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# Comparative Evaluation of Detection Yield of Truenat® Mycobacterium tuberculosis (MTB) Assay and Ziehl–Neelsen Smear Microscopy in Suspected Pulmonary Tuberculosis: A Retrospective Laboratory-Based Study

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## Abstract

Background: Tuberculosis (TB) remains a major public health concern, and timely diagnosis is essential for initiating treatment and reducing transmission. Ziehl-Neelsen (ZN) smear microscopy remains widely used in resource-limited settings because of its simplicity and low cost; however, its sensitivity is limited, particularly in patients with a low bacillary burden. Truenat® *Mycobacterium tuberculosis* (MTB) rapid molecular diagnostic assay (Molbio Diagnostics Limited, Verna, Goa, India) can detect *M. tuberculosis* directly from respiratory specimens and can be followed by testing for rifampicin (RIF) resistance.

Methodology: This retrospective comparative diagnostic study was conducted in the Department of Microbiology at N.K.P. Salve Institute of Medical Sciences & Research Centre and Lata Mangeshkar Hospital, Nagpur, Maharashtra, India, from April 2023 to March 2026. A total of 3,314 respiratory specimens (sputum samples) from patients clinically suspected of pulmonary TB were tested using both ZN smear microscopy and the Truenat MTB assay. Truenat-positive specimens were additionally tested for RIF resistance using the Truenat MTB-RIF assay. Diagnostic performance measures, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were calculated using ZN microscopy as a comparator because a definitive reference standard such as mycobacterial culture was unavailable.

Results: Among 3,314 respiratory sputum samples, ZN microscopy detected acid-fast bacilli in 73 (2.20%), while Truenat MTB detected *M. tuberculosis* in 117 (3.53%). All ZN-positive specimens were also positive by Truenat, while 44 (1.33%) additional cases were detected by Truenat among ZN-negative specimens. Agreement between the two methods was substantial (Cohen's $\kappa = 0.762$; 95% CI, 0.694-0.830), and McNemar's test showed a statistically significant difference in paired detection rates ($\chi^2 = 42.023$; $p < 0.001$; absolute difference 1.33 percentage points; 95% CI, 0.93-1.73). Because ZN microscopy is not a reference standard, these values represent concordance with microscopy only and not diagnostic accuracy against culture.

Conclusions: In this retrospective laboratory-based evaluation, Truenat MTB demonstrated a higher detection yield than ZN smear microscopy and identified additional cases that were missed by conventional microscopy. Its ability to provide rapid molecular detection and RIF resistance testing may offer important advantages for TB diagnosis and early identification of drug-resistant disease. Because no culture or other accepted reference standard was available, the diagnostic accuracy of Truenat MTB could not be independently established; prospective culture-referenced studies are warranted.

**Categories:** Pathology, Pulmonology, Infectious Disease
**Keywords:** molecular diagnosis, pulmonary tb, truenat mtb, tuberculosis, zn microscopy

## Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), remains an important global infectious disease and a major public health challenge. Despite the availability of effective antimicrobial therapy, delayed or missed diagnosis continues to contribute to disease transmission, progression, morbidity, and mortality [1]. Early identification of pulmonary TB is therefore essential not only for timely initiation of treatment but also for interrupting transmission and improving TB control. The continuing burden of TB, particularly in high-burden countries such as India, highlights the need for diagnostic approaches that are accurate, rapid, and accessible in routine healthcare settings [1,2].

Ziehl-Neelsen (ZN) smear microscopy for the detection of acid-fast bacilli has traditionally been one of the

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most widely used methods for diagnosing pulmonary TB, particularly in resource-limited settings [3]. Its low cost, technical simplicity, and relatively modest infrastructure requirements have contributed to its widespread use. However, conventional smear microscopy has limited sensitivity and generally requires a relatively high bacillary concentration for reliable detection [4]. Consequently, patients with paucibacillary disease may have negative smear results despite having active TB. This limitation can be particularly important in early disease and other clinical situations associated with a lower mycobacterial burden [5].

