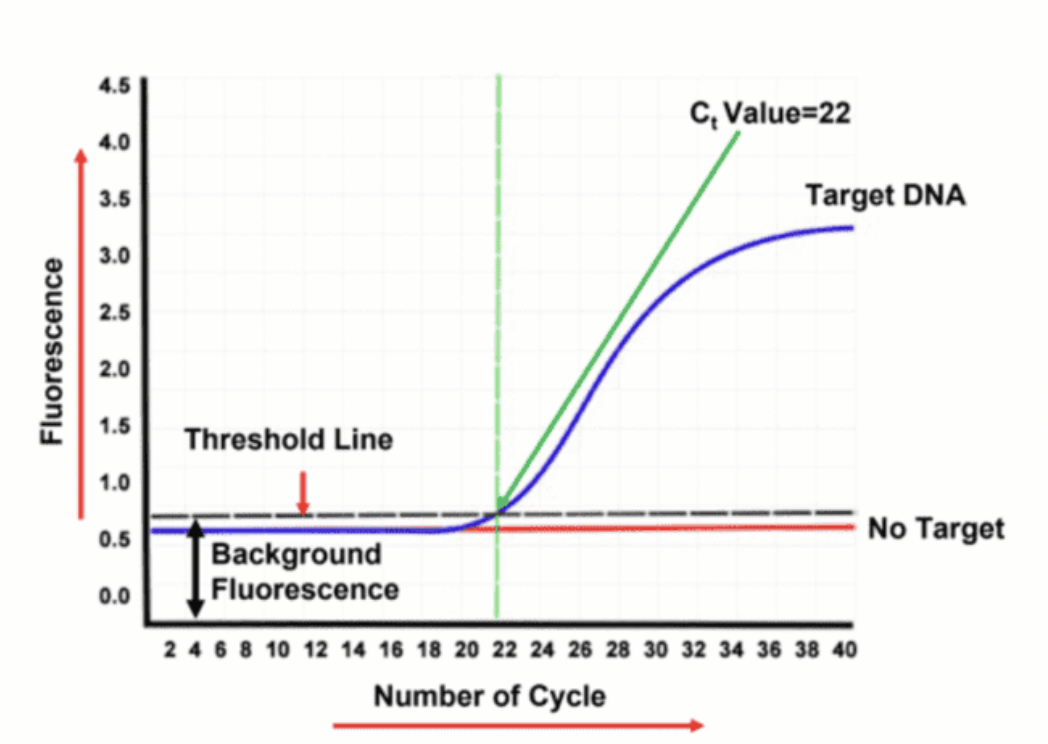


Figure 8: Signal detection and quantitation

Test Result | Analyte Result | Detail | Errors | History | Support

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	0.0	3	NEG	PASS
Probe C	0.0	9	NEG	PASS
Probe E	0.0	-1	NEG	PASS
Probe B	0.0	7	NEG	PASS
SPC	25.3	235	PASS	PASS
Probe A	0.0	0	NEG	PASS
QC-1	0.0	0	NEG	PASS
QC-2	0.0	0	NEG	PASS

Figure 9: CT values for all the probe values are zero

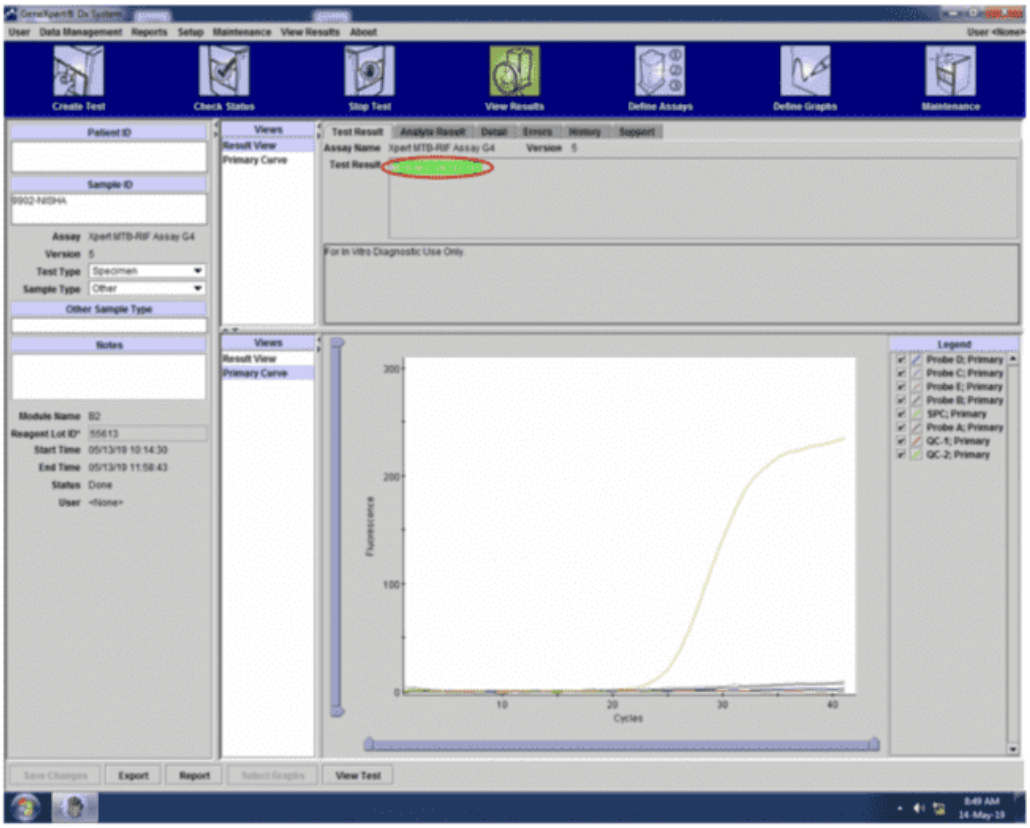


Figure 10: Result shown as MTB not detected since CT values for all the probe values are zero

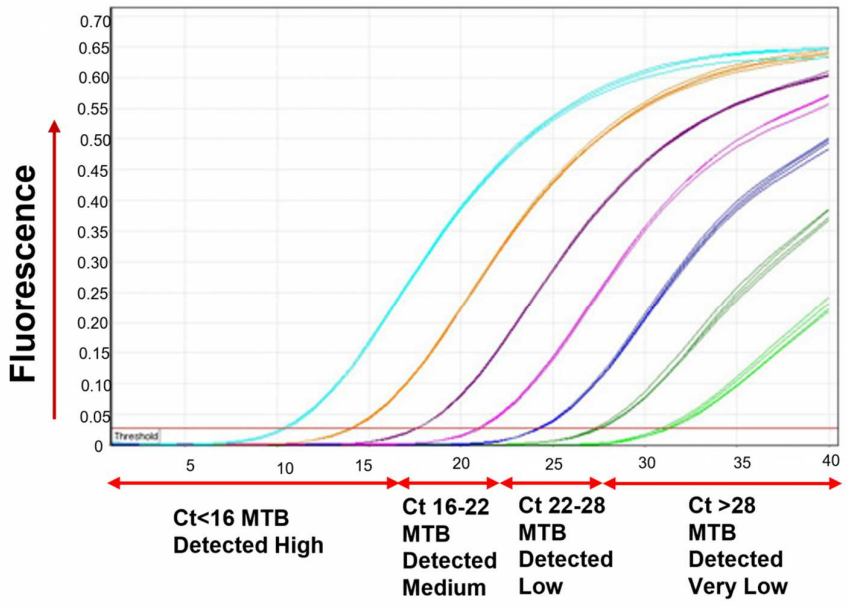


Figure 11: Load base analysis based on CT values

Nevertheless, the test was found to have a restricted ability to identify mutations linked to rifampicin resistance in mixed samples[43,44] and according to certain studies, it has a reduced ability to identify rpoB C533G mutations that are accountable for certain RIF-R instances.[45]Sometimes delays in the real-time signal produced by assay probes D and E cause Xpert to provide false-positive RIF-R calls for paucibacillary samples.[46] There have also been reports of its incorrect identification of a quiet mutation, rpoB F514F, as causing RIF-R. [46]

To overcome these limitations GeneXpert Ultra assay was developed.

GeneXpert Ultra assay

The cartridge loading and sample treatment procedures were the same as for Xpert assay.[38] In a nutshell, the difference is commercial sample reagent (SR; Cepheid, Sunnyvale, CA) containing sodium hydroxide and isopropanol is combined at a 2:1 ratio with each sample (clinical sample, spiked sputum, or cultivated M. tuberculosis CFU). Following a 15-minute incubation period during which shaking was sometimes applied, the mixture is then delivered to the cartridge's sample loading chamber for automated processing. The real-time signal generated from the probes targeting the multicopy IS6110 and IS1081 genes confirmed the presence of M. tuberculosis.[47–49] The presence of the wild-type or mutant RRDR DNA sequence is determined using the melting temperature (Tm) values produced by the rpoB SMB probes. For ultra's semiquantitative function, the cycle threshold (CT) of the first positive rpoB probe is also employed. The semiquantitative classifications of Xpert high, medium, low, and extremely low are comparable with Xpert assay. However, in GeneXpert ultra, a second semiquantitative "trace" category is included. The trace result indicated samples that tested positive for M. tuberculosis because they had molecular signals from IS6110 and/or IS1081

(CT ≤ 37) but no signal from at least three rpoB SMB's. Although the GeneXpert limit of detection was 115 CFU/ml, the new feature and trace category have dropped the test's limit of detection to 15.6 CFU/ml[50]. A customised version of GeneXpert Diagnostics software has been used to accomplish automated TB detection and susceptibility to RIF. During the second amplification phase, real-time PCR results revealed the presence of M. tuberculosis. For non-"trace" positive samples, post-PCR melting temperature (Tm) analysis was performed.

In a study conducted by Dorman et al[50], which had 462 pulmonary tuberculosis patients with sputum that tested positive for the disease on culture; Xpert Ultra and Xpert had sensitivity rates of 88 and 83 percent, respectively. [50]

In one review conducted by Zifodya et al [51] Nine studies were assessed to determine the diagnostic accuracy of Xpert ultra and Xpert MTB/RIF for the identification of pulmonary tuberculosis and rifampicin resistance in adults (seven of which were conducted in high-burden countries).

When compared to Xpert MTB/RIF, Xpert ultra showed a better sensitivity but a poorer specificity for TB identification. Combined Xpert ultra sensitivity and specificity among over 2800 culture-positive patients were 91% and 96 %, respectively, whereas Xpert MTB/RIF sensitivity and specificity were 85 and 98 percent, respectively. Pooled sensitivity and specificity for Xpert ultra were 78 and 96%, respectively, among smear-negative, culture-positive individuals, while sensitivity and specificity for Xpert MTB/RIF was 61 and 99 percent. The pooled sensitivity and specificity for Xpert ultra among HIV-positive individuals were 88 and 93 percent, respectively, whereas the corresponding values for Xpert MTB/RIF were 75 and 99 percent. The two tests' sensitivity and specificity for rifampicin resistance detection were comparable.[51] In our study, CT value is correlated with time to culture

positivity to know the significance of bacterial burden and it's correlation with other parameters also.

A significant inverse correlation was found between the cycle threshold (CT) values from cartridge-based nucleic acid amplification tests (NAATs) and the bacillary burden seen in sputum smear microscopy in a prospective observational study by Mohanraj et al. that assessed the usefulness of CT values as indicators of sputum bacillary burden in patients suspected of having tuberculosis (TB). Higher bacillary loads were associated with high and medium CT categories, and the mean CT value among Xpert positives was 22.18±6.69 cycles. Only samples in the high and medium CT categories in this study shown a moderate association between the CT of CBNAAT and the amount of time it took to show positive cultures; samples in the low and very low CT categories only demonstrated a weak link. Only a medium category or CT was statistically significant, despite the fact that high and medium category CT demonstrated modest connection.

One of the study's limitations is that, in contrast to our study, which combined the use of Gene Xpert and culture on a single sample, the investigation used separate samples for each.[5]

In a Retrospective cohort study conducted by Prakash et al, to investigate the relevance of cycle threshold (CT) values and to determine the correlation between CT values and culture growth outcomes in bronchial samples, pleural samples and others (CSF, lymph node biopsies, tissue samples) of suspected cases of Tuberculosis. This study revealed a strong inverse correlation between CT values and culture growth. It was interpreted that samples belonging to high CT value category yielded high culture positivity rates when compared to other categories and was opined that they can be initiated on treatment indefinitely without waiting for culture reports. Whereas the

sample belonging to very low CT category, it was advised to wait for culture reports before initiating treatment due to its poor positive culture yields seen in the study. However, in cases of samples which belonged to medium and low CT category, strong clinical correlation was advised before initiating the treatment. CT values were found to be effective predictors of culture positivity. [6]

In a cross sectional study conducted by Irene najjingo et al[7] to evaluate the mycobacterial load of sputum samples using culture and compare the CT values with smear microscopy across 5 referral hospitals in Uganda. The study revealed that the median days to culture positive were 15,13, 9 & 7 days respectively in very low, low, medium & high CT with cut off CT values being >28.01cycles, 22.01–28.0 cycles,16.01–22.0 cycles, <16.0 cycles for the above mentioned categories. Furthermore, study revealed a weak correlation of 0.37 between the Xpert CT values and MGIT TTCP.

In summary, genotypic methods offer rapid and specific detection of TB and known drug resistance mutations, making them suitable for quick clinical decision making, whereas phenotypic methods, even though are slower yet they provide comprehensive resistance profiles and are essential for confirming resistance and mono & polydrug resistant patterns which helps to treat cases. Combining both approaches in previous few studies in TB diagnosis.

Hence, in our study we aimed to study the correlation of Xpert CT values as a measure of bacillary burden with time to culture positivity primarily in BAL samples and also extrapulmonary samples. In this study, out of the 105 samples which are included, few were processed on Gene Xpert MTB RIF assay and few on Gene Xpert ultra to see if there would be any difference in link between culture positivity and CT value, which was also not done in earlier research.

MATERIALS AND METHODS

A one-year hospital based prospective observational Study from October 2022 to October 2023 at KLEs Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belagavi.

In this study, after taking thorough history, all relevant samples from suspected TB and EPTB patients i.e., BAL, sputum, pleural fluid, tissue, gastric Lavage, CSF, lymph node aspirate who visited outpatient or got admitted in the KLES Dr Prabhakar Kore Hospital & Medical research centre, Belagavi, and have fulfilled the inclusion criteria have been collected. The sample has been decontaminated using sample reagent and centrifuged for 15 minutes to concentrate it. Next, the Xpert MTB RIF/Xpert MTB RIF Ultra assay was used to examine the sample. Following MTB detection confirmation, the samples underwent additional processing in preparation for BACTEC MGIT 960 liquid culture. Weeks to positive on culture refers to the amount of time that elapsed from the time the sample was inoculated into the MGIT tube until it began to grow on the culture.

