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NEXT-GENERATION VERSUS CONVENTIONAL MOLECULAR TOOLS: A COMPARATIVE STUDY OF MOLBIO TRUENAT®, GENEXPERT® AND PCR IN TUBERCULOSIS DETECTION

Clinical Research

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ABSTRACT

Background: Tuberculosis (TB) remains a leading infectious cause of mortality worldwide, with approximately 10.6 million new cases annually. The disease, caused by the ***Mycobacterium tuberculosis*** **(MTB)** complex, poses significant challenges to global public health, particularly in resource-limited settings where traditional diagnostic methods often prove inadequate.[1] **Objective:** This comprehensive review systematically examines and compares three molecular diagnostic methodologies for TB detection: **the Molbio TrueNat® system, the GeneXpert® system, and conventional Polymerase Chain Reaction (PCR) techniques**. The analysis focuses on diagnostic performance metrics including sensitivity, specificity, predictive values, and overall accuracy. **Methods:** A systematic literature review was conducted across multiple **databases (PubMed, Scopus, Google Scholar) and institutional repositories from January 2015 to June 2025.** The review incorporates regulatory guidelines from the WHO, US FDA, CE marking authorities, and the ICMR. **Results:** Both **TrueNat** and **GeneXpert** demonstrated performance characteristics comparable to culture-based methods, offering rapid detection and rifampicin resistance identification. TrueNat showed sensitivity ranges of 73–100% and specificity of 87–99%. GeneXpert demonstrated sensitivity of 86–97% and specificity of 97–100%. Conventional PCR showed sensitivity of 77–95% with specificity exceeding 95%. **Conclusions:** Molecular diagnostic platforms represent a significant technological advancement in TB diagnostics. Their endorsement by major regulatory bodies confirms their critical role in contemporary infection control strategies and national elimination programs, providing the rapid and reliable detection capabilities essential for early treatment initiation.

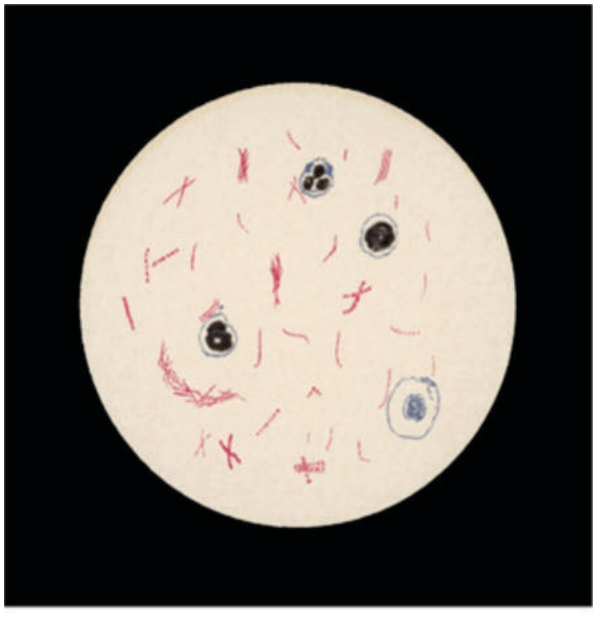
KEYWORDS

TrueNat, GeneXpert, CBNAAT, Conventional PCR, Tuberculosis, Mycobacterium tuberculosis, Diagnostic Accuracy, Molecular Diagnostics, WHO, ICMR.

INTRODUCTION

Global Burden Of Tuberculosis

Tuberculosis, caused primarily by ***Mycobacterium tuberculosis***, remains a formidable global health challenge despite decades of international control efforts.[2]



Mycobacterium tuberculosis.

According to the World Health Organization (WHO) Global Tuberculosis Report 2023, an estimated 10.6 million people developed TB worldwide, with 1.6 million deaths attributed to the disease. The burden is disproportionately concentrated in resource-limited settings, where traditional diagnostic infrastructure and healthcare delivery systems face significant constraints.[3]

The emergence of **drug-resistant TB (DR-TB), including multidrug-resistant (MDR-TB) and extensively drug-resistant TB (XDR-TB)**, has further complicated global control efforts.[4] Rapid and accurate diagnosis of TB and drug resistance patterns has become paramount for effective clinical management and public health response strategies.

Evolution Of Tuberculosis Diagnostic Technologies

Traditional diagnostic methods, including **sputum smear microscopy** and **mycobacterial culture**, have served as cornerstones of diagnosis for decades.[5] However, these conventional approaches present significant limitations, including suboptimal sensitivity (particularly in HIV co-infected individuals), prolonged turnaround times ranging from days to weeks, and substantial infrastructure requirements.