Mycobacterial culture provides greater sensitivity and remains an important reference method for confirming TB. Still, its major disadvantage is the prolonged time required for bacterial growth and subsequent drug susceptibility testing [5]. The delay associated with culture can postpone treatment decisions and potentially allow continued transmission. These limitations have encouraged the development and implementation of rapid molecular diagnostic tests capable of detecting MTB directly from clinical specimens within a much shorter time frame [6].

Nucleic acid amplification tests have substantially changed the diagnostic approach to TB by enabling rapid detection of MTB DNA and, in selected platforms, simultaneous assessment of drug resistance. Molecular assays can improve case detection among patients whose specimens are negative by conventional smear microscopy and can provide clinically relevant information within hours rather than the days or weeks required for culture-based methods [6].

Truenat® MTB assay (Molbio Diagnostics Limited, Verna, Goa, India) is a chip-based real-time polymerase chain reaction (PCR) platform developed for rapid molecular detection of MTB. The platform is designed for use in decentralized and resource-constrained settings and requires comparatively limited laboratory infrastructure. An additional Truenat MTB-rifampicin (RIF) assay can be used to detect RIF resistance, providing an opportunity for early identification of patients who may require further evaluation for drug-resistant TB. The availability of rapid molecular testing is particularly relevant in high-burden settings, where timely diagnosis and appropriate treatment are essential components of national TB control strategies [7].

Previous investigations have demonstrated the potential of Truenat and other molecular diagnostic platforms to improve TB case detection compared with conventional smear microscopy. However, diagnostic performance may vary according to the study population, specimen characteristics, disease burden, and reference standard used. In routine laboratory settings, it is therefore important to evaluate the additional diagnostic yield of molecular testing over conventional microscopy and to assess the feasibility of incorporating these approaches into existing diagnostic workflows [8,9].

The present retrospective, single-centre, laboratory-based study was undertaken to comparatively evaluate the detection yield of the Truenat MTB assay and conventional ZN smear microscopy among clinically suspected cases, using ZN microscopy as the comparator (not as a reference standard). The primary objective was to compare detection rates and quantify the additional yield of the Truenat assay over ZN microscopy. Secondary objectives were to assess agreement between the two methods, to describe RIF resistance among Truenat-positive specimens, and to explore the relationship between semiquantitative smear grade and Truenat cycle threshold (Ct) values. The study does not aim to establish diagnostic accuracy because no culture or other accepted reference standard was available.

# Materials And Methods

## Study design and setting

This retrospective, single-centre, laboratory-based comparative detection-yield study was conducted in the Department of Microbiology, N.K.P. Salve Institute of Medical Sciences & Research Centre and Lata Mangeshkar Hospital, Nagpur, Maharashtra, India. The study period extended from April 2023 to March 2026; March 2026 represents the end of data collection and laboratory record retrieval, not merely the date of result formulation. The study was designed to compare conventional ZN smear microscopy with the Truenat MTB molecular assay for the detection of MTB in respiratory specimens (sputum samples) obtained from patients clinically suspected of having pulmonary TB.

Mycobacterial culture and cartridge-based nucleic acid amplification test (CBNAAT) were not available for this retrospective dataset, and re-testing of stored specimens was not possible. ZN microscopy was therefore used as the comparator to quantify the incremental detection yield of Truenat MTB, not as a reference standard. This design cannot estimate sensitivity or specificity against culture-confirmed TB. It can, however, answer a programmatically relevant question: among specimens routinely submitted to a microscopy laboratory, how many additional MTB detections does a rapid molecular assay provide, and how closely do the two methods agree? These estimates are reported as detection yield and concordance.

## Study population and sample size

Respiratory specimens (sputum samples) obtained from patients clinically suspected of pulmonary TB and tested simultaneously by both ZN smear microscopy [10] and Truenat MTB [11] were eligible for inclusion.

Inadequate, insufficient, leaking, or duplicate specimens, as well as specimens that were not processed by both diagnostic methods, were excluded from the analysis.

A total of 3,314 respiratory specimens (sputum samples) received from patients clinically suspected of pulmonary TB during the study period were included. The specimens were received from both outpatient and inpatient departments and were processed using both ZN smear microscopy and the Truenat MTB assay.

