Indian J Med Res 161, May 2025, pp 482-490
DOI: 10.25259/IJMR_824_2025

Health Technology Innovation

Multicentric validation of the PathoDetect™ MTB RIF & INH assay for simultaneous detection of *Mycobacterium tuberculosis*, & drug resistance to rifampicin & isoniazid in presumptive pulmonary tuberculosis & drug-resistant TB patients

Hansraj Choudhary[1,#], Garima Malik[2,#], Devendra Singh Chauhan[5], Manpreet Bhalla[4], Azger Dusthackeer[6], Prabha Desikan[8], Sidhartha Giri[10], Sandeep Kumar[9], Madhumathi Jayaprakasam[1], Ajay Vir Singh[5], Prabhpreet Sethi[4], Md Shakir Reza[4], V. Mythily[6], V. Thiyagarajan[6], Nikita Panwalkar[8], Jyotismita Tripathy[10], Devdatt Mani[9], Diksha Singh[9], P. M. Ramesh[7], Manjeet Singh Chalga[3], Rajni Rani[2], Nivedita Gupta[1], Ravindra Mohan Pandey[#] & Manjula Singh[2]

Divisions of [1]Communicable Diseases, [2]Delivery Research, & [3]Division of Informatics and Data Centre, [#]Indian Council of Medical Research, [4]Department of Microbiology, National Institute for Tuberculosis and Respiratory Diseases, New Delhi, [5]Department of Microbiology & Molecular Biology, ICMR-National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra, Uttar Pradesh, [6]Department of Bacteriology, ICMR-National Institute for Research in Tuberculosis, [7]Department of Respiratory Medicine, Government Thiruvatteeswarar Hospital of Thoracic Medicine, Chennai, Tamil Nadu, [8]Department of Microbiology, ICMR-Bhopal Memorial Hospital and Research Centre, Bhopal, [9]Microbiology Laboratory, ICMR-National Institute of Research in Tribal Health, Jabalpur, Madhya Pradesh & [10]Department of NRL for Tuberculosis, ICMR-Regional Medical Research Centre, Bhubaneswar, India

Received April 3, 2025; Accepted May 30, 2025; Ahead of print June 20, 2025; Published June 30, 2025

***Background & objectives*: Tuberculosis (TB) remains a major global health concern, with India accounting for 26 per cent of the global burden. Despite advances, access to rapid molecular diagnostics is limited, and the assays currently used in National TB Elimination Programme (NTEP) do not detect isoniazid (INH) resistance upfront. PathoDetect™ MTB RIF & INH is an indigenous closed-system assay that simultaneously detects *Mycobacterium tuberculosis* (MTB) and resistance to rifampicin (RIF) and INH. This study evaluated its diagnostic characteristics.**

***Methods*: In this cross-sectional multicenter study conducted at six TB reference laboratories in India, 1039 participants were enrolled (718 presumptive pulmonary TB, 321 presumptive multidrug resistant TB; MDR-TB). PathoDetect™'s discriminatory ability was assessed using the measures sensitivity and specificity, and its diagnostic performance using positive predictive value (PPV) and negative predictive value (NPV). Liquid culture served as the reference standard for MTB detection, while phenotypic drug susceptibility testing (pDST) and line probe assay (LPA) as reference standards for RIF and INH resistance detection.**

