e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2025; 17(6); 1577-1583

Original Research Article

Comparative Analysis of Bacterial Load Detection Kinetics and Rifampicin Resistance: A Synergistic Evaluation of Liquid Culture (MGIT) and Cartridge-Based NAAT

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Received: 01-04-2025 Revised: 15-05-2025 / Accepted: 21-06-2025
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Conflict of interest: Nil

Abstract

Background: Effective global control of tuberculosis (TB) depends on the rapid and accurate detection of Mycobacterium tuberculosis (MTB) and the timely identification of rifampicin resistance, a key marker of multidrug-resistant TB. Phenotypic liquid culture using the Mycobacterial Growth Indicator Tube (MGIT) remains the reference standard for determining viability and performing comprehensive drug-susceptibility testing, although its diagnostic yield is constrained by variable time-to-positivity (TTP). In contrast, Cartridge-Based Nucleic Acid Amplification Tests (CBNAAT) provide rapid molecular detection of MTB and rifampicin resistance but cannot assess organism viability, thereby limiting their interpretation in certain clinical contexts.

Methods: We conducted a hybrid comparative analysis that combined a mathematical model of MGIT cumulative detection probabilities over a 21-day incubation period with a targeted synthesis of the published literature on CBNAAT performance. MGIT kinetics were modeled using exponential distribution functions calibrated to mean TTP values for four smear grades (scanty, 1+, 2+, 3+). CBNAAT performance across bacterial load categories and rifampicin resistance concordance was evaluated through review of major cohort studies and meta-analyses.

Results: Modelled MGIT kinetics demonstrated a strong dependence on baseline bacterial load. By day 14, cumulative positivity reached approximately 71% for smear 3+ samples but only 46% for scanty samples. Extending incubation to 21 days increased recovery of scanty samples to 60%, underscoring the risk of premature culture termination. Synthesized CBNAAT data showed that "High" and "Medium" semi-quantitative categories correlate closely with culture positivity (>95%), whereas "Low" and "Very Low" categories exhibit reduced culture recovery due to the detection of non-viable DNA and paucibacillary disease. Rifampicin resistance detection using CBNAAT displayed high overall specificity but occasional discordance, particularly in low-load specimens, driven by heteroresistance and silent rpoB mutations.

Conclusion: MGIT and CBNAAT serve complementary diagnostic roles. While CBNAAT provides rapid initial detection and resistance screening, MGIT remains essential for confirming viability and resolving discordant results, particularly in paucibacillary TB. Extending MGIT incubation beyond 21 days substantially improves yield in low-burden specimens and should be integrated into optimized diagnostic algorithms.

Keywords: Tuberculosis, Rifampicin Resistance, Heteroresistance, paucibacillary disease.

Introduction

Tuberculosis (TB) remains one of the leading causes of infectious mortality globally, continuing to challenge public health systems despite advances in diagnostics and treatment [1]. Effective TB control depends on the timely and accurate detection of Mycobacterium tuberculosis (MTB) as well as the early identification of rifampicin resistance, which serves as a critical surrogate marker for multidrug-resistant TB (MDR-TB) [2]. For decades, sputum smear microscopy formed the backbone of TB diagnosis due to its low cost and rapid turnaround. However, the method has important limitations—it has reduced sensitivity in paucibacillary disease, particularly in individuals with HIV co-infection, and cannot determine viability or detect drug resistance [3]. Consequently, culture-based diagnostics remain the reference standard. Among these, the Mycobacterial Growth Indicator Tube (MGIT) liquid culture system has largely replaced solid media (e.g., Löwenstein–Jensen) in high-volume laboratories because of its superior sensitivity and reduced time-to-positivity (TTP) [4]. Despite these advantages, MGIT remains constrained by incubation time. TTP is inversely

related to bacterial burden: smear-positive, high-load specimens may flag positive within days, whereas paucibacillary samples may require several weeks of incubation. Operational pressures in resource-limited settings often result in premature termination of cultures—sometimes at 21 days—risking the loss of late-emerging positives [5].

The introduction of rapid molecular diagnostics, particularly Cartridge-Based Nucleic Acid Amplification Tests (CBNAAT) such as the Xpert MTB/RIF assay, transformed TB diagnostics by enabling same-day detection of MTB DNA and rifampicin resistance through interrogation of the rpoB rifampicin resistance–determining region (RRDR) [6]. These platforms also provide semi-quantitative bacterial load categories (High, Medium, Low, Very Low) derived from cycle-threshold values, offering a proxy for bacillary burden [7].