## Data collection

Relevant demographic and laboratory information was retrieved retrospectively from laboratory records and entered into a structured data collection format. Each specimen was assigned a unique identification number to prevent duplication and maintain confidentiality. The recorded variables included sex, age group, ZN smear microscopy results, Truenat MTB results, and RIF resistance results among Truenat-positive specimens.

## Laboratory procedures

The following procedures were performed as part of the routine laboratory diagnostic workflow and are described for reproducibility of the diagnostic context; they were not study-specific interventions. ZN smear microscopy was performed to detect AFB according to standard ZN staining procedures [10].

The Truenat MTB assay was performed using the Truenat chip-based real-time PCR platform for molecular detection of MTB, according to the manufacturer's instructions [7,11]. Adult pulmonary sputum specimens measuring approximately 2-5 mL were collected in sterile sputum containers or 50-mL Falcon tubes. Two drops of liquefaction buffer were added to the specimen, followed by gentle mixing and incubation at room temperature for 10 minutes. When the specimen remained non-pipettable, incubation was extended for an additional five minutes with intermittent swirling.

Following liquefaction, 0.5 mL of processed sputum was transferred into the lysis buffer bottle, followed by the addition of two drops of liquefaction buffer and incubation for three to five minutes. The processed lysate was then loaded into the Truenat extraction cartridge. Automated DNA extraction was performed using the Trueprep® AUTO v2 system (Molbio Diagnostics Limited) for approximately 20 minutes. The extracted DNA eluate was collected in an elution tube (Molbio Diagnostics Limited) and subsequently used for amplification and detection of MTB DNA according to the assay procedure.

Sputum specimens positive for MTB by Truenat MTB were further tested with the Truenat MTB-RIF Dx assayto detect rifampicin resistance, according to the manufacturer's instructions [7,11].

## Outcome measures

*Primary Outcome*

The primary outcome of this study is the detection of MTB by Truenat MTB and by ZN smear microscopy, expressed as detection rates and as the number of additional Truenat-positive/ZN-negative specimens.

*Secondary Outcomes*

Secondary outcomes include: (i) agreement between ZN microscopy and Truenat MTB, quantified by Cohen's κ with 95% CI; (ii) difference in paired detection proportions, tested by McNemar's test with 95% CI for the absolute difference; (iii) proportion of rifampicin resistance among Truenat-positive specimens, with exact 95% CI; (iv) exploratory association between semiquantitative ZN smear grade (scanty, 1+, 2+, 3+) and Truenat Ct values.

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are not primary outcomes; if reported, they are presented explicitly as agreement statistics relative to the ZN comparator and not as diagnostic accuracy.

## Statistical analysis

Data were entered and analyzed using Epi Info™ software (Centers for Disease Control and Prevention, Atlanta, Georgia, United States). Categorical variables were summarized as frequency and percentage. A 2 × 2 contingency table was constructed to compare ZN smear microscopy and Truenat MTB results. Sensitivity, specificity, PPV, and NPV of Truenat MTB were calculated using ZN smear microscopy as the comparator because a definitive reference standard, such as mycobacterial culture or CBNAAT, was not available for this dataset. Agreement between the two diagnostic methods was assessed using Cohen's κ coefficient. The difference in paired test results was evaluated using McNemar's test. A p-value of ≤0.05 was considered statistically significant.

## Results

During the study period (April 2023 to March 2026), laboratory records identified 3,486 sputum samples from 3,102 unique patients with clinical suspicion of pulmonary TB. Of these, 172 specimens were excluded before analysis: 38 were inadequate or insufficient, 21 were leaking, 67 were duplicate specimens from the same patient within the same diagnostic episode, and 46 were not processed by both ZN microscopy and Truenat MTB. The remaining 3,314 specimens from 2,951 unique patients met the inclusion criteria and were analysed. The specimen, not the patient, was the unit of analysis.