To rule for contamination, all positive colonies were cultivated on blood agar. For samples processed with the Xpert MTB RIF assay, a semi-quantitative estimate of bacilli concentration was taken into consideration. This was determined by the range of CT High→ <16 cycles, Medium→ 16-22 cycles, Low →22-28 cycles), and Very low→ >28 cycles.

In contrast, semi-quantitative estimates of bacilli concentration were selected for samples processed on the Xpert MTB-RIF Ultra based on the range of rpoB CT values. Specifically, CT values between 15 and 18.9 were classified as CT-HIGH, 19 to 24.9 as CT-MEDIUM, 25 to 28.9 as CT-LOW, and 29 to 40 as CT-VERY LOW. In the presence of the TB detection probe signal (CT $\leq$ 37) and no more than

one rpoB SMB-positive signal (CT ≤ 40) along with the presence or absence of the IC signal, the result was considered as MTB trace detected but RIF resistance Indeterminate.

This study was approved by the department of ethical clearance committee and college dissertation and research committee, J. N. Medical College, Belagavi.

STATISTICAL ANALYSIS

The data obtained was coded and entered into Microsoft excel Worksheet(Annexure III). The data was analysed using statistical software SPSS version 20.0. Continuous variables were analysed for normality by the Shapiro Wilk test. The data was expressed in terms of mean± standard deviation (SD) for the data that followed normal distribution and the data which followed skewed distribution was expressed as median and interquartile range (IQR).

Depending on distribution of data, the comparison of continuous data was done using Mann Whitney U test and in case of more than two values Kruskal Wallis test was used to compare the data. The correlation between TTC and mean CT count was done using Spearman's correlation coefficient (ρ). At 95% confidence interval (CI), a probability value ('p' value) of less than or equal to 0.050 was considered to be statistically significant.

SAMPLE SIZE CALCULATION

The number of bronchoscopies done at the inpatient and outpatient levels at KLES Dr. Prabhakar Kore Hospital & Medical Research Centre, Belagavi, over the previous five years, as well as the prevalence rate of tuberculosis, were used to establish the sample size.

The minimum sample size formula used was

$$n = \frac{z_\alpha{}^2 P(1-P)}{d^2}$$

where P is the prevalence rate and d is the percentage likely difference in the prevalence. z α is linked with the level of significance. For 5% level of the significance z α = 1.96. The parameter considered in the calculation is the prevalence rate of PTB cases. With P = 48% and d = 20% of P = 9.6%, the sample size is 105.

Sample size is Calculated at 95% confidence interval with 20% tolerable error and 10% attrition rate.

INCLUSION CRITERIA:

1. All Cases of PTB with BAL for CBNAAT +VE
2. All Cases of PTB with Sputum for CBNAAT +VE
3. All cases of Extra pulmonary TB with CBNAAT +VE
4. Drug resistant cases with CBNAAT +VE
5. Age 18 years and above

EXCLUSION CRITERIA:

1. Age less than 18 years.
2. Failure to provide informed consent

RESULTS

TABLE 1 : SEX DISTRIBUTION

GENDER	PERCENTAGE
MALE	71.43%
FEMALE	28.57%

Total of 105 patients in this study met the eligibility criteria. Mean age was 45 years. Majority of our patients were males, 75 out of 105 patients(71.43%)whereas females were 30 out of 105 patients (28.57%).

GRAPH 1: SEX DISTRIBUTION

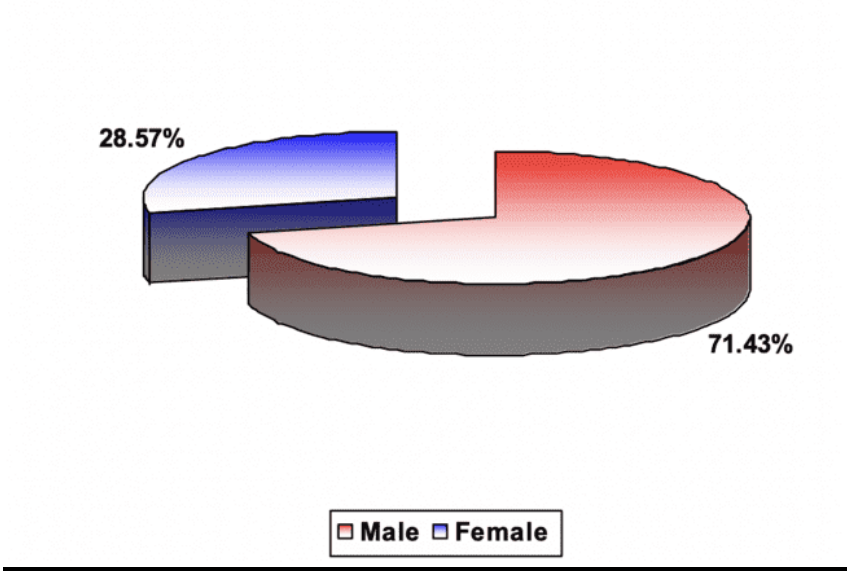


TABLE 2: AGE DISTRIBUTION

AGE GROUP (years)	DISTRIBUTION (%)
18 to 20	2.86%
21 to 30	19.05%
31 to 40	20%
41 to 50	19.05%
51 to 60	17.14%
61 to 70	17.14%
71 to 77	4.76%

The mean age of our study population was 45 years, with minimum being 18 and maximum being 77 years. People belonging to 18 to 20 years were 2.86%, 21 to 30 years were 19.05%, 31 to 40 years were 20.00%, 41 to 50 years were 19.05%, 51 to 60 years were 17.14%, 61 to 70 years were 17.14% and 71 to 77 years were 4.76% respectively.

GRAPH 2: AGE DISTRIBUTION

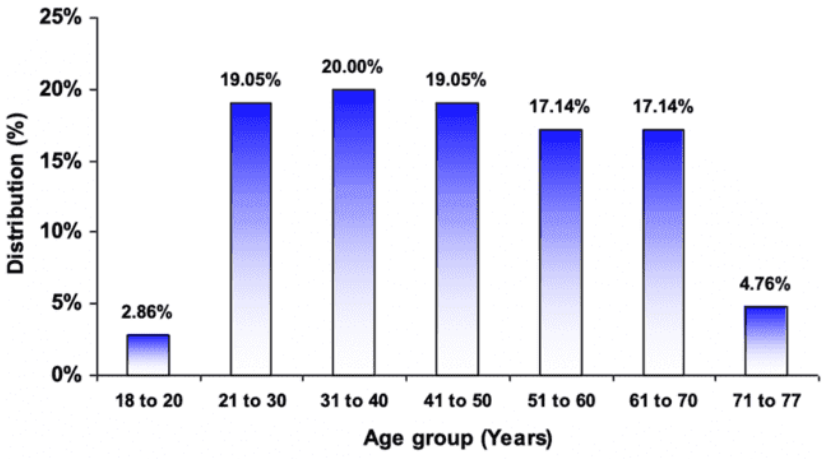


TABLE 3: BMI DISTRIBUTION

BMI Category (kg/m^2)	Percentage (%)
<18.50	32.28%
18.50 to 22.9	0.95%
23.00 to 24.99	45.71%
25.00 to 29.99	14.29%
≥30.00	6.67%

In our study, 45.71% of individuals belong to BMI 23.00 to 24.99 kg/m^2, the highest percentage among the categories. A significant portion, 32.28%, belong to BMI < 18.5099 kg/m^2, while only 0.95% belong to BMI 18.50 to 22.99 kg/m^2. patients that belong to BMI 25.00 to 29.99 kg/m^2 group and BMI ≥ 30.00 kg/m^2 account for 14.29% and 6.67% of the study population, respectively. The mean BMI of our study population was 20.22 with minimum being 12.6 kg/m^2 & maximum being 33.8 kg/m^2.

GRAPH 3: BMI DISTRIBUTION

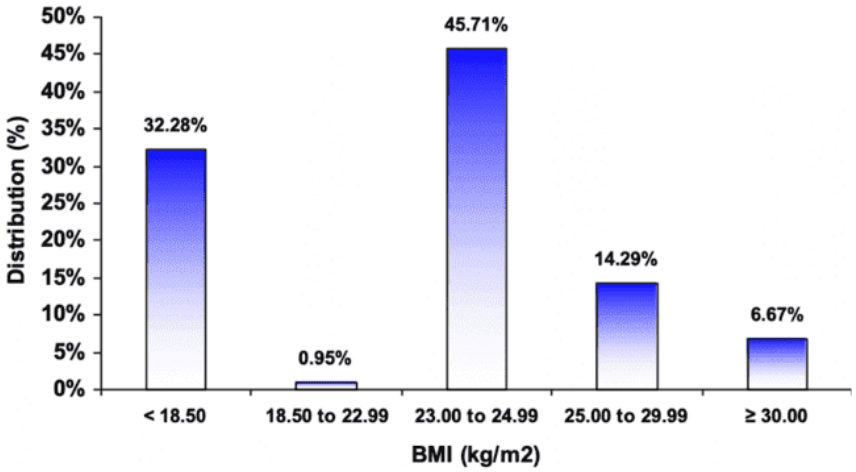


TABLE 4: COMORBIDITIES DISTRIBUTION

CATEGORY	DISTRIBUTION(%)
COMORBIDITIES	62.9%
NO COMORBIDITIES	37.1%

In our study population, 66 patients had comorbidities(62.9%) and 39 patients(37.1%) had nil comorbidities.

GRAPH 4: COMORBIDITIES DISTRIBUTION

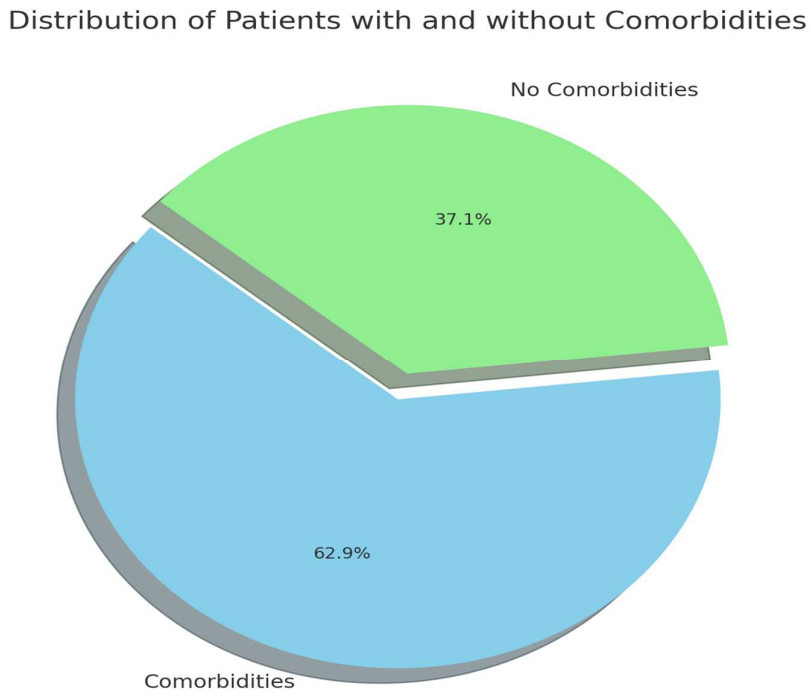


TABLE 5: COMORBIDITIES

COMORBIDITIES	DISTRIBUTION	%
T2DM	24	22.8
HTN	9	8.5
HTN/T2DM	3	2.8
HYPOTHYROIDISM	1	0.9
HIV	7	6.6
RIF RESISTANCE	9	8.5
H/O PTB	11	10.4
HBsAg+	2	1.9
ABSENT	39	37.1%
Total	**105**	**100.00**

In our study population, 24 patients(22.86%) had T2DM being the highest and 9 patients(8.57%) had HTN and 3 patients(2.86%) had both HTN&T2DM. Only one patient had Hypothyroidism . There were 11 patients(10.4%) with past history of TB in the current study. HIV positive status was seen in 7 patients(6.6%) and 2 patients had HBsAg status. 39 patients(64.76%) did not have any comorbidities.

GRAPH 5: COMORBIDITIES

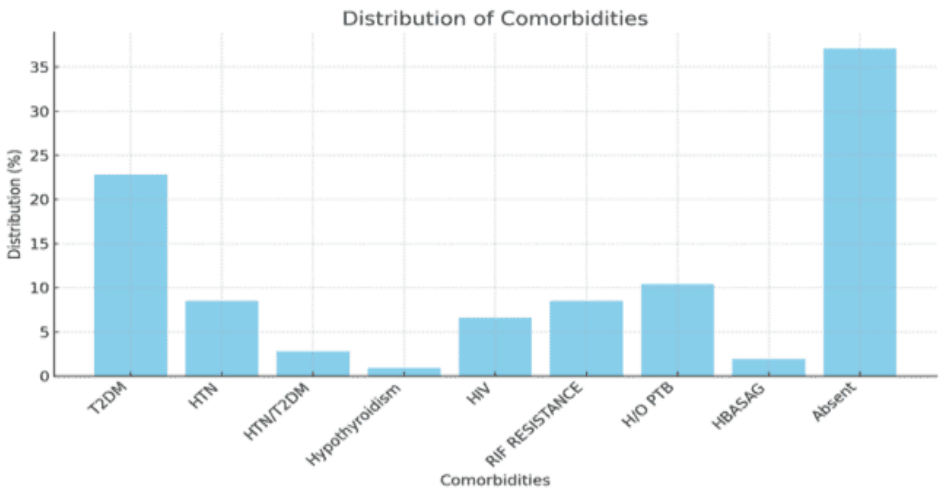


TABLE 6: DEMOGRAPHIC DATA DISTRIBUTION

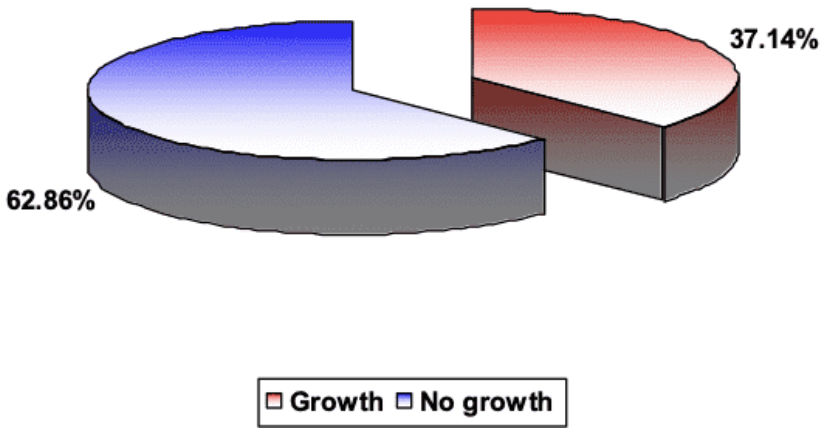
Parameters	**Mean (n=105)**		**Median**		**Range**		**p value**
	Mean	**SD**	**Median**	**IQR**	**Minimum**	**Maximum**	
Age (Years)	45.17	15.57	45.00	23.00	18.00	77.00	-
Height (CMS)	164.34	8.18	165.00	10.00	126.00	182.50	-
Weight (Kg)	54.37	9.85	55.00	12.00	23.00	78.00	-
Body mass Index (Kg/m^2)	20.22	3.46	20.50	4.90	12.60	33.80	-
Mean CT value	26.98	8.51	24.00	14.45	12.78	40.00	**<0.001**
TTC (Weeks)	3.00	1.60	3.00	2.00	1.00	6.00	**0.003**

The descriptive data of the patients enrolled is as depicted above. Based on the Kolmogorov–Smirnov test for normality. Mean age of the study population is 45.17 years, with a range of 23.00 to 77.00 years. **The** mean height in the study population is 164.34 cm, with a range of 126.00cms being minimum & 182.50 cm being the maximum. The mean weight of the study population is 54.37 kg, varying between 23.00 and 78.00 kg. Mean Body Mass Index is 20.22kg/m^2, with a range of 14.45 kg/m^2 to 33.80 kg/m^2. Mean CT value of the study population is 26.98, ranging from 12.78 to 40.00. Mean time to culture positivity in the study population is around 3.00 weeks, with a range of 1.00 to 6.00 weeks.