The advent of molecular diagnostic technologies utilizing nucleic acid amplification principles has revolutionized TB diagnostics. These methodologies enable rapid, highly specific detection of ***M. tuberculosis*** **DNA and simultaneous identification of drug resistance mutations.**

Molecular Diagnostic Platforms In Tuberculosis

Three primary molecular diagnostic platforms have gained prominence:

1. Truelab TrueNat® PCR System: A portable, microchip-based real-time PCR platform developed by Molbio Diagnostics.[6] It is capable of point-of-care (POC) deployment and battery operation, making it suitable for decentralized settings.[7]

2. GeneXpert® (Xpert MTB/RIF): An automated, cartridge-based nucleic acid amplification test (CBNAAT) developed by Cepheid, recognized for its standardized performance and global deployment.[8]

3. Conventional PCR: Laboratory-based nucleic acid amplification techniques utilizing various target genes and detection methodologies, requiring sophisticated infrastructure and trained personnel.

Literature Review And Methodology

Search Strategy And Data Sources

A comprehensive systematic search was conducted to identify relevant literature published between January 2015 and June 2025.[9] The strategy encompassed primary databases (**PubMed/MEDLINE, Scopus, Google Scholar, Cochrane Library, Web of Science**) and institutional repositories (**WHO, US FDA, EMA, ICMR, and National TB Programs**).

Search Terms And Inclusion Criteria

Search terms included **"Truelab TrueNat PCR," "CBNAAT," "Xpert MTB/RIF," "Conventional PCR,"** and **"Diagnostic accuracy."** Inclusion criteria focused on peer-reviewed original research, systematic reviews, and regulatory guidelines involving human subjects. Case reports, abstracts without full text, and duplicate publications were excluded.

Molecular Diagnostic Technologies: Technical Specifications

TrueNat® System

Technical Architecture

The Truelab TrueNat system represents an innovative approach to molecular diagnostics, incorporating microchip-based real-time PCR technology within a portable platform.[10]

The system utilizes proprietary chip-based thermocycling technology that enables rapid nucleic acid amplification and real-time fluorescence detection within a compact device weighing approximately 2.8 kg.

Assay Portfolio

- **TrueNat MTB:** Primary screening assay for qualitative detection of the pathogen.
- **TrueNat MTB Plus:** Enhanced sensitivity assay for low bacterial load specimens.
- **TrueNat MTB-RIF Dx:** Specialized assay for detecting rifampicin resistance mutations within the *rpoB* gene.[11]

Operational Advantages

Key features include battery operation (up to 8 hours), minimal infrastructure requirements, temperature stability, and a rapid turnaround time of approximately 60 minutes.[12]

GeneXpert® System

Platform Architecture

The Xpert MTB/RIF system is a fully automated, cartridge-based real-time PCR platform.[13]

The system integrates sample processing, nucleic acid amplification, and result interpretation within a single-use cartridge, minimizing contamination risks.[14]

Assay Variants

- **Xpert MTB/RIF:** Simultaneous detection of MTB and rifampicin resistance (~2 hours).[15]
- **Xpert MTB/RIF Ultra:** Enhanced version with improved sensitivity for paucibacillary disease (HIV co-infection, extrapulmonary TB).[16]

Conventional Polymerase Chain Reaction

Technical Approaches

Conventional PCR encompasses laboratory-based techniques targeting genes such as IS6110, 16S rRNA, and *hsp65*.

Detection Methods

Methods include gel electrophoresis with ethidium bromide staining, real-time PCR with fluorescent probes, and ELISA-based detection.

Diagnostic Performance Analysis

Sensitivity Analysis

Sensitivity varies based on specimen type and bacterial load. As shown in Table 1, Xpert Ultra and TrueNat MTB Plus demonstrate superior performance in smear-negative and pediatric cases.

Table 1: Comparative Sensitivity Analysis By Specimen Type

Diagnostic Method	Smear-Positive Sputum (%)	Smear-Negative Sputum (%)	Extrapulmonary Specimens (%)	Pediatric Cases (%)
TrueNat MTB	92.5 – 98.7	65.4 – 78.9	45.2 – 67.8	58.3 – 72.1
TrueNat MTB Plus	95.8 – 100.0	75.6 – 89.3	58.7 – 75.4	67.9 – 81.2
Xpert MTB/RIF	96.2 – 99.1	78.4 – 85.7	62.1 – 78.9	71.5 – 84.6
Xpert Ultra	97.8 – 99.8	85.3 – 92.1	75.4 – 86.7	78.9 – 89.3
Conventional PCR	89.7 – 95.4	58.9 – 72.3	41.7 – 59.8	52.4 – 65.7

Specificity Analysis

Specificity is consistently high across platforms due to the targeting of MTB-specific genetic sequences.