A total of 3,314 sputum samples from patients clinically suspected of pulmonary TB were included in the study. The majority of the specimens (n=2,265, 68.3%) were from male patients, whereas female patients accounted for 1,049 (31.7%). The largest proportion of specimens was obtained from patients aged 21-40 years, comprising 2,488 (75.1%) of the study population, followed by the age group of 41-60 years with 785 (23.7%) specimens. The remaining age groups accounted for a small proportion of the study population (Table *1*).

| Characteristic | | Frequency (Percentage) |
|---|---|---|
| Sex | Male | 2,265 (68.3) |
| | Female | 1,049 (31.7) |
| Age group (years) | 0–20 | 15 (0.5) |
| | 21–40 | 2,488 (75.1) |
| | 41–60 | 785 (23.7) |
| | 61–80 | 23 (0.7) |
| | 81–100 | 3 (0.1) |

**TABLE 1: Demographic characteristics of the study population (N=3,314)**

Among the 3,314 sputum specimens examined by ZN smear microscopy, 73 (2.2%) were positive for AFB, while 3,241 (97.8%) were negative. In comparison, Truenat MTB detected MTB in 117 (3.5%) specimens, while 3,197 (96.5%) were negative. Thus, Truenat MTB demonstrated a higher detection yield than conventional ZN smear microscopy (Table *2*).

| Diagnostic method | Positive, n (%) | Negative, n (%) |
|---|---|---|
| Ziehl–Neelsen microscopy | 73 (2.2) | 3,241 (97.8) |
| Truenat MTB | 117 (3.5) | 3,197 (96.5) |

**TABLE 2: Results of Ziehl–Neelsen smear microscopy and Truenat MTB assay* (N=3,314)**

*Molbio Diagnostics Limited, Verna, Goa, India

Figure *2* demonstrates the representative microscopic appearance of AFB on ZN staining. AFB are visualized as red-staining bacilli against the blue background produced by the counterstain, illustrating the microscopic basis for identification of smear-positive specimens.

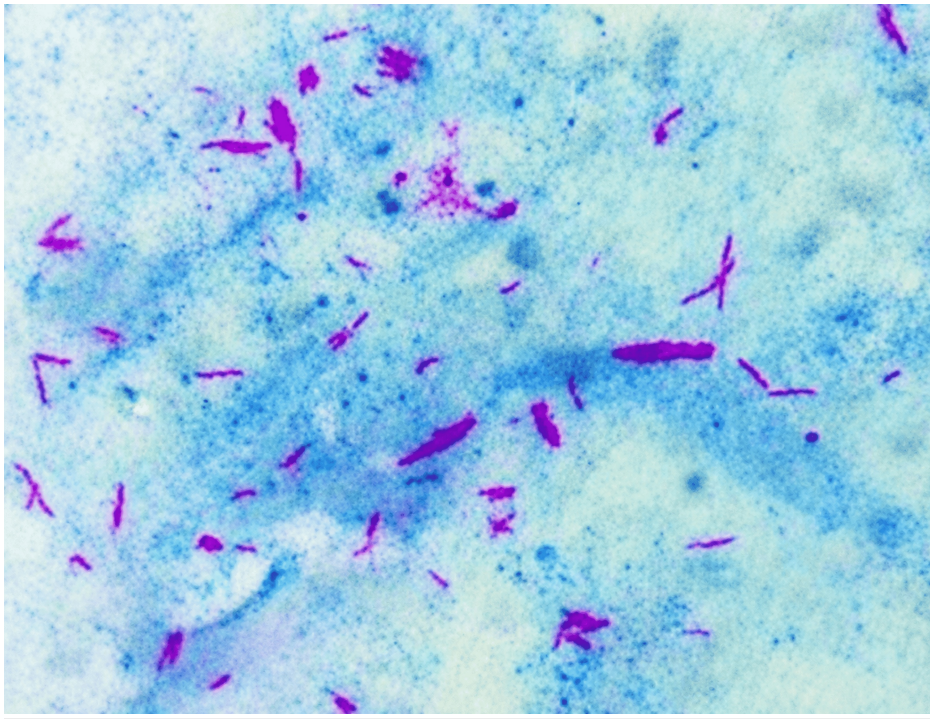


**FIGURE 1: Acid-fast bacilli in Ziehl–Neelsen Staining (100 X)**

Representative microscopic field showing red-staining acid-fast bacilli against a blue background following Ziehl-Neelsen staining. The image demonstrates the characteristic microscopic appearance used to identify smear-positive specimens

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Figure 3 shows a representative Truenat MTB amplification plot, demonstrating the molecular amplification pattern used for detection of MTB DNA in a positive specimen.