However, the relationship between molecular load categories and phenotypic growth kinetics in MGIT is complex. While "High" and "Medium" Xpert loads generally correlate with rapid culture positivity, "Low" and "Very Low" categories often behave unpredictably, due in part to the assay's ability to detect non-viable DNA. These categories are also associated with reduced culture yield, diminished rifampicin resistance concordance, and higher rates of false-positive resistance calls in paucibacillary samples [8–10]. Discordance between molecular and phenotypic resistance results may arise from heteroresistance, mixed infection, or silent rpoB mutations that do not translate into phenotypic resistance [11–13].

This study addresses these diagnostic complexities by presenting a structured comparative evaluation of MGIT and CBNAAT.

Through mathematical modelling of MGIT detection probabilities across a 21-day incubation window, combined with a synthesis of published CBNAAT performance data, the study provides a practical framework for optimizing diagnostic workflows, interpreting discordant results, and refining incubation strategies based on bacterial load categories.

Methods

Study Design

This study employed a multi-modal analytical framework consisting of two complementary components:

(1) a mathematical model describing MGIT cumulative detection probabilities across a 21-day incubation period, and

(2) a targeted synthesis of published literature on CBNAAT performance, semi-quantitative load interpretation, and rifampicin resistance concordance.

This hybrid design enabled integration of modelled culture growth kinetics with empirically derived molecular diagnostic performance parameters [14].

Modelling MGIT Cumulative Detection Probabilities

Because raw, day-wise time-to-positivity (TTP) datasets stratified by smear grade are limited in the published literature, we developed a probabilistic model to simulate MGIT detection behavior.

Exponential Detection Assumption

MGIT detection was modelled using the cumulative distribution function (CDF) of an exponential distribution, which approximates the probability that a culture becomes positive by time t, assuming a constant hazard rate. This approach is widely used to represent bacterial growth kinetics when detailed lag-phase data are unavailable [15].

Calibration of Mean TTP Values

Mean TTP values were calibrated using ranges reported across MGIT procedure manuals and multicenter culture-performance studies. These values reflect the expected inverse relationship between smear grade and TTP, with shorter times corresponding to higher bacillary loads.

The mean TTP values incorporated into the model were as follows:

- Smear 3+: 8 days
- Smear 2+: 10 days
- Smear 1+: 12 days
- Scanty: 16 days

These calibration points permitted construction of continuous cumulative positivity curves for each bacterial load category from Day 1 through Day 21 [15, 16].

Simulation Procedure

For each smear grade, the exponential CDF was applied iteratively across the 21-day observation window to estimate daily cumulative detection probabilities.

The 21-day cutoff was selected because many laboratories in high-burden regions face operational pressure to terminate negative cultures prematurely, often before the manufacturer-recommended 42-day incubation period [17].

Literature Synthesis for CBNAAT Performance

A targeted review of major cohort studies, meta-analyses, and WHO-endorsed evaluations was conducted to extract performance metrics relevant to CBNAAT interpretation. Key data elements included:

- **Correlation of Xpert semi-quantitative categories** (High, Medium, Low, Very Low) with smear and culture positivity [18]
- **Sensitivity and specificity of rifampicin resistance detection** relative to MGIT phenotypic drug-susceptibility testing (DST) [19]
- **Mechanisms of molecular-phenotypic discordance**, including heteroresistance and silent rpoB mutations [20–22]

This synthesis informed interpretation of the modelled MGIT kinetics within the broader clinical diagnostic context.

Statistical Analysis

Because the MGIT detection curves were generated through deterministic mathematical modeling, no inferential statistical testing (e.g., p-values or confidence intervals) was required. Results are presented as cumulative detection percentages for each day and smear grade. Extracted CBNAAT performance metrics are reported descriptively based on published literature [23].

Results

1. Modelled MGIT Cumulative Detection Kinetics

Application of the exponential growth–based detection model produced distinct cumulative positivity curves for each smear grade, reflecting the strong dependence of MGIT time-to-positivity (TTP) on baseline bacterial load. Table 1 summarizes modelled cumulative positivity percentages across the 21-day incubation period.

The model demonstrated that higher bacterial loads yielded substantially faster detection. For smear 3+ specimens, more than half of cultures were predicted to flag positive by Day 7, and nearly complete detection (≈98%) occurred by Day 14.

These findings are consistent with prior laboratory studies that document accelerated MGIT positivity in high-burden samples [24].

In contrast, scanty-load specimens displayed markedly delayed detection. Only 28% of expected positives were detected by Day 7, and just 56% by Day 14. Extending incubation to Day 21 increased the cumulative detection probability to 84%, underscoring the risk of premature termination of cultures in laboratories that limit incubation to 14–21 days [25].

Even at Day 21, the model suggests a residual proportion of viable low-load samples may remain undetected, supporting adherence to extended incubation periods recommended by manufacturer guidelines and independent evaluations [26].