TABLE 7**:** DISTRIBUTION OF PATIENTS ACCORDING TO THE CULTURE

Culture	**Distribution (n=105)**	
	No	**%**
Growth	39	37.14
No growth	66	62.86
Total	**105**	**100.00**

GRAPH 6: DISTRIBUTION OF PATIENTS ACCORDING TO THE CULTURE

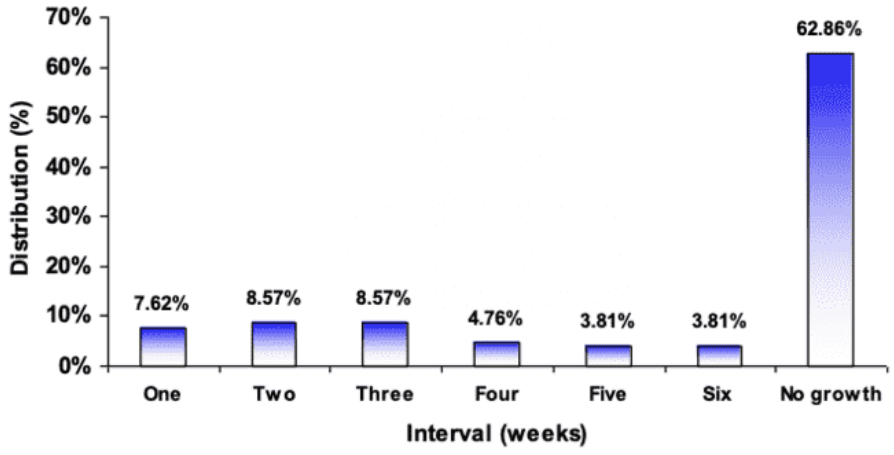


In the present study, positive culture growth was noted in 39(37.14%) patients. There was no growth in 66(62.86%)patients.

TABLE 8: DISTRIBUTION OF PATIENTS ACCORDING TO THE INTERVAL AT CULTURE GROWTH

Interval (weeks)	**Distribution (n=105)**	
	No	**%**
One	8	7.62
Two	9	8.57
Three	9	8.57
Four	5	4.76
Five	4	3.81
Six	4	3.81
No growth	66	62.86
Total	**105**	**100.00**

GRAPH 7: DISTRIBUTION OF PATIENTS ACCORDING TO THE INTERVAL AT CULTURE GROWTH

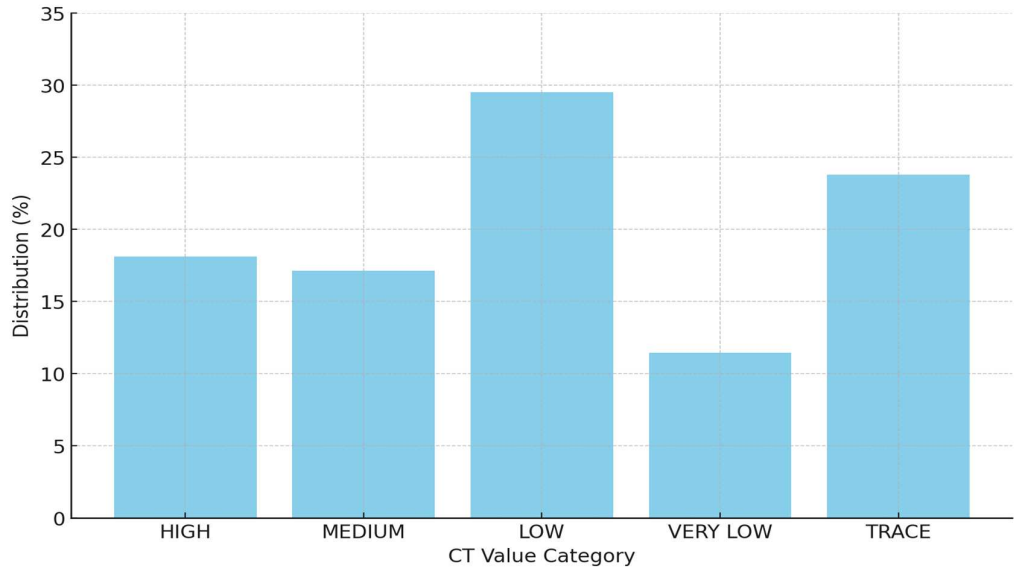


In this study, out of 39 patients who had positive culture growth, 8 patients showed growth at 1 week, 9 patients each showed growth at 2nd and 3rd weeks respectively. 4 patients showed growth at 5 weeks and 4 patients each showed growth at 5th and 6th weeks respectively.

TABLE 9: DISTRIBUTION OF PATIENTS ACCORDING TO THE CT VALUE CATEGORY

CT value Category (XpertMTB RIF/ XpertUltra)	**Distribution (n=105)**	
	No	**%**
HIGH **(XpertMTB RIF-<16)& (XpertUltra-15-18.9)**	19	18.10
MEDIUM **(XpertMTB RIF-16-22) (XpertUltra-19-24.9)**	18	17.14
LOW **(XpertMTB RIF-22-28) (XpertUltra-25-28.9)**	31	29.52
VERY LOW **(XpertMTB RIF->28) (XpertUltra-29-40)**	12	11.43
TRACE **(≤37- 40)**	25	23.81
Total	**105**	**100.00**

GRAPH 8: DISTRIBUTION OF PATIENTS ACCORDING TO THE CT VALUE CATEGORY

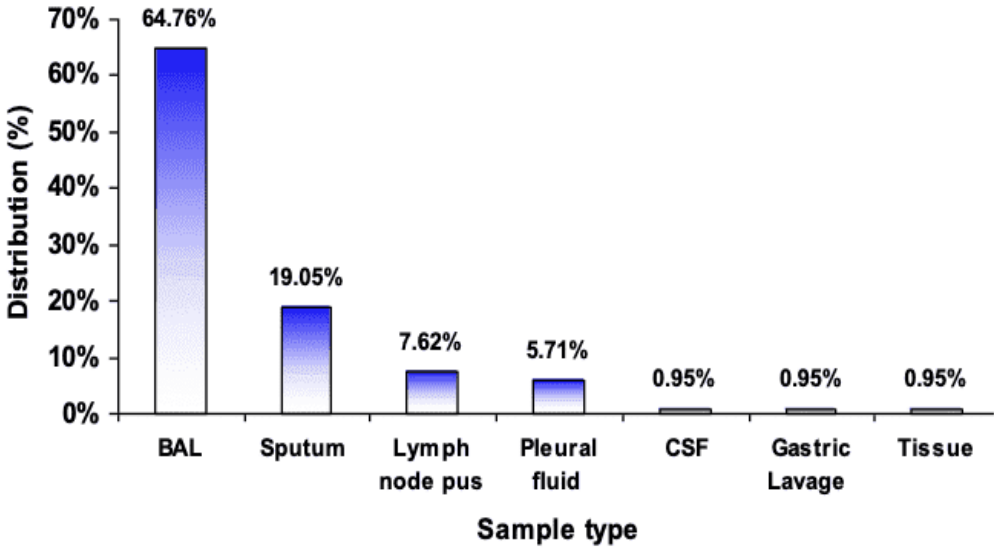


In the present study, out of 105 patients, 19 patients belonged(18.10%) belonged to CT HIGH category and 18 patients belonged(17.14%) to CT MEDIUM category. Majority of the population i.e., 31 patients(29.52%) belonged to CT LOW category followed by 25 patients(23.81%) belonged to TRACE category. Least number of patients i.e., 12(11.43%) belonged to VERY LOW category.

TABLE 10: DISTRIBUTION OF PATIENTS ACCORDING TO THE SAMPLE TYPE AND ITS ASSOCIATION WITH CULTURE

Sample type	Culture				Total		P VALUE
	Positive		Negative		No.	%	
	No	%	No	%			
BAL	24	35.29	44	64.71	68	64.76	
CSF	0	0.00	1	100.00	1	0.95	
Gastric Lavage	0	0.00	1	100.00	1	0.95	**0.002**
Lymph node Pus	0	0.00	8	100.00	8	7.62	
Pleural fluid	1	16.67	5	83.33	6	5.71	
Sputum	14	70.00	6	30.00	20	19.05	
Tissue	0	0.00	1	100.00	1	0.95	
Total	**39**	37.14	**66**	62.86	**105**	**100.00**	

GRAPH 9: DISTRIBUTION OF PATIENTS ACCORDING TO THE SAMPLE TYPE AND ITS ASSOCIATION WITH CULTURE

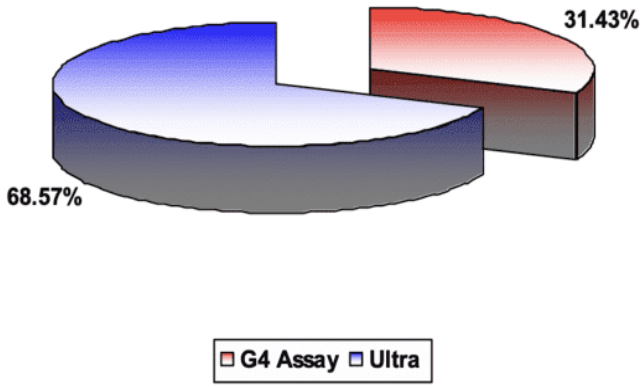


In the present study, 68 samples(64.76%) consisted of BAL samples, out of which 24(35.29%) had positive culture growth and 44 samples(64.71%) had no culture growth. 20 samples (19.05%) consisted of Sputum samples out of which 14 samples (70%) showed positive culture growth and 6 samples(30%) showed no culture growth. 8 samples (7.62%) of the present study consisted of lymph node aspirate out of which, no samples yielded culture growth. 6 samples(5.71%) of the study consisted of pleural fluid, out of which 1 sample(16.67%) yielded culture growth and 5 samples(83.33%) had no culture growth. 1 sample each(0.95%) in the present study consisted of CSF, GASTRIC LAVAGE and TISSUE samples out which none of them have shown to yield culture growth. There was statistical significance noted between type of sample and culture growth with **p value of 0.002.**

TABLE 11: DISTRIBUTION OF PATIENTS ACCORDING TO THE ASSAY TYPE AND ITS ASSOCIATION WITH CULTURE

Assay type	Culture				Total		P VALUE
	Positive		Negative		No.	%	
	No	%	No	%			
G4 Assay	13	39.39	20	60.61	33	31.43	0.829
Ultra	26	36.11	46	63.89	72	68.57	
Total	**39**	37.14	**66**	62.86	**105**	**100.00**	

GRAPH 10: DISTRIBUTION OF PATIENTS ACCORDING TO THE ASSAY TYPE AND ITS ASSOCIATION WITH CULTURE

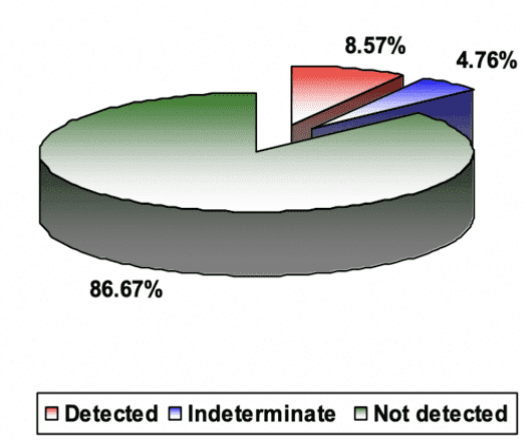


In the present study, out of 105 samples, 72 samples(68.57%) were processed on GeneXpert Ultra assay out of which 26 samples(36.11%) showed positive culture growth and 46(63.89%) showed no culture growth. 33 samples(31.43%) were processed on GeneXpert G4 assay out of which 13(39.39%) were culture positive and 20 samples(60.61%) were culture negative. There is no statistical significance noted with type of assay used and culture growth. P value is 0.829.