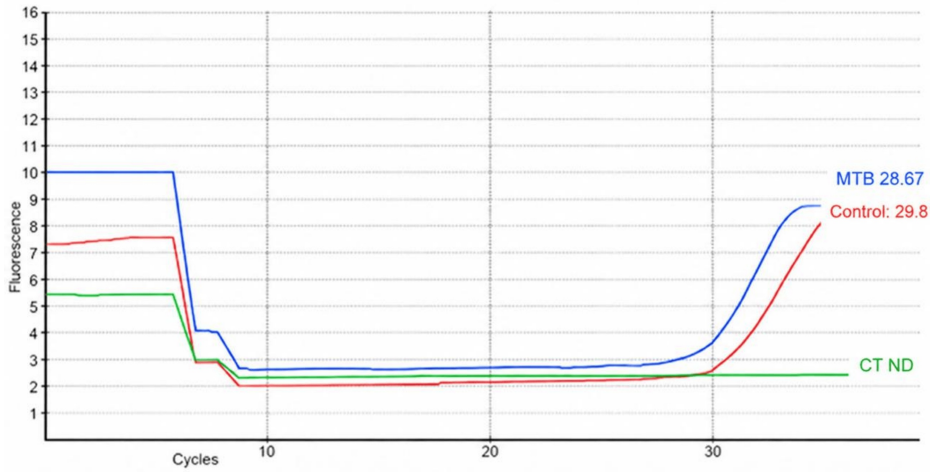



**FIGURE 2: Trunatassy amplification plot**

Representative real-time PCR amplification plot from a Truenat MTB (Molbio Diagnostics Limited, Verna, Goa, India)-positive specimen, demonstrating the amplification pattern used for molecular detection of MTB DNA.

PCR: polymerase chain reaction; MTB: *Mycobacterium tuberculosis;* CT: cycle threshold; ND: not detected

The cross-tabulation demonstrated that all 73 (100.0%) specimens positive by ZN microscopy were also positive by Truenat MTB. In addition, Truenat MTB detected 44 (1.3%) specimens that were negative by ZN microscopy. No specimen was positive by ZN microscopy and negative by Truenat MTB. Overall, Truenat

MTB detected 117 (3.5%) positive specimens compared with 73 (2.2%) detected by ZN microscopy, demonstrating an increased detection yield with molecular testing (Table 3).

| ZN microscopy | Truenat MTB positive, n (%) | Truenat MTB negative, n (%) | Total, N (%) |
|---|---|---|---|
| Positive | 73 (2.2) | 0 (0.0) | 73 (2.2) |
| Negative | 44 (1.3) | 3,197 (96.5) | 3,241 (97.8) |
| Total | 117 (3.5) | 3,197 (96.5) | 3,314 (100.0) |

**TABLE 3: Cross-tabulation of Ziehl–Neelsen microscopy and Truenat MTB* results**

*Molbio Diagnostics Limited, Verna, Goa, India

MTB: *Mycobacterium tuberculosis*; ZN: Ziehl–Neelsen

Using ZN microscopy as the comparator, Truenat MTB demonstrated a sensitivity of 100.0%, specificity of 98.6%, positive predictive value of 62.4%, and negative predictive value of 100.0% (Table 4). These findings indicate strong concordance of Truenat MTB with ZN-positive specimens while also identifying additional specimens that were negative by conventional microscopy. However, these diagnostic performance estimates should be interpreted cautiously because ZN microscopy is not a reference standard; the reported sensitivity of 100% merely indicates that no smear-positive specimen was missed by Truenat and does not represent the sensitivity of Truenat for culture-confirmed TB.

| Diagnostic parameter (vs ZN comparator) | Estimate (%) | 95% CI |
|---|---|---|
| Sensitivity | 100.00 | 95.0–100.0 |
| Specificity | 98.64 | 98.3–98.9 |
| Positive predictive value | 62.39 | 53.1–70.9 |
| Negative predictive value | 100.00 | 99.9–100.0 |

**TABLE 4: Agreement of Truenat MTB with ZN microscopy (comparator-based agreement statistics)**

These values are agreement statistics relative to ZN microscopy and do not represent diagnostic accuracy against mycobacterial culture or any accepted reference standard. The 100% sensitivity estimate indicates only that no ZN-positive specimen was missed by Truenat (Molbio Diagnostics Limited, Verna, Goa, India).