[#]Equal contribution

***Results*: For MTB detection in presumptive pulmonary TB (PTB), PathoDetect™ showed a sensitivity of 98.1 per cent [95% confidence interval (CI): 96.1-99.2], specificity of 94.2 per cent (95% CI: 91-96.5), PPV of 94.9 per cent (95% CI: 92.2-96.9), and NPV of 97.8 per cent (95% CI: 95.5-99.1) with near-perfect agreement with Truenat® (k=0.89). Among 514 confirmed TB cases, PathoDetect™ detected RIF resistance with a sensitivity of 86.5 per cent (95% CI: 80.2-91.5), specificity of 91.6 per cent (95% CI: 88.2-94.3), PPV of 82.3 per cent (95% CI: 75.6-87.8), and NPV of 93.8 per cent (95% CI: 90.7-96.1). For INH resistance, sensitivity was 88.9 per cent (95% CI: 84.1-92.6), specificity 87 per cent (95% CI: 82.4-90.8), PPV 85.6 per cent (95% CI: 80.5-89.8), and NPV 90 per cent (95% CI: 85.7-93.4) using pDST as reference. Truenat® MTB-RIF showed comparable performance for RIF resistance detection (k=0.75). Compared to line probe assay (LPA), PathoDetect™ demonstrated higher sensitivity (93.4 *vs.* 88.8%), specificity (98.2 *vs.* 93.9%), PPV (96.1 *vs.* 86.8%) and NPV (97 *vs.* 94.9%) for RIF resistance detection over Truenat®.**

***Interpretation & conclusions*: PathoDetect™ is a reliable molecular diagnostic tool for detection of MTB and resistance to RIF & INH. The assay showed better RIF resistance detection compared to INH. Its high sensitivity and specificity indicate strong discriminatory ability, while PPV and NPV demonstrate reasonably good diagnostic performance in the study population. These findings support PathoDetect™ as a promising alternative for rapid TB diagnosis, particularly in high-burden settings.**

Key words High throughput assay- isoniazid - MDR-TB- pathodetect - rifampicin - tuberculosis

Tuberculosis (TB) is a chronic airborne disease caused by *Mycobacterium tuberculosis* (MTB) and has reclaimed its position as the leading cause of death from infectious diseases, surpassing coronavirus disease (COVID-19) in 2023[1]. In 2023, an estimated 10.8 million new TB cases and 1.3 million deaths were reported worldwide[1]. India accounted for 26 per cent of the global TB burden, the largest among all high-burden countries[1,2]. In 2023, India reported over 2.55 million TB cases, demonstrating the recovery of its TB program to pre-pandemic level[2]. Despite India's advancements in TB diagnostics, nucleic acid amplification testing (NAAT) at 26.5 per cent remains significantly lower than smear microscopy at 73.5 per cent, highlighting a gap in access to rapid and accurate molecular diagnostictests[2]. Expanding NAAT coverage is crucial for early detection, drug resistance screening, and better treatment outcomes, particularly in high-burden and rural areas. With the Government of India setting ambitious targets to end TB ahead of the Sustainable Development Goal (SDG), there is an urgent need for more reliable and accurate tools for diagnosing TB, including detecting RIF and INH resistance.

Culture-based methods are the gold standard for TB diagnosis but are slow, contamination-prone, and require well-equipped laboratories and highly skilled human resources. Smear microscopy, though widely used, has low sensitivity[3]. In 2022, among 13.9 million tested, 4.5 per cent were smear-positive, while NAAT detected MTB in 18.2 per cent[4] of those tested. To overcome the limitations of these conventional diagnostic methods, the World Health Organization (WHO) has recommended rapid molecular tests, such as Truenat® MTB-RIF Dx (Molbio Diagnostics, India) and Xpert® MTB/RIF (Cepheid, USA), as initial diagnostic tools for all patients presenting with TB symptoms[5]. Molecular tests are preferred for their high sensitivity, specificity, and rapid turnaround time. However, these assays have low throughput for testing samples per day and offer only rifampicin (RIF) susceptibility testing along with MTB detection. WHO-endorsed Line Probe Assays (LPAs) detect specific drug-resistance mutations for first- and second-line TB drugs[6]. Despite their accuracy, LPAs have long turnaround times and are only available at reference laboratories. Culture-based phenotypic drug susceptibility tests (pDSTs), the gold standard, are slow and challenging to implement in resource-limited settings.