TABLE 12: DISTRIBUTION OF PATIENTS ACCORDING TO THE RIF RESISTANCE AND ITS ASSOCIATION WITH CULTURE

RIF resistance	Culture				Total		P VALUE
	Positive		Negative		No.	%	
	No	%	No	%			
Detected	7	77.78	2	22.22	9	8.57	**0.009**
Indeterminate	0	0.00	5	100.00	5	4.76	
Not detected	32	35.16	59	64.84	91	86.67	
Total	**39**	37.14	**66**	62.86	**105**	**100.00**	

GRAPH 11: DISTRIBUTION OF PATIENTS ACCORDING TO THE RIF RESISTANCE AND ITS ASSOCIATION WITH CULTURE

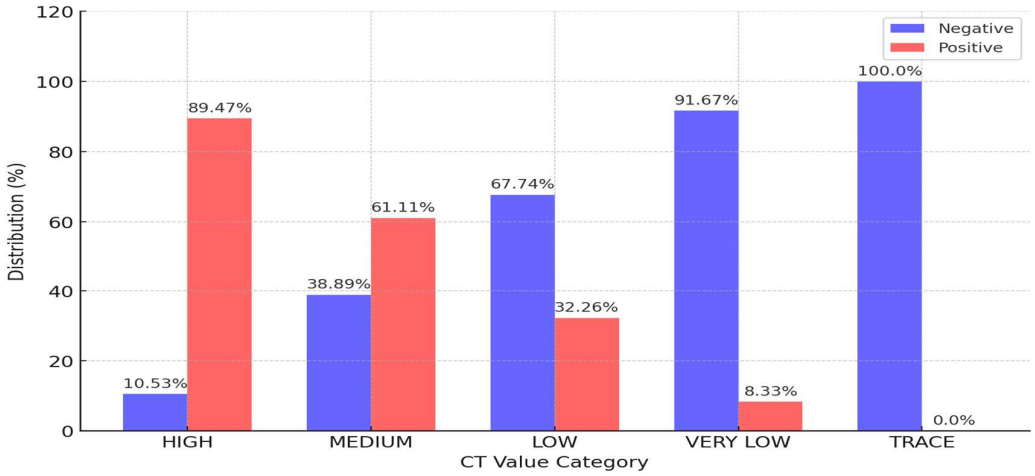


In the present study, RIF resistance was detected in 9 patient(8.57%)of the patients, out of which 7 patients(77.78%) were culture positive and 2 patients(22.22%) were culture negative. Out of 91 patients who had no RIF resistance, 32 patients(35.16%) were culture positive and 59 patients(64.84%) were culture negative. 5 patients(4.76) of study population had indeterminate RIF resistance, showed no growth in culture. Statistical significant association was found between RIF resistance and culture positivity with **p value of 0.009**

TABLE 13: DISTRIBUTION OF PATIENTS ACCORDING TO THE CT VALUE AND ITS ASSOCIATION WITH CULTURE

CT value Category (XpertMTB RIF/ XpertUltra)	**Culture**				**Total**		**P VALUE**
	Positive		**Negative**		**No.**	**%**	
	No	**%**	**No**	**%**			
HIGH (<16/15-18.9)	17	89.47	2	10.53	19	18.10	
MEDIUM (16-22/19-24.9)	11	61.11	7	38.89	18	17.14	
LOW (22-28/25-28.9)	10	32.26	21	67.74	31	29.52	**< 0.001**
VERY LOW (>28/28-40)	1	8.33	11	91.67	12	11.43	
TRACE (≤37- 40)	0	0.00	25	100.00	25	23.81	
Total	**39**	37.14	**66**	62.86	**105**	**100.00**	

GRAPH 12: DISTRIBUTION OF PATIENTS ACCORDING TO THE CT VALUE AND ITS ASSOCIATION WITH CULTURE



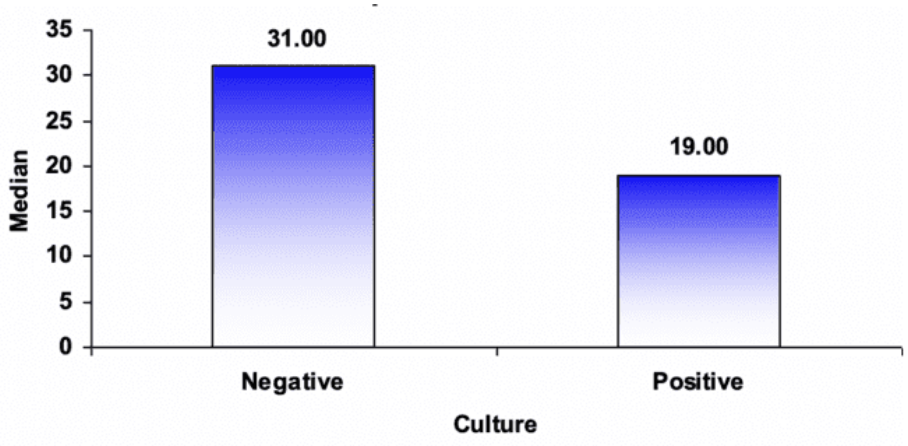
In our study 19 patients(18.1%) belonged to HIGH CT value category out of which 17(89.47%) yielded positive culture growth and only 2(10.53%) had no growth in culture. 18 patients(17.14%) patients belonged to MEDIUM CT category out of which 11(61.11%) yielded positive culture growth and 7 patients(38.389%) showed no growth in culture growth. Out of 31 patients(29.52%) belonging to LOW CT category, only 10(32.26%) had positive culture growth and 21(67.74%) yielded no culture growth. In 12 patients who belonged to VERY LOW CT category, only 1 patient(8.33%) yielded positive culture and 11(91.67%) showed no culture growth. All 25 samples(23.81%) belonging to TRACE CT category showed no culture growth. A statistically significant correlation ($p<0.001$) was seen between the CT value category and the development of the culture.

TABLE 14: COMPARISON OF MEAN CT COUNT WITH RESPECT TO CULTURE

Parameters	**Culture**				**p value**
	Positive (n=39)			**Negative (n=66)**	
	Median	**IQR**	**Median**	**IQR**	
Mean CT Count	19.00	5.60	31.00	16.1	**<0.001**

Mann-Whitney U test

GRAPH 13: COMPARISON OF MEAN CT COUNT WITH RESPECT TO CULTURE

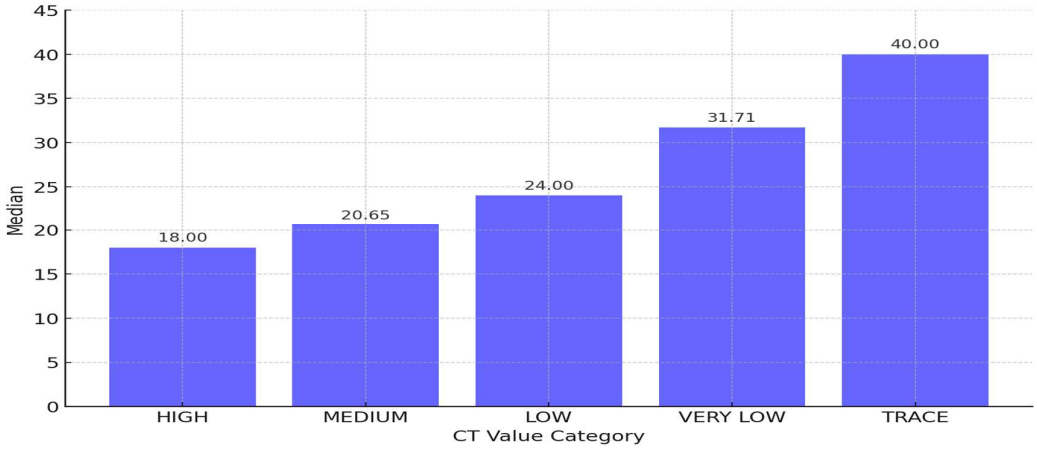


In our study, it was observed that the mean CT count in samples which yielded positive culture growth was 19, whereas the mean CT count was 31 in negative cultures. A p-value of less than 0.001 indicated a statistically significant correlation between the mean CT count and culture growth.

TABLE 15: COMPARISON OF MEAN CT COUNT WITH RESPECT TO CT VALUE

CT value	n	Mean CT value	
		Median	IQR
HIGH (<16/15-18.9)	19	18.00	2.00
MEDIUM (16-22/19-24.9)	18	20.65	2.43
LOW (22-28/25-28.9)	31	24.00	2.80
VERY LOW (>28/28-40)	12	31.71	1.83
TRACE (≤37- 40)	25	40.00	-
p value	**<0.001**		
Kruskal Wallis Test			

GRAPH 14: COMPARISON OF MEAN CT COUNT WITH RESPECT TO CT VALUE

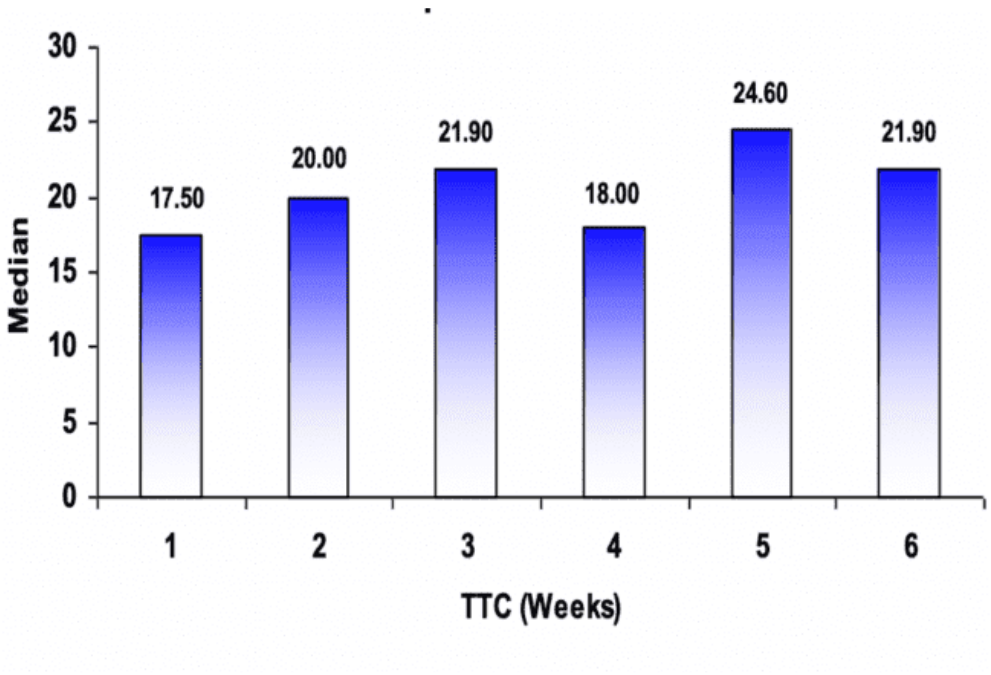


In our study it was observed that the mean CT count of 19 high CT category samples was 18. The mean CT count of 18 medium CT category samples was 20.6. the mean CT value of 31 low CT category was 24 and 12 very low CT category was 31.7. the mean CT value of 35 trace CT category was 40. it was observed that mean CT value was in accordance simultaneously with the baseline reference CT value category used in the study with statistically significant association of **p value<0.001.**

TABLE 16: COMPARISON OF MEAN CT COUNT WITH RESPECT TO TTC

TTC (Weeks)	**n**	**Mean CT value**	
		Median	**IQR**
1	8	17.50	2.70
2	9	20.00	5.40
3	9	21.90	4.75
4	5	18.00	10.24
5	4	24.60	5.80
6	4	21.90	7.65
p value	**0.014**		
Kruskal Wallis Test			

GRAPH 15: COMPARISON OF MEAN CT COUNT WITH RESPECT TO TTC



In the present study it was observed that 8 samples with mean CT count of 17.50 showed culture positivity within 1 week and samples with mean CT COUNT of 20(9 samples) and 21.9 (9 samples) showed growth in 2 and 3 weeks. However, there were 5 samples(10.24%) showed growth in 4 weeks. There were 4 samples each with Mean CT values 24.6 and 21.90 showed growth in 5 & 6 weeks respectively. It can be observed that the time to culture positive increases in concordance with the mean CT value, and that there is a statistically significant correlation between the two ($p = 0.014$).

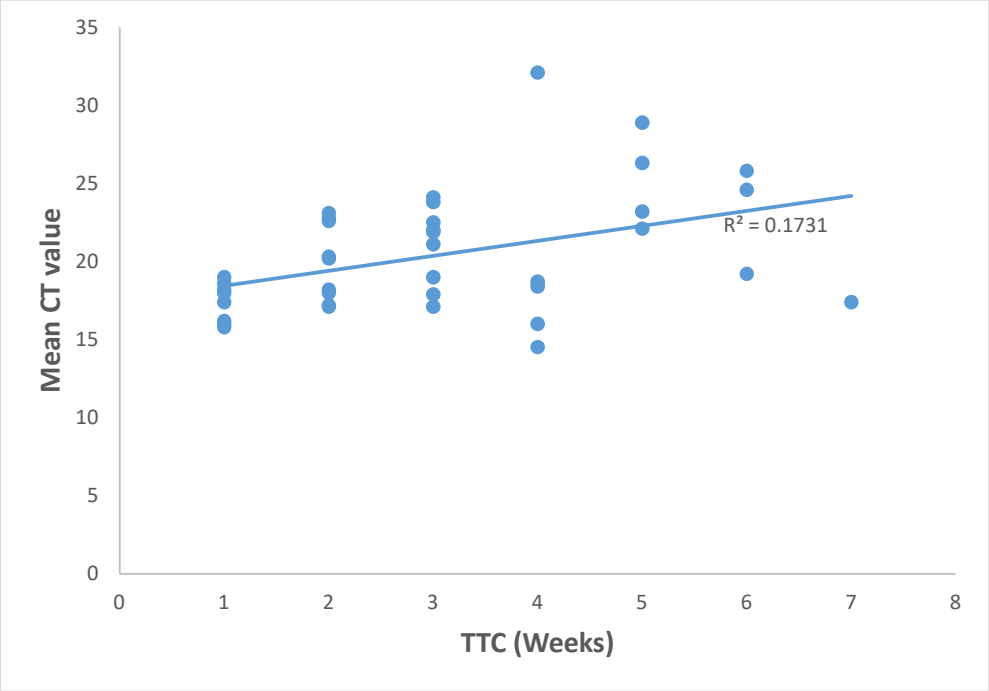
TABLE 17: DISTRIBUTION OF PATIENTS ACCORDING TO THE CT VALUE CATEGORY AND TTCP

CT value category	TTC (Weeks)														Total	
	1		2		3		4		5		6		No growth		No.	%
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
High	7	36.84	3	15.79	3	15.79	2	10.53	0	0.00	2	10.53	2	10.53	19	18.10
Medium	1	5.56	3	16.67	4	22.22	2	11.11	1	5.56	0	0.00	7	38.89	18	17.14
Low	0	0.00	3	9.68	2	6.45	0	0.00	3	9.68	2	6.45	21	67.74	31	29.52
Very low	0	0.00	0	0.00	0	0.00	1	8.33	0	0.00	0	0.00	11	91.67	12	11.43
Trace	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	25	0.00	25	23.81
Total	**8**	7.62	**9**	8.57	**9**	8.57	**5**	4.76	**4**	3.81	**4**	3.81	**66**	62.86	**105**	**100.00**

The above table depicts the samples belonging to different CT value categories and their culture growth in weeks. Out of 19 samples(18.10%) belonging to HIGH CT category, 7 samples(36.84%) showed culture growth in 1st week, 3 samples(15.79%) showed culture growth in 2 weeks, 3 samples(15.79%) showed growth in 3 weeks, 2 samples(10.53%) showed growth in 4 weeks, and 2 samples(10.53%) showed growth in 6 weeks and 2 samples(10.52%) showed no culture growth. Out of 18 samples(17.14%) which belonged to MEDIUM CT category, 1 sample(5.56%) showed culture growth in 1st week, 3 samples(16.67%) showed culture growth in 2 weeks, 4 samples(22.22%) showed culture growth in 3 weeks, 2 samples(11.11%) showed culture growth in 4 weeks, 1 sample(5.56%) showed culture growth in 5 weeks, and 7 samples(38.89%) showed no culture growth. Out of 31 samples

belonging to LOW CT category, none of the samples showed culture growth in 1st week. 3 samples(9.68%) showed culture growth in 2 weeks, 2 samples(6.45%) showed culture growth in 3 weeks, 3 samples(9.68%) showed culture growth in 5 weeks, 2 samples(6.45%) showed culture growth in 6 weeks and 21 samples(67.74%) showed no culture growth. Out of 12 samples which belonged to VERY LOW CT category, 1 sample(8.33%) showed culture growth in 4 weeks and 11 samples(91.67%) showed no culture growth. All 25 samples(23.81%) belonging to TRACE CT category showed no culture growth by end of 6 weeks

GRAPH 16: SPEARMAN COREELATION GRAPH CORRELATING MEAN CT VALUE AND TTCP



ρ=0.444; R^2=0.173; p=0.005

The spearman correlation graph between TTCP and mean CT value is depicted in the above graph. Statistically significant weak positive correlation was noted between TTC and mean CT count (ρ=0.444; R^2=0.173; p=0.005).