Table 2: Comparative Specificity Analysis

Diagnostic Method	Overall Specificity (%)	Specificity in HIV+ Patients (%)	Specificity in High TB Burden Settings (%)
TrueNat MTB	87.4 – 99.2	85.7 – 96.8	89.3 – 98.7
TrueNat MTB Plus	95.1 – 99.4	92.6 – 98.1	94.8 – 99.1
Xpert MTB/RIF	97.2 – 99.8	95.8 – 99.3	96.7 – 99.6
Xpert Ultra	95.6 – 98.9	94.1 – 97.8	95.2 – 98.4
Conventional PCR	95.7 – 99.1	93.4 – 97.9	94.8 – 98.6

Drug Resistance Detection Capabilities

Rifampicin Resistance

Rifampicin resistance (RR) serves as a proxy marker for MDR-TB.[17] Both TrueNat and GeneXpert utilize molecular beacons to detect mutations in the rifampicin resistance-determining region (RRDR) of the *rpoB* gene.

Table 3: Rifampicin Resistance Detection Performance

Diagnostic Method	Sensitivity for RIF Resistance (%)	Specificity for RIF Resistance (%)	Turnaround Time
TrueNat MTB-RIF Dx	94.2 – 98.7	97.8 – 99.4	60–90 min
Xpert MTB/RIF	96.8 – 99.2	98.4 – 99.7	90–120 min
Xpert Ultra	97.1 – 99.6	98.1 – 99.5	90–120 min
Conventional PCR + Seq	98.9 – 99.8	99.2 – 99.9	2–5 days

Global Regulatory Approvals And Endorsements

World Health Organization (WHO)

- **TrueNat:** Endorsed in 2020 as an initial diagnostic test replacing smear microscopy, particularly for decentralized settings.[18]
- **GeneXpert:** Endorsed in 2010 (Xpert MTB/RIF) and 2017 (Ultra) as the initial diagnostic test for adults and specific populations (HIV, pediatric).[19]

US FDA And Other Bodies

- **GeneXpert:** FDA 510(k) clearance (2013).
- **TrueNat:** Utilized under WHO authorization and national approvals (e.g., India); currently holds CE marking but no FDA clearance.
- **ICMR:** Validated TrueNat (2017–2019), leading to its integration into India's National TB Elimination Programme (NTEP).

Table 4: Regulatory Status Summary

Platform	WHO Endorsement	FDA Approval	CE Marking	Global Deployment
TrueNat Series	Yes (2020)	No	Yes	40+ countries
Xpert Series	Yes (2010/2017)	Yes (2013/2017)	Yes	130+ countries

Operational Characteristics And Implementation

Infrastructure And Cost Analysis

Molecular platforms demonstrate favorable cost-effectiveness when considering reduced diagnostic delays and transmission prevention, though initial capital costs are higher than microscopy.

Table 5: Cost Analysis per Test (Indian Rupees - INR)

Cost Component	TrueNat	GeneXpert	Conventional PCR
Instrument Cost	□6.6L – 12.4L	□14.0L –	□41.3L – 82.6L
Test Kit Cost	□660 – 990	□825 – 1,405	□415 – 1,240
Total Cost/Test	**□990 – 1,650**	**□1,240 –**	**□2,065 – 4,130**

Note: Costs are approximate and subject to currency fluctuation and

subsidy programs.

Integration Into National Programs

India's NTEP experience with TrueNat (2019–2024) involved deploying over 3,000 machines to peripheral areas, testing over 15 million specimens. GeneXpert remains a staple in intermediate and central laboratories globally.[20]

CONCLUSIONS

Summary Of Key Findings

This comprehensive analysis demonstrates that molecular diagnostic platforms-TrueNat, GeneXpert, and conventional PCR-represent significant technological advancements. All three demonstrate sensitivity ranges of 73–100% and specificity exceeding 95% in most settings.

Future Directions

The TrueNat system offers distinct advantages for decentralized deployment due to portability and battery operation.[21] GeneXpert provides standardized, high-throughput performance suitable for hospital settings.[22] Future developments should focus on next-generation assays with broader drug-resistance panels and digital health integration to further accelerate global TB elimination goals.

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Conflicts Of Interest

The authors declare no conflicts of interest related to this research.

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