MTB: *Mycobacterium tuberculosis*; ZN: Ziehl–Neelsen

There was substantial agreement between ZN microscopy and Truenat MTB (Cohen's $\kappa = 0.762$; 95% CI, 0.694-0.830). McNemar's test demonstrated a statistically significant difference in the paired detection results between the two methods ($\chi^2 = 42.023$; $p < 0.001$), indicating that Truenat MTB detected significantly more positive specimens than ZN microscopy. The absolute difference in detection rates was 1.33 percentage points (95% CI, 0.93-1.73) (Table 5).

| Statistical measure | Value |
|---|---|
| Cohen's κ | 0.762 |
| 95% CI for κ | 0.694–0.830 |
| Absolute difference in detection rates | 1.33 percentage points |
| 95% CI for difference | 0.93–1.73 |
| McNemar's $\chi^2$ | 42.023 |
| p-value | <0.001 |

**TABLE 5: Statistical comparison and agreement between ZN microscopy and Truenat MTB**

McNemar's test assessed the difference in paired detection results between ZN microscopy and Truenat MTB (Molbio Diagnostics Limited, Verna, Goa, India). A p-value ≤0.05 was considered statistically significant.

MTB: *Mycobacterium tuberculosis*; ZN: Ziehl–Neelsen

Among 117 Truenat-positive specimens, rifampicin resistance was detected in four (3.4%; exact 95% CI, 0.9-8.5). Because multidrug-resistant TB is typically associated with a higher bacillary burden, ZN positivity would be expected in most such cases. Given only four resistant isolates, this proportion is too small to support any inference about the prevalence of rifampicin resistance in the source population and must not be generalised (Table *6*).

| Rifampicin status | Frequency (Percentage) | Exact 95% CI |
|---|---|---|
| Resistant | 4 (3.4) | 0.9–8.5 |
| Sensitive/non-resistant | 113 (96.6) | 91.5–99.1 |
| Total | 117 (100.0) | — |

**TABLE 6: Rifampicin resistance among Truenat MTB*-positive specimens**

*Molbio Diagnostics Limited, Verna, Goa, India

MTB: *Mycobacterium tuberculosis*

## Discussion

TB remains an important public health problem, and timely laboratory diagnosis is essential for appropriate treatment and reduction of transmission [1,2]. ZN smear microscopy remains widely used for its simplicity, low cost, and suitability for routine laboratory settings; however, its sensitivity is limited, particularly in specimens with a low bacillary burden [3-5]. Molecular diagnostic methods have therefore become increasingly important in the diagnosis of pulmonary TB because they can directly detect MTB nucleic acid from respiratory specimens and, on some platforms, provide information on drug resistance [6,7]. The present study compared the detection yield of Truenat MTB and ZN microscopy among patients clinically suspected of pulmonary TB and additionally assessed rifampicin resistance among Truenat-positive specimens.

In the present study, ZN smear microscopy detected AFB in 73 (2.2%) specimens, whereas Truenat MTB detected MTB in 117 (3.5%) specimens. Thus, Truenat identified 44 (1.3%) additional positive specimens among those that were negative by ZN microscopy. Importantly, all 73 ZN-positive specimens were also Truenat-positive, and no specimen was ZN-positive and Truenat-negative. The higher detection yield observed with Truenat is consistent with the established limitations of smear microscopy and the greater analytical sensitivity of nucleic acid amplification assays [3,4,7]. In the multicentre prospective diagnostic accuracy study by Penn-Nicholson et al., Truenat MTB showed high sensitivity and specificity against culture, with performance varying by site and reference standard [7]. Subsequent systematic reviews and meta-analyses have reported pooled sensitivity of approximately 87% and specificity of approximately 86% for Truenat MTB against culture, with wide confidence intervals reflecting heterogeneity across settings [7,9,12]. The present study cannot confirm whether the 44 additional Truenat-positive specimens are true

positives, because neither culture nor clinical follow-up was available; they must be regarded as unresolved discordants rather than confirmed cases. Prospective culture-referenced studies are needed to establish the independent accuracy of Truenat in this setting.