TABLE 18: EXTRA PULMONARY SAMPLES AND CULTURE GROWTH

SAMPLE TYPE	TOTAL NO	CT HIGH	CULTURE GROWTH	CT MEDIUM	CULTURE GROWTH	CT LOW	CULTURE GROWTH	CT VERY LOW	CULTURE GROWTH
PLEURAL FLUID	6	1	0	2	1(3 weeks)	1	0	2	0
L.N ASPIRATE	8	0	0	1	0	6	0	1	0
TISSUE	1	0	0	0	0	1	0	0	0
GASTRIC LAVAGE	1	0	0	0	0	1	0	0	0
CSF	1	0	0	0	0	1	0	0	0
TOTAL	17	1	0	3	1	10	0	3	0

The above table depicts the extra pulmonary samples and its association with culture growth. Out of 6 samples which belonged to pleural fluid, only 1 sample which belonged to medium CT category showed culture growth at 3 weeks. Other samples which included lymph node aspirate, tissue, gastric lavage and csf had not shown any culture growth at the end of 6 weeks.

DISCUSSION

TB still remains a threat in many developing nations, including India, despite quick advancements in detection and treatment. It is one of the top ten leading causes of death worldwide. Undoubtedly, the battle against tuberculosis has produced noteworthy outcomes. 53 million lives have been saved from the grip of tuberculosis in the past 17 years, mostly as a result of prompt diagnosis and efficient treatment. Though over the years the newer diagnostic methods have come in practice, it still remains the cause of concern due to its impact on mankind. Initiatives like RNTCP and DOTS are effectively addressing this problem as a top priority, reducing obstacles in the path to a great extent. Furthermore, since 2003, the Foundation for Innovative Diagnostics (FIND) has enhanced impartial access to novel diagnostic techniques for all countries while striving to improve TB diagnostics.[52] In RNTCP, the backbone of the programme success was the sputum microscopy for the diagnosis and treatment of tuberculosis. The sputum culture for AFB remained the gold standard for the diagnosis and treatment. The liquid culture facilities were not available everywhere in a resource burden country like India. Government could implement the programme at district level where all the facilities were available. As culture requires well equipped laboratories like reference laboratories, the liquid culture ideally takes 1-2 weeks to give the results whereas solid culture takes even longer that is 4-6 weeks. After culture the drug susceptibility testing (DST) still takes 1-2 weeks to process the test and give results. DST will give the sensitivity to rifampicin, isoniazid & multidrug resistance detection. Because of these drawbacks in the diagnostic delays and treatment initiation, tuberculosis remained a challenge to treat. Emerging drug resistance due to delay in diagnosis of MDR TB was a challenge then. The breakthrough for the RNTCP programme was the discovery of newer diagnostic

method, CBNAAT in 2009, which gives the result within 2 hrs. The test also gives the rifampicin sensitivity and resistant pattern which helps to diagnose MDR TB at the earliest. Earlier CBNAAT was available at few centres across nation. The RNTCP strengthened the system by upgrading CBNAAT to remote places covering the large population that changed the perspective of tuberculosis treatment and management. Cartridge based nucleic acid amplification test is a rapid test that identifies tuberculosis and rifampicin drug resistance within 2 hours using a semiquantitative hemi-nested reverse transcription-polymerase chain reaction technology (rt-PCR). It integrates and automates the processing, amplification, and detection of target sequences. In 2009, there were 9.4 million cases of tuberculosis worldwide, and an estimated 1.3 million people died from it. CBNAAT, when employed as a replacement or add-on test to microscopy, boosted TB case detection by around 30%. Its application as a replacement for traditional culture and DST. It significantly increased multidrug resistance case finding roughly by threefold which led to WHO in 2010, recommending it as a first line test in suspected tuberculosis patients. CBNAAT also provides a comprehensive semi quantitative understanding about the bacillary load in the sample based on the cycle threshold values (CT) of the targets that are unique to MTB. It correlates to the number of PCR cycles taken to detect the target DNA. It is believed that in a sample with high bacillary burden, it would take less number of PCR cycles to detect the minimum concentration the target DNA and inversely, more number of cycles to detect in less bacillary burden sample. This CT value gives us the fair idea of the bacillary burden in the specimen. However, the detailed examination of the CT values of CBNAAT and its therapeutic implications has some knowledge gaps. While some researches have attempted to investigate the relationship between CT categories and time to culture positivity, others have

compared the CT value of CBNAAT with smear microscopy.[7,53,54] However, there might be direct association of CBNAAT CT value and time to culture positivity depending on the bacillary burden or the other factors responsible for time to show culture positivity. The studies are done to know the correlation between CT value of CBNAAT for sputum samples and time to culture positivity. [6] There hasn't been much research done for CBNAAT CT values from the GeneXpert MTB RIF assay and GeneXpert ultra assay and time to culture positivity in bronchoalveolar lavage (BAL) samples primarily and also extrapulmonary and multidrug resistant samples.

In our study, total of 105 patients were studied. All the patients were CBNAAT positive whose CT values were taken. They were categorised in to High, Medium, Low, Very Low & Trace Categories. All the samples were subjected to culture. The time taken to show culture positivity were compared with CT value.

Our study comprised 105 patients, with a significant male predominance, 71.43% of the patients were male (75 out of 105), and 28.57% were female (30 out of 105). This result was comparable to the study conducted by Irene Najjingo et.al where males accounted for 67.4% which aimed at studying the Xpert CT readings are compared with smear microscopy and culture as a way to quantify the bacillary burden.[7]This gender disparity might suggest a higher prevalence or susceptibility to TB. Various factors including occupational exposure, lifestyle, geographical distribution & habits such as smoking, alcohol consumption and tobacco chewing could contribute to this disparity.

The age distribution in this study sample revealed a mean age of 45 years, with the youngest patient being 18 and the oldest 77. Peng Lu et al. found a similar mean age of 50 years in his study while investigating the relationship between

diabetes and body mass index and the likelihood of developing tuberculosis. This overall middle-aged distribution could be linked to decreased immunity as a result of comorbidities, as opposed to younger people who have a stronger immunological response.

In this study, patients' BMI distribution varied significantly, with majority of the study group having BMI between 23.00 and 24.99 kg/m²(45.71%). A significant proportion (32.28%) of patients had a BMI < 18.50 kg/m². Overall many studies have shown that less BMI poor nutrition status has shown more risk of developing Tuberculosis.

The most prevalent comorbidity in this study was Type 2 Diabetes Mellitus (T2DM), seen in 24 individuals (22.86%) which was followed by 11 patients (10.48%) having past history of tuberculosis and 39 patients(37.14%) had no concomitant comorbidities. TB and T2DM association has been established from many studies where it has shown bidirectional association, leading to inclusion of diabetes screening in RNTCP like HIV patients for Tuberculosis.

This study included seven HIV-positive specimens, although only two exhibited culture positivity. One sputum sample from the low CT group exhibited culture growth at 6 weeks, whereas one from the medium CT category showed growth at 3 weeks. This was consistent with the findings of Blakemore et al's[43] study examined the capabilities quantitatively of Xpert MTB/RIF assay, which found that lower culture positivity rates and higher CT values were attributable to reduced bacillary burden due to poor immunological response in HIV patients. Only two patients in our study tested positive for culture, which could not determine the association with CT value.

In this study, growth on the culture was seen in 39 out of 105 patients. Similarly, in a study where Mohanraj Set al[5] studied CT values of CBNAAT as a measure of sputum bacillary burden and correlated with culture positivity, 32.21% among Xpert positive samples out of 149 samples studied were culture positive. In a similar study done by Ashish K Prakash et al[6] on determining clinical utility of CT value of CBNAAT and its diagnostic accuracy in extra-pulmonary and pulmonary samples in association with culture positivity found a lower overall culture positivity (16.7%), highlighting the variability of the results across different populations and sample types.

In our study, 8 of the 39 patients with positive culture growth (7.62%) showed growth within one week. Nine individuals (8.57%) demonstrated growth in the second and third weeks, respectively. Growth was seen in 5 individuals (4.76%) in the fourth week and in 4 patients (3.81%) in the fifth and sixth weeks. The remaining 66 patients (62.86%) demonstrated no growth. This variance reflects the diversity in bacterial load and growth rates in various samples. There could be several causes for the variation in time to cultural positive. It is possible that bacterial virulence and genecity influence the time to culture positive. The type of sample could also attribute to the negative cultures especially in extrapulmonary samples where the culture yield will be poor. However, in this study only time to culture positivity was noted but the factors affecting the culture positivity was not considered in detail.

This study showed specimens with high CT value category (<16 cycles) showing 89.47% culture positive rates, medium CT value category (16-22 cycles), 61.11% culture positive rates, low CT value category (22-28 cycles), 32.26% culture positive rates, very low CT value category (>28 cycles), 8.33% culture positive rates

and trace CT value category with no culture positive rates showing significant association. Similar trends were observed by Mohanraj S et al in his study[5] showing high CT value category with significant culture positivity correlation, medium CT value category with moderate culture positivity, low CT value category with lower culture positivity, very low with minimal culture positivity. So from this it can be inferred that high CT values show more culture positivity and low CT values show low culture positivity outcomes. This has got direct relation with CT value and bacillary burden. Further similarity between both the studies provide valuable insight about bacillary burden and the utility of CT values in predicting bacterial load.

In this study, sputum sample showed 70% culture positivity rates, BAL showed 35.29% culture positive rates, whereas no growth in cultures were seen in samples of cerebrospinal fluid, gastric lavage, lymph node pus, tissue were noted. Similarly, Ashish K Prakash et al [6]in his study found highest yield in sputum specimens (29.4%) and lowest in pleural fluid (4.2%). This study is comparable with other studies showing the higher rates of culture positivity in sputum samples compared to BAL and showed least positivity in extrapulmonary samples.

This study compared the GeneXpert Ultra and G4 assays, which showed identical culture positive rates (36.11% for Ultra and 39.39% for G4), demonstrating that both assays are highly successful and equally efficient in diagnosing tuberculosis, though the methods are different. The minor difference in culture positivity rates implies that, while the ultra assay is intended to improve sensitivity and identify lower bacterial loads, the G4 assay is still an effective tool for TB detection. This study emphasises the diagnostic relevance of both assay types in clinical settings, where precise and fast TB detection is critical for optimal patient care. The considerable

culture positivity rates for both assays reinforce the necessity of adopting modern molecular diagnostics in TB control programmes, ensuring that even individuals with lower bacterial burdens are appropriately diagnosed and treated.

In our study, 8.57% of patients had rifampicin resistance, with higher culture positive (77.78%) among resistant cases. Similarly, in a study by Mohan Raj S et al[5] discovered that rifampicin resistant specimens had a significantly greater culture positivity. The greater culture positivity rate in resistant cases highlights the necessity of recognising drug resistance cases earlier. However, the values are not statistically significant enough to apply this conclusion to the research area.

This study provides a comparative analysis of mean CT counts between culture-positive and culture-negative samples, aiming to find the relationship between CT values and culture outcomes, emphasizing the diagnostic significance of CT values in predicting bacterial load and culture positivity. In this study, the mean CT count for culture-positive samples was 19 cycles, indicating a high bacterial load that correlates strongly with the ability to grow bacteria in culture. In contrast, the mean CT count for culture-negative samples was significantly higher at 31 cycles, reflecting a lower bacterial load, which likely falls below the threshold necessary for successful culture growth. The statistical significance of this difference, with a p-value of less than 0.001, reinforces the diagnostic value of CT counts, confirming that lower CT values (indicative of higher bacterial loads) are predictive of positive culture results. In a similar study done by Mohanraj et al[5], There was a strong correlation between the mean CT value of Xpert positive samples, which was 22.18±6.69 cycles, and the time taken to show positive cultures indicating that lower CT values are associated with positive culture outcomes and higher CT values correlating with lower culture

positivity rates aligning with our study, showing a clear inverse relationship between CT values and culture positivity. Overall, CT values are a reliable predictor of bacterial load and culture outcomes highlighting the use of CT values as a crucial tool in TB diagnostics, emphasizing their utility in predicting bacterial load and improving the accuracy of TB detection and management. This finding can be utilized in the clinical practise as depending on CT value the patient can be confidently started on early initiation of the treatment.

In our study, we found a clear inverse association between CT values and TTCP. Samples with an average CT count of 17.5 showed culture growth within one week, indicating a high bacterial load and rapid culture positivity. In contrast, samples with higher mean CT counts showed delayed culture growth i.e with mean CT counts of 24.6 and 21.9 showed culture positivity at five and six weeks, respectively. This significant statistical correlation (p=0.014) between CT value categories and TTCP highlights the usefulness of CT values in forecasting the time it takes for cultures to become positive. This result is consistent with the findings of Mohanraj et al[5],who also investigated the association between CT values and TTCP, giving a relevant comparison to our study. They found an inverse association between CT values and TTCP. Samples with low CT values demonstrated quick culture positivity, often within two to three weeks, whereas samples with higher CT values took longer to grow. In a similar study done by Blakemore et al, this trend was consistent where higher CT values (less bacillary burden) took longer time to show culture positivity[43]. Both the studies emphasise the predictive relevance of CT values in TB diagnosis. This consistency accentuates the diagnostic importance of CT values in predicting TTCP and guiding clinical decisions.