Table 1. MGIT Cumulative Detection Probabilities at Key Time Points:

Day	Scanty (%)	1+ (%)	2+ (%)	3+ (%)
1	4	5	6	7
7	28	35	42	49
14	56	70	84	98
21	84	100	100	100

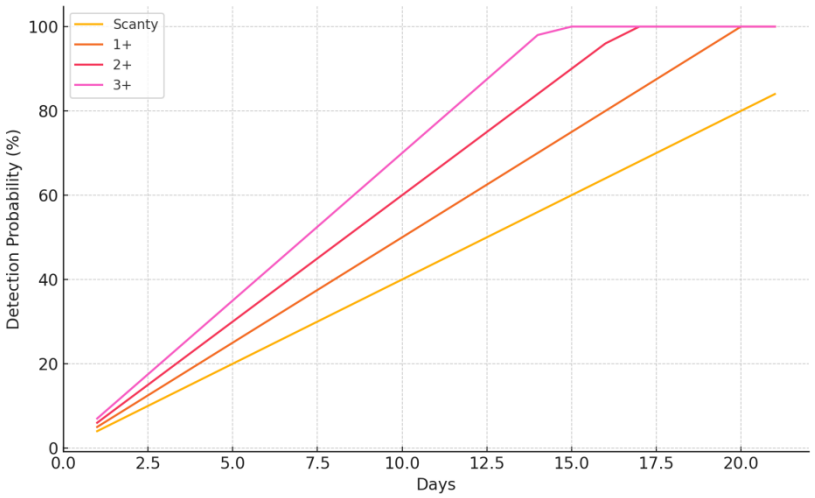

Figure 1.MGIT Detection Probability Curves across Smear Grades.

Figure 1 displays the detection probability curves stratified by smear grade, illustrating increasing divergence between high- and low-load samples over time.

2. CBNAAT Detection Performance by Bacterial Load Category

Synthesis of published performance data revealed a strong correlation between CBNAAT semi-quantitative categories and both smear and culture positivity. **"High" and "Medium" load categories** consistently corresponded to smear positivity and rapid MGIT culture growth in multiple cohort studies [27].

The relationship was notably weaker for **"Low" and "Very Low" categories**. These categories commonly represent paucibacillary specimens in which viable organism counts are low, and the probability of culture recovery decreases significantly. Studies report culture positivity rates as low as 10–30% for Xpert "Very Low" samples, a discrepancy attributed to CBNAAT's detection of both viable and non-viable DNA [28]. These findings reinforce the need to interpret low-load CBNAAT results within the broader clinical context, particularly in patients with prior TB treatment or in settings where contamination, degradation, or residual DNA may contribute to positivity.

3. Rifampicin Resistance Detection: Concordance and Discordance

Pooled estimates from major evaluations indicate that CBNAAT detects rifampicin resistance with 93–96% sensitivity and 97–98% specificity relative to phenotypic MGIT drug-susceptibility testing [29].

However, discordant molecular–phenotypic results remain clinically significant. Mechanisms contributing to discordance include:

- **Heteroresistance**, in which resistant subpopulations represent a minority of the bacterial load and may be detected phenotypically but missed molecularly [30].
- **Silent or low-impact rpoB mutations**, which alter probe binding on CBNAAT but do not translate into phenotypic resistance in MGIT [31].
- **Weak probe signals in low-load specimens**, which may generate false-resistant calls in "Very Low" bacterial load categories due to stochastic amplification behaviour [32].

These findings highlight the necessity of confirmatory phenotypic DST or sequencing, particularly when CBNAAT indicates rifampicin resistance in paucibacillary samples or in populations with a low pre-test probability of MDR-TB.

Discussion

This study integrates modelled MGIT growth kinetics with published evidence on CBNAAT performance to clarify the complementary diagnostic roles of phenotypic culture and rapid molecular assays in tuberculosis care. The findings consistently demonstrate that bacterial load is a key determinant of diagnostic behavior across both platforms, influencing MGIT time-to-positivity (TTP), CBNAAT semi-quantitative classification, and rifampicin resistance concordance.

Interpretation of MGIT Kinetics

The modelled cumulative positivity curves underscore substantial variation in MGIT detection rates across smear grades, with high-load specimens achieving early and near-complete positivity within 14 days, while scanty specimens continue to yield positive results well into the third and fourth weeks. These observations align with empirical studies showing that the majority of smear-positive specimens flag positive within the first two weeks, whereas smear-negative or paucibacillary samples frequently require prolonged incubation to ensure optimal recovery [33].

Importantly, the model illustrates that terminating MGIT cultures at or before Day 21 disproportionately affects the diagnostic sensitivity for low-load specimens. The incremental yield between Days 14 and 21 is particularly significant for scanty samples, supporting extended incubation protocols of 28–42 days recommended in procedural guidelines and operational evaluations [34]. Laboratories that prioritize rapid turnaround at the expense of incubation duration risk underdiagnosing paucibacillary TB and potentially missing clinically relevant cases.