The additional 44 Truenat-positive/ZN-negative specimens are an important finding of this study. Smear microscopy generally requires a relatively high concentration of bacilli for reliable microscopic detection. Therefore, a negative smear does not exclude pulmonary TB, particularly when the bacillary burden is low [3-5]. Molecular assays detect mycobacterial nucleic acid and can therefore identify specimens that may not contain sufficient bacilli for visualization by conventional microscopy [6,7]. The findings of the present study support the potential value of incorporating molecular testing into diagnostic workflows, particularly for patients with smear-negative respiratory specimens in whom pulmonary TB remains clinically suspected.

Published data on the persistence of MTB DNA detection after completion of anti-tubercular therapy (ATT) are limited and platform-dependent. In the absence of treatment history and clinical follow-up in this dataset, the duration of post-ATT Truenat positivity could not be assessed and may have contributed to the additional detections.

Using ZN microscopy as the comparator, Truenat MTB detected all 73 ZN-positive specimens and 44 additional ZN-negative specimens, corresponding to agreement statistics of 100.0% sensitivity, 98.6% specificity, 62.4% PPV, and 100.0% NPV relative to microscopy. Because ZN microscopy is not a reference standard, the reported sensitivity of 100% merely indicates that no smear-positive specimen was missed by Truenat; it does not represent the sensitivity of the Truenat assay for culture-confirmed TB. Likewise, the PPV of 62.4% reflects the imperfect sensitivity of the comparator and cannot be interpreted as a true positive predictive value. These values should therefore be read as agreement statistics only.

The PPV of 62.4% should likewise be interpreted in the context of the comparator used. The 44 Truenat-positive/ZN-negative specimens were considered discordant results rather than being automatically classified as false-positive molecular results. Because culture confirmation or another independent reference standard was unavailable, the present study cannot determine whether these specimens represented true additional detections of pulmonary TB or molecular false-positive results. This limitation is particularly relevant when comparing diagnostic tests in a setting where the conventional test has imperfect sensitivity. Studies evaluating the diagnostic accuracy of molecular TB assays against appropriate microbiological reference standards have demonstrated that their performance estimates depend on the population studied and the reference standard applied [7]. Accordingly, the additional Truenat-positive results observed in this study should be interpreted as an increased molecular detection yield rather than as definitively confirmed additional TB cases.

Another important finding was the detection of RIF resistance among Truenat-positive specimens. Of the 117 specimens positive by Truenat MTB, four (3.4%) were reported as RIF-resistant, while 113 (96.6%) were reported as sensitive/non-resistant. Rapid molecular detection of RIF resistance can provide clinically relevant information early in laboratory evaluation and help identify patients who require further assessment for drug-resistant TB [6,7,13,14]. However, the relatively small number of RIF-resistant specimens in the present study limits interpretation of the observed proportion. It precludes conclusions about the prevalence of RIF resistance in the wider population, and these data should be regarded as descriptive only.

The findings also have practical implications for TB diagnostic services, particularly in settings where access to more complex laboratory infrastructure may be limited. Truenat is designed as a portable molecular diagnostic platform that can support decentralized testing, and previous studies have demonstrated its potential utility for near-care TB diagnosis [7,8]. The ability to combine molecular detection of MTB with assessment of rifampicin resistance may complement conventional smear microscopy and strengthen laboratory-based TB diagnostic workflows [7,8,15-17]. In settings with a substantial burden of TB, improving detection among smear-negative patients may facilitate earlier identification and appropriate clinical management of TB. However, the effect of increased molecular detection on treatment initiation, patient outcomes, or TB transmission was not assessed in the present study.