This study examined how CT value categories correlate with time to culture positivity (TTCP). The high CT value category showed the quickest culture positivity, with 36.84% of samples becoming positive within one week, indicating a high bacterial load. The medium CT value category had culture positivity mainly within 2-3 weeks, reflecting substantial but slightly delayed growth. The low CT value category had a 32.26% positivity rate, with growth typically in 4-6 weeks, indicating a moderate bacterial load. The very low CT value category had minimal positivity (8.33%), with growth after six weeks, and the trace category showed no culture positivity. In similar study done by Ashish K Prakash et al[6], the study results were comparable to our study. In an another relevant study done by Bineeta Kashyap et al[55] to determine the association of CBNAAT grading on the basis of CT value with conventional microbiological diagnosis, it was noted that there was 100% culture positivity in samples which belonged to high & medium CT value category in the range of 2-3 weeks majorly. Whereas only 58% of low category samples showed culture positivity in 5-8 weeks predominantly and 100% of very low samples did not show any culture growth.[55]The above studies demonstrate that lower CT values, indicative of higher bacterial loads, result in quicker culture growth, highlighting the prognostic value of CT values in TB diagnostics. This consistent correlation highlights the importance of using CT values to predict culture outcomes and guide timely clinical interventions.

In the present study, 17 extrapulmonary samples were evaluated, with only pleural fluid sample showed culture positivity after three weeks. This study emphasises the difficulties of growing cultures in extrapulmonary samples and diagnosing them through culture, as low culture positivity rates indicates the need for more sensitive diagnostic procedures for these types of specimens. In a comparable

manner, Ashish K Prakash et al[6] in his study, showed poorer sensitivity for extrapulmonary samples than for pulmonary samples. Pleural fluid produced a lower yield, with a culture positive rate of 66.7%, which was significantly lower than that of pulmonary specimens. This supports the finding that extrapulmonary tuberculosis pose diagnostic challenges, necessitating more advanced and sensitive diagnostic procedures. Both studies highlight the difficulties of obtaining positive culture results from extrapulmonary samples, emphasizing the need for better diagnostic methods to effectively detect TB in these specimens.

This study depicts the Spearman correlation between mean CT value and time taken to show positive cultures, showing a statistically significant weak positive correlation (ρ=0.444; R^2=0.173; p=0.005). This correlation suggests that as the mean CT value increases, indicating a lower bacterial load, the TTCP also increases, meaning that it takes longer for cultures to become positive. This finding underlines the importance of CT values in predicting the speed of culture growth and highlights their prognostic value in TB diagnostics. This finding in our study correlates with findings of Mohanraj et al[5] research that also shown a favourable relationship between the mean CT value and the amount of time it takes to grow in culture, though the correlation strength varied by CT value categories. The overall Spearman correlation between CT value and TTCP was reported as moderate (r=0.46), with an R^2 of 0.22 from linear regression analysis, indicating a moderate association between these variables. Subgroup analyses showed a moderate positive correlation for high and medium CT ranges (r=0.47 and r=0.54, respectively) but a weaker correlation for low and very low CT ranges (r=0.22 and r=0.25, respectively). Blakemore et al, in his study demonstrated strong correlation(r=0.68) between CT values and time taken to show culture positivity.[54] Similarly Bineeta Kashyap et al demonstrated a statistically

significant inverse correlation(r=0.64) between CBNAAT CT value grading and time to culture positivity in her study.[55] These outcomes amplify to support the use of CT values as a key diagnostic and prognostic tool in TB management, by predicting the bacillary burden in the given sample which helps us understanding the disease severity and ensuring more accurate and timely treatment for patients.

LIMITATIONS

- This study primarily focused on BAL samples, which may differ significantly from sputum samples in bacterial load and characteristics, limiting the use of study to generalise the conclusion.
- Sample size is small to conclude any cut off for CT values.
- Different bacterial loads and growth characteristics in extrapulmonary samples might have influenced the overall correlation between CT values and culture positivity.
- The extra pulmonary sample size is small with only 17 samples making it difficult to conclude about culture negativity and paucibacillary reason in these cases.
- A higher proportion of samples in the TRACE category might have weakened the observed correlation between CT values and TTCP compared to other studies.
- This study was conducted in a specific hospital setting, making the findings not directly applicable to other geographic regions or clinical settings with different patient demographics and TB prevalence rates.

SUMMARY

- This study examined the relationship between cycle threshold (CT) values from Xpert MTB/RIF and Xpert MTB/RIF Ultra assays and the probability and timing of culture positivity in patients suspected of having tuberculosis (TB).
- It aimed to determine if CT values could serve as indicators of bacterial load, infectivity and transmissibility.
- The study included 105 patients, with a male predominance of 75 males patients and 30 female patients.
- The mean age of the study population was 45 years, with patients ranging from 18 to 77 years. The largest age group was 31 to 40 years (20%), followed by 21 to 30 years and 41 to 50 years (both 19.05%).
- The mean BMI was 20.22 kg/m^2, with significant variability among patients. The highest percentage of patients (45.71%) had a BMI between 23.00 to 24.99 kg/m^2. A substantial portion (32.28%) had a BMI of less than 18.50 kg/m^2.
- 62.9% of the patients had comorbidities. The most common was Type 2 Diabetes Mellitus (22.86%) followed by past history of tuberculosis (10%), hypertension (8.57%). HIV positive status was noted in 6.67% of the patients.
- Patients were categorized based on CT values: High (18.10%), Medium (17.14%), Low (29.52%), Very Low (11.43%), and Trace (23.81%). Lower CT values correlated with higher culture positivity rates: High (89.47%), Medium (61.11%), Low (32.26%), Very Low (8.33%), and Trace (0%).
- Findings revealed that higher CT values (indicating lower bacterial loads) were generally associated with lower rates of culture positivity, whereas lower CT values (indicating higher bacterial loads) correlated with earlier and more frequent culture positivity.

- Specifically, high CT values showed a diverse distribution of growth times, while medium and low CT values presented varied patterns with significant portions of samples showing no growth.
- Very low and trace CT values were mostly associated with no growth.
- The study confirms the inverse relationship between CT values and TTCP using spearman's correlation.
- This study detailed the relationship between CT values and TTCP. Lower CT values were associated with faster culture positivity. For instance, high CT value categories showed earlier culture positivity compared to medium and low CT value categories.
- 8.57% of the patients had rifampicin resistance, with a higher culture positivity rate of 77.78% among resistant cases. This highlights the importance of early detection of drug resistance for effective TB management.

CONCLUSION

The study concluded that CT values are inversely related to the probability of microbial growth in culture over time. Lower CT values are associated with higher bacterial loads and earlier culture positivity, making them potentially valuable indicators of mycobacterial burden and infectivity. These findings support the use of CT values as useful markers in management of TB, particularly in differentiating between high and low bacterial loads.

BIBLIOGRAPHY

1. World Health Organization. Global tuberculosis report 2023. Pg.no 2
2. World Health Organization. Global tuberculosis report 2017. Pg.no 1
3. World Health Organization. Global tuberculosis report 2022. Pg.no 2
4. World Health Organization. Global tuberculosis report 2021. Pg.no 7
5. Mohanraj S, Vijayakumar KV, Indhu S, Suryakumari V, Preethi, Chaitanya KV, et.al. The cycle of threshold value of cartridge based nucleic acid amplification as a measure of sputum bacillary burden. IOSR J Dent Med Sci. 2020 Dec;19(12):6-12.
6. Prakash AK, Datta B, Jaiswal A, Goyal P, Chatterji P, Mittal S, et.al. The cycle of threshold value of cartridge based nucleic acid amplification test and its correlation with time to positivity in AFB culture. In: Tuberculosis. European Respiratory Society; 2017. p. PA2744.
7. Najjingo I, Muttamba W, Kirenga BJ, Nalunjogi J, Bakesiima R, Olweny F, et al. Comparison of GeneXpert cycle threshold values with smear microscopy and culture as a measure of mycobacterial burden in five regional referral hospitals of Uganda: A cross-sectional study. PLoS One. 2019 May 15;14(5)
8. World Health Organization. The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: policy guidance. Geneva: World Health Organization; 2016.
9. World Health Organization. The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin: policy update. Geneva: World Health Organization; 2016.
10. Scarparo C, Piccoli P, Rigon A, Ruggiero G, Nista D, Piersimoni C, et.al. Direct identification of mycobacteria from MB/BacT Alert 3D bottles: Comparative

evaluation of two commercial probe assays. J Clin Microbiol. 2001 Sep;39(9):3222-7.

11. Rossau R, Traore H, De Beenhouwer H, Mijs W, Jannes G, De Rijk P, et al. Evaluation of the INNO-LiPA Rif. TB assay, a reverse hybridization assay for the simultaneous detection of Mycobacterium tuberculosis complex and its resistance to rifampin. Antimicrob Agents Chemother. 1997 Oct;41(10):2093-8.
12. Sam IC, Drobniewski F, More P, Kemp M, Brown T. Mycobacterium tuberculosis and rifampin resistance, United Kingdom. Emerg Infect Dis. 2006 May;12(5):752-9.
13. Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M. List of Prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. Int J Syst Evol Microbiol. 2020 Nov 1;70(11):5607-12.
14. Madigan M, Martinko J, Stahl DA, Clark DP. Koch, infectious disease and pure culture microbiology. In: Brock biology of microorganisms. Boston: Pearson Education; 2012. p. 15.
15. Mir M, Rasool U, Aisha S, Alshehri B, Hamadani S. Human pathogenic microbes (bacterial and fungal) and associated diseases. Hum Pathog Microbes. 2022;1-30.
16. Allen BW, Mitchison DA. Counts of viable tubercle bacilli in sputum related to smear and culture gradings. Med Lab Sci. 1992 Jun;49(2):94-8.
17. World Health Organization. Frieden T. Toman's Tuberculosis: Case detection, treatment and monitoring – questions and answers. 2nd ed. Geneva: World Health Organization; 2004.
18. Zainuddin ZF, Dale JW. Does Mycobacterium tuberculosis have plasmids? Tubercle. 1990 Mar;71(1):43-9.

19. Nagai Y, Iwade Y, Katayama M, Yamaguchi T, Hayakawa E, Nakano M, et al. High resolution melting curve assay for rapid detection of drug-resistant Mycobacterium tuberculosis. J Infect Chemother. 2013;19(6):1116-25.
20. Morgan MA, Horstmeier CD, DeYoung DR, Roberts GD. Comparison of a radiometric method (BACTEC) and conventional culture media for recovery of mycobacteria from smear-negative specimens. J Clin Microbiol. 1983 Aug;18(2):384-8.
21. Ichiyama S, Shimokata K, Takeuchi J. Comparative study of a biphasic culture system (Roche MB Check system) with a conventional egg medium for recovery of mycobacteria. Tubercle Lung Dis. 1993 Oct;74(5):338-41.
22. Centers for Disease Control and Prevention. Tuberculosis: Drug susceptibility testing;2003.
23. Migliori GB, Zellweger JP, Abubakar I, Ibraim E, Caminero JA, De Vries G, et al. European Union standards for tuberculosis care. Eur Respir J. 2012 Apr;39(4):807-19.
24. World Health Organization. De Kantor IN, Kim SJ, Frieden T, Laszlo A, Luelmo F, Norval PY, et al. Laboratory services in tuberculosis control. WHO Global Tuberculosis Programme. Geneva: WHO; 1998.
25. World Health Organization. Mycobacteriology laboratory manual. 2014.
26. Sharma M. The mycobacteria, chapter 6, 2nd edition, page 46. 2009.
27. American Thoracic Society. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med. 2000 Apr 1;161(4):1376-95.
28. Cohen RA, Muzaffar S, Schwartz D, Bashir S, Luke S, McGartland LP, et al. Diagnosis of pulmonary tuberculosis using PCR assays on sputum collected

within 24 hours of hospital admission. Am J Respir Crit Care Med. 1998 Jan 1;157(1):156-61.

29. Centers for Disease Control and Prevention. Nucleic acid amplification tests for tuberculosis. MMWR Morb Mortal Wkly Rep. 1996 Nov 1;45(43):950-2.
30. CaneanZaro A. Rapid diagnostic tests for tuberculosis: What is the appropriate use? Am J Respir Crit Care Med. 1997;155:1804.
31. Perry S, Catanzaro A. Use of clinical risk assessments in evaluation of nucleic acid amplification tests for HIV/tuberculosis [The Pittsfield Lecture]. Int J Tuberc Lung Dis. 2000 Feb;4(2).
32. Laraque F, Griggs A, Slopen M, Munsiff SS. Performance of nucleic acid amplification tests for diagnosis of tuberculosis in a large urban setting. Clin Infect Dis. 2009 Jul;49(1):46-54.
33. Havlir DV, Barnes PF. Tuberculosis in patients with human immunodeficiency virus infection. N Engl J Med. 1999 Feb 4;340(5):367-73.
34. Catanzaro A, Perry S, Clarridge JE, Dunbar S, Goodnight-White S, LoBue PA, et al. The role of clinical suspicion in evaluating a new diagnostic test for active tuberculosis. JAMA. 2000 Feb 2;283(5):639.
35. Campos M, Quartin A, Mendes E, Abreu A, Gurevich S, Echarte L, et al. Feasibility of shortening respiratory isolation with a single sputum nucleic acid amplification test. Am J Respir Crit Care Med. 2008 Aug 1;178(3):300-5.
36. Lawn SD, Nicol MP. Xpert® MTB/RIF assay: Development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol. 2011 Sep 30;6(9):1067-82.

37. Pai M, Minion J, Sohn H, Zwerling A, Perkins MD. Novel and improved technologies for tuberculosis diagnosis: Progress and challenges. Clin Chest Med. 2009 Dec;30(4):701-16.
38. World Health Organization. Xpert MTB/RIF implementation report. World Health Organization; 2014.
39. Scott LE, Beylis N, Nicol M, Nkuna G, Molapo S, Berrie L, et al. Diagnostic accuracy of Xpert MTB/RIF for extrapulmonary tuberculosis specimens: Establishing a laboratory testing algorithm for South Africa. J Clin Microbiol. 2014 Jun;52(6):1818-23.
40. Sumangala V, Venkatesha D T, Chennaveerappa R K, Gayathree L. Role of GeneXpert® MTB/RIF assay for early diagnosis of pulmonary tuberculosis in people living with HIV. J Int Med Dent. 2017 Oct 14;4(2):56-60.
41. Barnard DA, Irusen EM, Bruwer JW, Plekker D, Whitelaw AC, Deetlefs JD, et al. The utility of Xpert MTB/RIF performed on bronchial washings obtained in patients with suspected pulmonary tuberculosis in a high prevalence setting. BMC Pulm Med. 2015 Dec 16;15(1):103.
42. National TB Elimination Programme (NTEP). CP semi-quantitative results interpretation - CBNAAT.
43. Blakemore R, Story E, Helb D, Kop J, Banada P, Owens MR, et al. Evaluation of the analytical performance of the Xpert MTB/RIF assay. J Clin Microbiol. 2010 Jul;48(7):2495-501.
44. Zetola NM, Shin SS, Tumedi KA, Moeti K, Ncube R, Nicol M, et al. Mixed Mycobacterium tuberculosis complex infections and false-negative results for rifampin resistance by GeneXpert MTB/RIF are associated with poor clinical outcomes. J Clin Microbiol. 2014 Jul;52(7):2422-9.