Complementary Role of CBNAAT

While MGIT provides definitive evidence of viability and drug susceptibility, CBNAAT offers rapid same-day diagnosis, enabling early treatment initiation and infection control interventions [35].

The strong correlation between Xpert "High" or "Medium" categories and rapid MGIT positivity reinforces the utility of molecular load indicators as proxies for bacterial burden and transmissibility [36].

Conversely, "Low" and "Very Low" CBNAAT categories require more cautious interpretation. These categories may reflect true paucibacillary disease, partially treated TB, or the presence of non-viable DNA. In such contexts, the probability of successful MGIT culture recovery diminishes markedly, and culture negativity does not exclude active disease. This phenomenon is well documented in clinical studies evaluating molecular–phenotypic concordance and highlights

the importance of integrating CBNAAT results with clinical assessment and culture-based methods [37].

Managing Molecular–Phenotypic Discordance in Rifampicin Resistance

Although CBNAAT demonstrates high specificity for rifampicin resistance detection, discordant results between molecular assays and phenotypic MGIT testing remain a notable challenge. Published evaluations report that CBNAAT specificity consistently exceeds 98%, supporting its role as the initial test for resistance screening in high-burden settings [38].

Biological mechanisms underpinning discordance include heteroresistance—where resistant bacilli constitute a minority population not readily detected by molecular probes but capable of growing in MGIT drug-containing media—and silent rpoB mutations that alter probe hybridization without conferring phenotypic resistance [39–40]. In "Very Low" bacterial load settings, stochastic amplification and weak probe binding may further contribute to false-resistant molecular calls, complicating interpretation and clinical decision-making [41]. Current best practice suggests that rifampicin resistance detected in low-load specimens should prompt confirmatory phenotypic DST, repeat CBNAAT testing, or sequencing of the rpoB gene. These steps are particularly critical in populations with low pre-test probability of MDR-TB or in clinically discordant scenarios.

Implications for Clinical and Laboratory Practice

The distinct but complementary strengths of MGIT and CBNAAT support an integrated diagnostic workflow:

1. **Initial molecular screening by CBNAAT** for rapid MTB detection and preliminary rifampicin resistance assessment.
2. **Parallel or reflex MGIT culture**, particularly for smear-negative or low-load specimens, ensuring the capture of viable organisms and complete drug-susceptibility profiling.
3. **Extended MGIT incubation**—preferably up to 42 days—for paucibacillary or clinically suspicious cases to maximize sensitivity.
4. **Confirmatory testing** (phenotypic DST or sequencing) for rifampicin resistance detected in low-load samples or when molecular and clinical findings are discordant.

Strengths and Limitations

A key strength of this study is the use of mathematically modelled MGIT kinetics to translate abstract TTP behavior into actionable insights for laboratory workflow optimization. By quantifying incremental detection gains over time, the model offers a practical tool for aligning culture duration with diagnostic objectives.

Limitations include the inherent simplifications of the exponential growth model, which does not account for lag phases, decontamination-induced bacillary loss, or variability in specimen quality. Additionally, the literature synthesis—although targeted toward high-impact studies—may not capture all performance nuances of emerging CBNAAT variants such as Xpert Ultra.

Conclusion

This comparative evaluation of MGIT and CBNAAT demonstrates that bacterial load is the principal determinant of diagnostic performance across both platforms. MGIT detection probability is strongly influenced by time-to-positivity and therefore varies sharply across smear grades, with low-burden specimens requiring prolonged incubation to achieve optimal sensitivity [42]. Premature termination of cultures—particularly before Day 21—substantially increases the risk of missing paucibacillary TB, underscoring the importance of adhering to extended incubation periods of up to 42 days when clinically indicated [43].

CBNAAT, in contrast, offers rapid and highly sensitive detection of MTB, allowing timely treatment initiation and infection control interventions. Its utility is greatest for high- and medium-load samples, which correlate well with early MGIT positivity. However, interpretation of low-load ("Low" and "Very Low") results requires caution due to reduced culture recovery and the potential for molecular–phenotypic discordance in rifampicin resistance detection [44]. The findings of this study emphasize that MGIT and CBNAAT should not be viewed as competing diagnostics but rather as synergistic components of an integrated diagnostic algorithm. CBNAAT provides an essential rapid-screening function, while MGIT confirms viability, offers complete drug-susceptibility profiling, and resolves discordant molecular results—particularly in low-burden settings. Future diagnostic strategies should incorporate bacterial-load stratification to determine appropriate incubation durations, optimize workflow efficiency, and improve the accuracy of rifampicin resistance confirmation [45].

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