This study has several limitations. First, it was a retrospective, single-centre, laboratory-based study, and sampling was not explicitly consecutive, so selection bias cannot be excluded, and the findings may not be generalisable to other populations and healthcare settings. Second, mycobacterial culture or another independent reference standard was not available; ZN microscopy was therefore used only as a comparator, and the agreement statistics reported here must not be interpreted as diagnostic accuracy. Third, the Truenat-positive/ZN-negative specimens were not confirmed by culture or clinical follow-up, preventing definitive classification of these discordant results as true additional detections or as false-positive molecular results. Fourth, the unit of analysis was the specimen rather than the patient, so repeat specimens from the same patient may have contributed disproportionately; in addition, the proportion of non-sputum respiratory specimens was not recorded. Fifth, prior history of TB and recent ATT could not be systematically ascertained from the retrospective laboratory records. Because molecular assays may remain

positive for variable periods after mycobacterial death or during post-treatment follow-up, some Truenat-positive/ZN-negative discordants may reflect residual DNA rather than active disease, which is an important source of potential misclassification in the additional-yield estimate. Sixth, semiquantitative smear grades and Truenat Ct values were not available for analysis, so the relationship between bacillary load and molecular amplification could not be assessed. Finally, the study evaluated laboratory test results only and did not assess clinical outcomes, treatment initiation, or treatment response, so the clinical significance of the additional detections remains unknown. These limitations should be considered when interpreting the concordance estimates and the additional detections observed with Truenat.

Despite these limitations, the present study provides real-world laboratory evidence that Truenat MTB detected more MTB-positive specimens than conventional ZN smear microscopy in a cohort of patients clinically suspected of pulmonary TB. The detection of 44 additional Truenat-positive specimens among ZN-negative specimens, together with substantial agreement between the two methods, supports the potential value of molecular testing as a complement to conventional microscopy. The detection of RIF resistance in a small subset of Truenat-positive specimens further demonstrates the potential clinical utility of molecular testing for providing early resistance information, although the number of resistant isolates was too small to support any inference about resistance prevalence. Prospective studies incorporating mycobacterial culture or another accepted reference standard, together with systematic recording of prior ATT history, smear grade and Ct values, and clinical follow-up with treatment outcomes, are warranted to determine the independent diagnostic accuracy and clinical impact of the Truenat MTB assay.

## Conclusions

In this retrospective, single-centre, laboratory-based comparative detection-yield study, the Truenat MTB assay demonstrated a higher detection yield than conventional ZN smear microscopy among patients clinically suspected of pulmonary TB, identifying 44 additional specimens that were negative by smear microscopy. Agreement between the two methods was substantial, and the difference in paired detection rates was statistically significant. Truenat also identified RIF resistance in a small subset of positive specimens, providing clinically relevant information for early management.

Because ZN microscopy was used as a comparator and no culture or other accepted reference standard was available, the study does not establish the diagnostic accuracy or clinical impact of Truenat MTB; the agreement statistics reported here should not be read as accuracy estimates. Prospective, culture-referenced studies with clinical follow-up and treatment outcomes are warranted to establish the independent diagnostic accuracy and programmatic utility of the Truenat MTB assay in this setting.

## Additional Information

### Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

**Concept and design:** Vishwajit Paikrao, Madhusudan Parasram Ganvir, Kalpana Date, Javed Khan

**Acquisition, analysis, or interpretation of data:** Vishwajit Paikrao, Madhusudan Parasram Ganvir, Kalpana Date, Javed Khan

**Drafting of the manuscript:** Vishwajit Paikrao, Madhusudan Parasram Ganvir, Kalpana Date, Javed Khan

**Critical review of the manuscript for important intellectual content:** Vishwajit Paikrao, Madhusudan Parasram Ganvir, Kalpana Date, Javed Khan

**Supervision:** Vishwajit Paikrao, Madhusudan Parasram Ganvir, Kalpana Date, Javed Khan

### Disclosures

**Human subjects:** Informed consent for treatment and open access publication was obtained or waived by all participants in this study. Institutional Ethics Committee, N.K.P. Salve Institute of Medical Sciences and Research Centre and Lata Mangeshkar Hospital issued approval NKPSIMS & RC and LMH/ IEC / 28 / 2026. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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