45. Rufai SB, Kumar P, Singh A, Prajapati S, Balooni V, Singh S. Comparison of Xpert MTB/RIF with line probe assay for detection of rifampin-monoresistant Mycobacterium tuberculosis. J Clin Microbiol. 2014 Jun;52(6):1846-52.
46. Williamson DA, Basu I, Bower J, Freeman JT, Henderson G, Roberts SA. An evaluation of the Xpert MTB/RIF assay and detection of false-positive rifampicin resistance in Mycobacterium tuberculosis. Diagn Microbiol Infect Dis. 2012 Oct;74(2):207-9.
47. Eisenach KD, Crawford JT, Bates JH. Repetitive DNA sequences as probes for Mycobacterium tuberculosis. J Clin Microbiol. 1988 Nov;26(11):2240-5.
48. Thierry D, Cave MD, Eisenach KD, Crawford JT, Bates JH, Gicquel B, et al. IS6110, an IS-like element of Mycobacterium tuberculosis complex. Nucleic Acids Res. 1990 Jan 11;18(1):188.
49. Van Soolingen D, Hermans PW, de Haas PE, van Embden JD. Insertion element IS1081-associated restriction fragment length polymorphisms in Mycobacterium tuberculosis complex species: A reliable tool for recognizing Mycobacterium bovis BCG. J Clin Microbiol. 1992 Jul;30(7):1772-7.
50. Dorman SE, Schumacher SG, Alland D, Nabeta P, Armstrong DT, King B, et al. Xpert MTB/RIF Ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: A prospective multicentre diagnostic accuracy study. Lancet Infect Dis. 2018 Jan;18(1):76-84.
51. Zifodya JS, Kreniske JS, Schiller I, Kohli M, Dendukuri N, Schumacher SG, et al. Xpert Ultra versus Xpert MTB/RIF for pulmonary tuberculosis and rifampicin resistance in adults with presumptive pulmonary tuberculosis. Cochrane Database Syst Rev. 2021 Feb 22;2021(5).
52. Foundation for Innovative Diagnostics. FIND Tuberculosis Project portfolio.

53. Hanrahan CF, Theron G, Bassett J, Dheda K, Scott L, Stevens W, et al. Xpert MTB/RIF as a measure of sputum bacillary burden. Variation by HIV status and immunosuppression. Am J Respir Crit Care Med. 2014 Jun 1;189(11):1426-34.

54. Blakemore R, Nabeta P, Davidow AL, Vadwai V, Tahirli R, Munsamy V, et al. A Multisite Assessment of the Quantitative Capabilities of the Xpert MTB/RIF Assay. Am J Respir Crit Care Med. 2011 Nov 1;184(9):1076–84.

55. Kashyap B, Gupta N, Dewan P, Singh N, Khanna A. Association of Cartridge Based Nucleic Acid Amplification Test (CBNAAT) Grading on the Basis of Cycle Threshold Value with Conventional Microbiological Diagnosis in Pediatric Tuberculosis. Bangladesh J Infect Dis. 2021 Jan 20;7(2):72–7.

ANNEXURE I – CONSENT FORM

ASSOCIATION OF BAL CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST GRADING ON THE BASIS OF CYCLE THRESHOLD VALUE WITH TIME TO CULTURE POSITIVITY

Name of Student/Principal Investigator: REG NO: BR0121005

Name of Guide/ Co Investigators:

PURPOSE OF THE STUDY: This novel study aims to investigate the correlation between CT values from CBNAAT and time to culture positivity (TTCP) in bronchoalveolar lavage (BAL) and extrapulmonary samples, highlighting factors influencing bacillary burden and drug resistance. By examining these relationships, this research addresses unexplored areas offering valuable insights for future TB management and research.

PROCEDURES INVOLVED: If you agree to enroll yourself in my study, you will be subjected to clinical examination which will involve assessment of your vitals, general physical examination and focussed systemic examination. Samples like Bronchoalveolar lavage, Pleural fluid, Lymph node aspirate, Gastric lavage, csf will be collected if needed.

RISKS AND BENEFITS: There are no potential risks involved in this study.

BENEFITS OF TAKING PART IN THIS RESEARCH: By taking part in this study, you will contributing to the growing body of evidence in TB diagnosis and management which will further add to diagnostic accuracy of tuberculosis.

VOLUNTARY PARTICIPATION / WITHDRAWAL FROM THE STUDY: Taking part in the study is voluntary. You may choose not to enroll yourself in this study and may choose to leave the study anytime in between.

ALTERNATIVES: Your decision regarding participation in study will not change present or future health care services offered to you at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. You would simply be excluded from the study if you wish to, and all your details shall be kept confidential and you will get the routine line of management.

PRIVACY AND CONFIDENTIALITY: All data collected or disclosed by you during the course of participation of study, will be kept fully confidential. If, however during the course it becomes necessary for the progress of the course to disclose the identity, it would be done so only after your informed & written consent. The only people to know that you are a research subject are members of the research team. No information about you will be disclosed to other without your written permission except: In emergency to protect your rights AND welfare.

If required by law.

AUTHORIZATION TO PUBLISH RESULT: The results of the study may be used to publish an article. When the results of research are published or discussed in a conference, no information will be displayed that would disclose your identity. Any information obtained in connection with this study and that can be identified with you will remain confidential.

FINANCIAL INCENTIVES FOR PARTICIPATION: No additional costs shall be incurred upon you for the purpose of this study. It is purely being done with the idea of research and all the cost of study will be borne by the investigator.

COMPENSATION: In the event that you become injured as a result of taking part in this study, treatment will be offered to you at KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum, or you will be given information about where to receive medical care. However, no reimbursement, compensation or free medical care will be given.

QUESTIONS/CONTACT DETAILS: You shall be free to contact the below mentioned name & addresses anytime during the study period for any clarification or help as you may desire for.

If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052

CONSENT STATEMENT

I am making a voluntary decision to participate in the study "**ASSOCIATION OF BAL CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST GRADING ON THE BASIS OF CYCLE THRESHOLD VALUE WITH TIME TO CULTURE POSITIVITY"** My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:	Signature or left thumb Impression of the participant:
Name of the witness:	Signature or left thumb Impression of the witness:
Name of the investigator:	Signature of the investigator:

ANNEXURE II-CASE PROFORMA

"ASSOCIATION OF BAL CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST GRADING ON THE BASIS OF CYCLE THRESHOLD VALUE WITH TIME TO CULTURE POSITIVITY"

NAME OF THE PATIENT:

AGE:

AGE GROUP:

SEX:

HEIGHT:

WEIGHT:

BMI:

COMORBIDITIES:

MEDICAL HISTORY:

SMOKING HISTORY:

ALCOHOL HISTORY

SAMPLE TYPE:

ASSAY TYPE:

CT VALUE:

MEAN CT VALUE:

CULTURE GROWTH IN WEEKS

RIF RESISTANCE:

MASTER CHART

Serial Number	Age (Yeears)	Age group (Years)	Sex	SAMPLE TYPE	ASSAY TYPE	GENE X PERT	CT VALUE	MEAN CT VALUE	CULTURE (WEEKS)	RIF RESISTANCE	HEIGHT(cm)	WEIGHT(kg)	BMI (KG/m2)	BMI (KG/m2)	COMORBIDITIES	Connorbidies	Medical history	SMOKING HISTORY	ALCOHOL HISTORY	Culture	Coded Sex	Coded CT value	Coded TTC
1	30	21 to 30	M	BAL	GENE XPERT ULTRA	DETECTED	VERY LOW	31	NO GROWTH	NOT DETECTED	170.8	55	19	18 ot 22.99	-	-	-	YES	YES	0	1	1	0
2	31	31 to 40	F	SPUTUM	GENE XPERT ULTRA	DETECTED	HIGH	18	1 WEEK	DETECTED	162.5	43	16.3	<18.50	Hypothyroidism	+	+	NO	NO	1	0	4	1
3	21	21 to 30	F	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	INDETERMINATE	160	49	18.4	<18.50	-	-	-	NO	NO	0	0	0	0
4	38	31 to 40	M	BAL	GENE XPERT ULTRA	DETECTED	MEDIUM	20	2 WEEKS	NOT DETECTED	175.2	56	18.9	18 ot 22.99	T2DM	+	-	YES	YES	1	1	3	2
5	60	51 to 60	M	BAL	GENE XPERT ULTRA	DETECTED	MEDIUM	21	NO GROWTH	NOT DETECTED	167	57	20.2	18 ot 22.99	HTN/T2DM, Hbsag+ve	+	+	YES	YES	0	1	3	0
6	35	31 to 40	M	BAL	GENE XPERT ULTRA	DETECTED	LOW	25	NO GROWTH	NOT DETECTED	172.7	57	19.5	18 ot 22.99	T2DM	+	+	NO	YES	0	1	2	0
7	25	21 to 30	F	BAL	GENE XPERT ULTRA	DETECTED	LOW	23	2 WEEKS	NOT DETECTED	152	50	21	18 ot 22.99	-	-	+	NO	NO	1	0	2	2
8	55	51 to 60	M	BAL	GENE XPERT ULTRA	DETECTED	MEDIUM	24	3 WEEKS	NOT DETECTED	170	50	17.3	<18.50	T2DM	+	+	YES	NO	1	1	3	3
9	67	61 to 70	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	INDETERMINATE	172	61	20.38	18 ot 22.99	HTN/T2DM	+	+	YES	No	0	1	0	0
10	65	61 to 70	M	BAL	GENE XPERT ULTRA	DETECTED	MEDIUM	20	NO GROWTH	NOT DETECTED	164	52	19.1	18 ot 22.99	T2DM, HIV	+	+	NO	YES	0	1	3	0
11	52	51 to 60	F	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	157	49	19.8	18 ot 22.99	T2DM	+	+	NO	NO	0	0	0	0
12	36	31 to 40	F	BAL	GENE XPERT ULTRA	DETECTED	HIGH	18	4 WEEKS	DETECTED	154	52	21.6	18 ot 22.99	T2DM	+	+	NO	NO	1	0	4	4
13	34	31 to 40	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	160.2	57	22.7	18 ot 22.99	-	-	+	NO	NO	0	1	0	0
14	28	21 to 30	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	165	45	16.5	<18.50	HTN	+	+	NO	NO	0	1	0	0
15	63	61 to 70	F	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	152	55	23.8	23.00 to 24.99	HTN	+	+	NO	NO	0	0	0	0
16	25	21 to 30	F	BAL	GENE XPERT ULTRA	DETECTED	VERY LOW	32	NO GROWTH	DETECTED	157.4	43	17.4	<18.50	-	-	+	NO	NO	0	0	1	0
17	70	61 to 70	F	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	162.4	56	21	18 ot 22.99	HTN	+	+	NO	NO	0	0	0	0
18	51	51 to 60	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	170.8	60	20.76	18 ot 22.99	HTN	+	+	NO	NO	0	1	0	0
19	46	41 to 50	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	165	56	20.5	18 ot 22.99	-	-	-	YES	NO	0	1	0	0
20	62	61 to 70	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	167	67	23.7	23.00 to 24.99	HTN	+	+	YES	YES	0	1	0	0
21	23	21 to 30	F	BAL	GENE XPERT ULTRA	DETECTED	LOW	23	2 WEEKS	NOT DETECTED	157.4	58	23.5	23.00 to 24.99	-	-	-	NO	NO	1	0	2	2
22	23	21 to 30	M	BAL	GENE XPERT ULTRA	DETECTED	HIGH	19	1 WEEK	NOT DETECTED	170	50	17.3	<18.50	-	-	-	NO	NO	1	1	4	1
23	36	31 to 40	M	BAL	GENE XPERT ULTRA	DETECTED	VERY LOW	32	NO GROWTH	NOT DETECTED	172	60	20.5	18 ot 22.99	H/O PTB	-	+	NO	NO	0	1	1	0
24	42	41 to 50	M	BAL	GENE XPERT ULTRA	DETECTED	HIGH	18	1 WEEK	NOT DETECTED	165	53	19.4	18 ot 22.99	T2dm	+	+	NO	YES	1	1	4	1
25	63	61 to 70	M	BAL	GENE XPERT ULTRA	DETECTED	HIGH	17	1 WEEK	NOT DETECTED	170	42	14.5	<18.50		-	+	YES	NO	1	1	4	1
26	75	71 to 77	F	BAL	GENE XPERT ULTRA	DETECTED	HIGH	18	3 WEEKS	DETECTED	160	43	16.8	<18.50	HTN/T2DM	+	-	NO	NO	1	0	4	3
27	52	51 to 60	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	INDETERMINATE	170	60	20.7	18 ot 22.99	T2DM	+	+	YES	YES	0	1	0	0
28	42	41 to 50	M	BAL	GENE XPERT ULTRA	DETECTED	LOW	24	3 WEEKS	NOT DETECTED	171	60	20.7	18 ot 22.99	-	-	-	NO	YES	1	1	2	3
29	35	31 to 40	F	BAL	GENE XPERT ULTRA	DETECTED	LOW	24	NO GROWTH	NOT DETECTED	162.5	49	18.4	<18.50	H/O PTB	-	+	NO	NO	0	0	2	0
30	34	31 to 40	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	INDETERMINATE	170.6	52	17.9	<18.50	HTN, H/O PTB	+	+	YES	YES	0	1	0	0
31	40	31 to 40	M	BAL	GENE XPERT ULTRA	DETECTED	LOW	24	NO GROWTH	NOT DETECTED	175	70	22.7	18 ot 22.99	-	-	-	YES	NO	0	1	2	0
32	31	31 to 40	F	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	160	60	23.4	23.00 to 24.99	H/O PTB/HBSAG+VE	-	+	NO	NO	0	0	0	0
33	50	41 to 50	F	BAL	GENE XPERT ULTRA	DETECTED	VERY LOW	32	4 WEEKS	DETECTED	162	65	24	23.00 to 24.99	-	-	-	NO	NO	1	0	1	4
34	35	31 to 40	F	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	165	60	22.4	18 ot 22.99	H/O PTB	-	+	NO	NO	0	0	0	0
35	55	51 to 60	M	BAL	GENE XPERT ULTRA	DETECTED	MEDIUM	17	2 WEEKS	NOT DETECTED	172	67	22.3	18 ot 22.99	H/O PTB	-	+	YES	NO	1	1	3	2
36	75	71 to 77	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	167	70	24.8	23.00 to 24.99	-	-	+	YES	YES	0	1	0	0
37	29	21 to 30	M	BAL	GENE XPERT ULTRA	DETECTED	HIGH	17	3 WEEKS	NOT DETECTED	171	52	17.3	<18.50	-	-	-	YES	YES	1	1	4	3
38	63	61 to 70	F	BAL	GENE XPERT ULTRA	DETECTED	VERY LOW	31	NO GROWTH	NOT DETECTED	165	55	20.2	18 ot 22.99	H/O PTB	-	+	NO	NO	0	0	1	0
39	50	41 to 50	F	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	162.5	46	17.3	<18.50	-	-	+	NO	NO	0	0	0	0
40	65	61 to 70	M	SPUTUM	GENE XPERT ULTRA	DETECTED	HIGH	19	4 WEEKS	NOT DETECTED	170	64	22	18 ot 22.99	T2DM	+	-	YES	YES	1	1	4	4
41	25	21 to 30	F	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	165	40	14.69	<18.50	H/O PTB	-	+	NO	NO	0	0	0	0
42	48	41 to 50	M	BAL	GENE XPERT ULTRA	DETECTED	HIGH	17	2 WEEKS	NOT DETECTED	172	45	15.05	<18.50	T2DM	+	-	NO	YES	1	1	4	2
43	65	61 to 70	M	BAL	GENE XPERT ULTRA	DETECTED	HIGH	18	2 WEEKS	NOT DETECTED	170	64	22.15	18 ot 22.99	HTN	+	+	YES	NO	1	1	4	2
44	64	61 to 70	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	INDETERMINATE	172	66	22.05	18 ot 22.99	-	-	-	NO	NO	0	1	0	0
45	45	41 to 50	M	BAL	GENE XPERT ULTRA	DETECTED	LOW	26	5 WEEKS	NOT DETECTED	175	70	22.8	18 ot 22.99	T2DM	+	+	YES	YES	1	1	2	5
46	23	21 to 30	F	TISSUE	GENE XPERT ULTRA	DETECTED	LOW	24	NO GROWTH	NOT DETECTED	162.5	54	20.3	18 ot 22.99	-	-	-	NO	NO	0	0	2	0
47	34	31 to 40	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	175.2	63	20.57	18 ot 22.99	-	-	+	YES	YES	0	1	0	0
48	62	61 to 70	F	BAL	GENE XPERT ULTRA	DETECTED	VERY LOW	29	NO GROWTH	NOT DETECTED	167	65	23.3	23.00 to 24.99	H/O PTB	-	+	NO	NO	0	0	1	0
49	41	41 to 50	M	BAL	GENE XPERT ULTRA	DETECTED	LOW	26.6	NO GROWTH	NOT DETECTED	165	55	20.2	18 ot 22.99	-	-	-	NO	NO	0	1	2	0
50	40	31 to 40	F	BAL	GENE XPERT ULTRA	DETECTED	LOW	27.7	NO GROWTH	NOT DETECTED	166	43	16.3	<18.50	-	-	-	NO	NO	0	0	2	0
51	50	41 to 50	M	BAL	GENE XPERT ULTRA	DETECTED	HIGH	17.9	NO GROWTH	NOT DETECTED	170	60	20.7	18 ot 22.99	T2DM	+	-	YES	YES	0	1	4	0
52	50	41 to 50	M	BAL	GENE XPERT ULTRA	DETECTED	HIGH	17.4	6 WEEKS	NOT DETECTED	172	60	25.8	25 to 29.99	T2DM	+	-	YES	YES	1	1	4	6
53	53	51 to 60	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	172.5	55	23.7	23.00 to 24.99	HTN, H/O PTB	+	+	YES	YES	0	1	0	0
54	39	31 to 40	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	171	60	25.8	25 to 29.99	-	-	-	YES	YES	0	1	0	0
55	54	51 to 60	M	BAL	GENE XPERT ULTRA	DETECTED	VERY LOW	31.4	NO GROWTH	NOT DETECTED	170	50	21.5	18 ot 22.99	T2DM	+	-	NO	YES	0	1	1	0

56	23	21 to 30	M	BAL	GENE XPERT ULTRA	DETECTED	LOW	22	NO GROWTH	NOT DETECTED	182.5	65	19.6	18 ot 22.99	-	-	-	NO	NO	0	1	2	0
57	26	21 to 30	F	SPUTUM	GENE XPERT ULTRA	DETECTED	HIGH	19	3 WEEKS	NOT DETECTED	165	50	18.4	<18.50	-	-	-	NO	NO	1	0	4	3
58	44	41 to 50	M	BAL	GENE XPERT ULTRA	DETECTED	LOW	22.8	2 WEEKS	NOT DETECTED	170	62	21.3	18 ot 22.99	T2DM	+	-	NO	YES	1	1	2	2
59	36	31 to 40	M	BAL	GENE XPERT ULTRA	DETECTED	HIGH	19	1 WEEK	NOT DETECTED	172	55	18.2	<18.50	-	-	-	YES	YES	1	1	4	1
60	45	41 to 50	M	LYMPH NODE ASPIRATE	GENE XPERT ULTRA	DETECTED	LOW	24.5	NO GROWTH	NOT DETECTED	165	50	18.4	<18.50	T2DM	+	+	YES	YES	0	1	2	0
61	45	41 to 50	F	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	175	60	19.6	18 ot 22.99	HTN, H/O PTB	+	+	YES	NO	0	0	0	0
62	47	41 to 50	M	BAL	GENE XPERT ULTRA	DETECTED	LOW	22.5	3 WEEKS	NOT DETECTED	177	60	19.6	18 ot 22.99	T2DM	+	+	YES	YES	1	1	2	3
63	43	41 to 50	M	SPUTUM	GENE XPERT ULTRA	DETECTED	HIGH	19.2	6 WEEKS	NOT DETECTED	172	65	22	18 ot 22.99	-	-	-	YES	YES	1	1	4	6
64	63	61 to 70	M	BAL	GENE XPERT ULTRA	DETECTED	LOW	22.1	5 WEEKS	NOT DETECTED	170	63	21.1	18 ot 22.99	T2DM	+	-	YES	YES	1	1	2	5
65	57	51 to 60	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	160	50	19.5	18 ot 22.99	-	-	-	NO	NO	0	1	0	0
66	57	51 to 60	M	SPUTUM	GENE XPERT ULTRA	DETECTED	LOW	23.2	NO GROWTH	NOT DETECTED	162	40	15.3	<18.50	-	-	-	NO	NO	0	1	2	0
67	52	51 to 60	F	BAL	GENE XPERT ULTRA	DETECTED	HIGH	18.2	2 WEEKS	NOT DETECTED	158	53	21.4	18 ot 22.99	-	-	-	NO	NO	1	0	4	2
68	73	71 to 77	M	BAL	GENE XPERT ULTRA	DETECTED	LOW	22.8	NO GROWTH	NOT DETECTED	165	64	15.1	<18.50	-	-	-	NO	NO	0	1	2	0
69	72	71 to 77	F	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	158	48	20.8	18 ot 22.99	-	-	-	NO	NO	0	0	0	0
70	53	51 to 60	M	SPUTUM	GENE XPERT ULTRA	DETECTED	VERY LOW	29.2	NO GROWTH	NOT DETECTED	158	53	21.4	18 ot 22.99	-	-	-	NO	NO	0	1	1	0
71	77	71 to 77	M	BAL	GENE XPERT ULTRA	DETECTED	TRACE	40	NO GROWTH	NOT DETECTED	168	65	23	23.00 to 24.99	-	-	-	NO	NO	0	1	0	0
72	55	51 to 60	M	PLEURAL FLUID	GENE XPERT ULTRA	DETECTED	LOW	23.1	NO GROWTH	NOT DETECTED	165	64	25.8	25 to 29.99	-	-	-	NO	NO	0	1	2	0
73	27	21 to 30	F	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	22	3 WEEKS	NOT DETECTED	153	39	16.7	<18.50	-	-	+	NO	NO	1	0	3	3
74	41	41 to 50	M	LYMPH NODE ASPIRATE	GENE XPERT G4 ASSAY	DETECTED	LOW	26	NO GROWTH	NOT DETECTED	160	60	23.4	23.00 to 24.99	HIV	-	+	NO	NO	0	1	2	0
75	70	61 to 70	M	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	LOW	25.9	NO GROWTH	NOT DETECTED	150	40	17	<18.50	-	-	-	YES	YES	0	1	2	0
76	65	61 to 70	M	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	LOW	24.8	NO GROWTH	NOT DETECTED	152	48	20.8	18 ot 22.99	-	-	-	YES	yes	0	1	2	0
77	41	41 to 50	M	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	LOW	25.8	6 WEEKS	NOT DETECTED	158	32	12.6	<18.50	-	-	-	NO	NO	1	1	2	6
78	66	61 to 70	M	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	20.3	2 WEEKS	NOT DETECTED	160	50	19.5	18 ot 22.99	-	-	-	NO	NO	1	1	3	2
79	55	51 to 60	M	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	LOW	24.6	6 WEEKS	NOT DETECTED	170	54	18.3	<18.50	T2DM, HIV	+	+	NO	NO	1	1	2	6
80	50	41 to 50	M	BAL	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	21.1	3 WEEKS	NOT DETECTED	167	54	19.3	18 ot 22.99	T2DM, HIV	+	-	NO	YES	1	1	3	3
81	38	31 to 40	M	PLEURAL FLUID	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	21.9	3 WEEKS	NOT DETECTED	160	44	17.3	<18.50	HIV	-	+	NO	NO	1	1	3	3
82	61	61 to 70	M	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	VERY LOW	36	NO GROWTH	NOT DETECTED	162	40	15.3	<18.50	-	-	-	NO	NO	0	1	1	0
83	18	18 to 20	M	LYMPH NODE ASPIRATE	GENE XPERT G4 ASSAY	DETECTED	LOW	27.1	NO GROWTH	NOT DETECTED	158	53	21.4	18 ot 22.99	-	-	+	NO	NO	0	1	2	0
84	62	61 to 70	M	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	16	4 WEEKS	DETECTED	161	34	13.1	<18.50	-	-	-	YES	NO	1	1	3	4
85	52	51 to 60	M	BAL	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	20.7	NO GROWTH	NOT DETECTED	165	73	26.8	25 to 29.99	-	-	-	NO	NO	0	1	3	0
86	46	41 to 50	M	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	LOW	28.9	5 WEEKS	NOT DETECTED	165	48	15.1	<18.50	-	-	-	YES	YES	1	1	2	5
87	26	21 to 30	M	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	16.2	1 WEEK	DETECTED	165	64	25.8	25 to 29.99	-	-	-	YES	NO	1	1	3	1
88	68	61 to 70	M	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	14.52	4 WEEKS	DETECTED	168	65	23	23.00 to 24.99	T2DM	+	-	YES	YES	1	1	3	4
89	34	31 to 40	F	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	HIGH	15.8	1 WEEK	NOT DETECTED	158	48	20.8	18 ot 22.99	-	-	-	NO	NO	1	0	4	1
90	37	31 to 40	F	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	HIGH	16	1 WEEK	NOT DETECTED	162	36	13.7	<18.50	-	-	-	NO	NO	1	0	4	1
91	28	21 to 30	M	SPUTUM	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	20.6	NO GROWTH	NOT DETECTED	172	48	16.2	<18.50	-	-	-	NO	NO	0	1	3	0
92	25	21 to 30	M	BAL	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	23.2	5 WEEKS	NOT DETECTED	151	51	23.2	23.00 to 24.99	-	-	-	NO	NO	1	1	3	5
93	56	51 to 60	M	PLEURAL FLUID	GENE XPERT G4 ASSAY	DETECTED	HIGH	12.78	NO GROWTH	NOT DETECTED	155	56	23.3	23.00 to 24.99	-	-	-	YES	YES	0	1	4	0
94	34	31 to 40	M	LYMPH NODE ASPIRATE	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	21.6	NO GROWTH	NOT DETECTED	149	55	21.6	18 ot 22.99	-	-	+	YES	YES	0	1	3	0
95	22	21 to 30	M	GASTRIC LAVAGE	GENE XPERT G4 ASSAY	DETECTED	LOW	23.4	NO GROWTH	NOT DETECTED	163	47	14.9	<18.50	-	-	-	NO	NO	0	1	2	0
96	52	51 to 60	M	BAL	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	20.6	NO GROWTH	NOT DETECTED	165	73	26.8	25 to 29.99	T2DM	+	-	YES	YES	0	1	3	0
97	19	18 to 20	F	LYMPH NODE ASPIRATE	GENE XPERT G4 ASSAY	DETECTED	LOW	24.1	NO GROWTH	NOT DETECTED	158	53	21.2	18 ot 22.99	-	-	-	NO	NO	0	0	2	0
98	39	31 to 40	F	PLEURAL FLUID	GENE XPERT G4 ASSAY	DETECTED	MEDIUM	21.1	NO GROWTH	NOT DETECTED	160	44	17.2	<18.50	HIV	-	+	NO	NO	0	0	3	0
99	49	41 to 50	M	BAL	GENE XPERT G4 ASSAY	DETECTED	LOW	23.5	NO GROWTH	NOT DETECTED	165	78	33.8	>30	-	-	-	NO	NO	0	1	2	0
100	23	21 to 30	M	LYMPH NODE ASPIRATE	GENE XPERT G4 ASSAY	DETECTED	LOW	25.3	NO GROWTH	NOT DETECTED	156	63	25.9	25 to 29.99	T2DM	+	-	NO	YES	0	1	2	0
101	25	21 to 30	M	LYMPH NODE ASPIRATE	GENE XPERT G4 ASSAY	DETECTED	LOW	21	NO GROWTH	DETECTED	145	36	21.3	18 ot 22.99	-	-	-	NO	NO	0	1	2	0
102	58	51 to 60	M	PLEURAL FLUID	GENE XPERT G4 ASSAY	DETECTED	VERY LOW	33.8	NO GROWTH	NOT DETECTED	168	52	18.1	<18.50	T2DM	+	-	YES	YES	0	1	1	0
103	30	21 to 30	M	LYMPH NODE ASPIRATE	GENE XPERT G4 ASSAY	DETECTED	VERY LOW	33.1	NO GROWTH	NOT DETECTED	180	75	23.1	23.00 to 24.99	-	-	-	NO	NO	0	1	1	0
104	34	31 to 40	F	PLEURAL FLUID	GENE XPERT G4 ASSAY	DETECTED	VERY LOW	31	NO GROWTH	NOT DETECTED	146	50	23.1	23.00 to 24.99	-	-	-	NO	NO	0	0	1	0
105	19	18 to 20	M	CSF	GENE XPERT G4 ASSAY	DETECTED	LOW	25.08	NO GROWTH	NOT DETECTED	126	23	14.5	<18.50	HIV	-	+			0	1	2	0