Mylab Discovery Solutions Pvt. Limited (Pune, Maharashtra) has developed a multiplex real-time polymerase chain reaction (qPCR) assay for the simultaneous detection of MTB and resistance to RIF and INH[7]. The assay targets specific primer and probe regions, including IS6110 and rrs for MTB detection, inhA and katG for INH resistance, and rifA, rifC, and rifE encompass the 81 bp hotspot region of the rpoB gene for RIF resistance. This two-tube assay (Tube 1-MTB & INH targets; Tube 2-RIF targets) runs on the Compact XL/Q platform, which uses lyophilised reagents and pre-filled extraction cartridges for automated sample processing. The Compact XL system can simultaneously process up to 32 tests, serving as a fully automated nucleic acid extractor.

In contrast, the Compact Q system handles real-time PCR for up to eight samples simultaneously. The assay demonstrated 100 per cent sensitivity and specificity in a limited number of sputum samples, highlighting its potential for rapid and accurate MTB and drug-resistance detection[7].

The present multicenter study was conducted to evaluate the diagnostic characteristics of PathoDetect™ MTB RIF & INH assay (PathoDetect™) for detecting MTB in sputum samples from individuals suspected of pulmonary TB, using liquid culture (BD BACTEC MGIT960) as the reference standard. The study also assessed the diagnostic characteristics of PathoDetect™ for detecting RIF and INH resistance, using pDST and LPA as two separate reference standards.

Materials & Methods

Study design & settings: This cross-sectional, masked, multicentre study was conducted from March 2022 to October 2022 at six sites in India. The study was conducted at six reference laboratories for tuberculosis across various regions of the country: Indian Council of Medical Research (ICMR)-National JALMA Institute for Leprosy and other Mycobacterial Diseases (ICMR-NJIL & OMD) at Agra and National Institute for Tuberculosis and Respiratory Diseases (NITRD) at New Delhi (northern region); ICMR-National Institute for Research in Tuberculosis (ICMR-NIRT) at Chennai (southern region); ICMR-Regional Medical Research Centre (ICMR-RMRC) at Bhubaneswar (eastern region); ICMR-National Institute of Research in Tribal Health (ICMR-NIRTH) at Jabalpur and ICMR-Bhopal Memorial Hospital and Research Centre (BMHRC) at Bhopal (central region). The study received approval from the Institutional Ethics Committees at each site, and written informed consent was obtained from all participants.

The study had two primary objectives. The first was to evaluate the sensitivity and specificity of PathoDetect™ in detecting MTB in sputum samples, using MGIT liquid culture as the reference standard. The second primary objective was to assess the sensitivity and specificity of PathoDetect™ in detecting resistance to Rifampicin (RIF) and Isoniazid (INH), using phenotypic drug susceptibility testing (pDST) and line probe assay (LPA) as two separate reference standards. We also compared the diagnostic characteristics of the 'PathoDetect™' with those of the Truenat® MTB-RIF for detecting MTB and RIF resistance.

Study population: The study population consisted of adult men and women (>18 yr) who presented to tertiary care TB hospitals attached to reference laboratories with a high clinical suspicion of pulmonary TB (PTB). Participant recruitment sites included Pulmonary OPDs at S.N. Medical College, Agra (NJIL&OMD); NITRD, New Delhi; Capital Hospital and PGIMER, Bhubaneswar (ICMR-RMRC); DMC and People's Hospital, Bhopal, and Pulmonary Medicine OPD at BMHRC, Bhopal (BMHRC); Netaji Subhash Chandra Bose Medical College, Jabalpur (ICMR-NIRTH); and the Institute of Thoracic Medicine and GHTM, Otteri, Chennai (ICMR-NIRT).

Presumptive PTB patients with any of the symptoms and sign suggestive of TB, including cough > 2 wk, fever > 2 wk, weight loss, haemoptysis, any abnormality in chest X-ray according to programmatic guideline[8], who consented to provide a minimum of 3 ml sputum samples were recruited at each center after obtaining informed consent. Pregnant women and individuals on immunosuppressive drugs, or those who were on anti-tuberculosis treatment (ATT) or had received ATT for more than seven days, were excluded. Participants who have failed treatment with first-line drugs, contacts of known MDR-TB cases or RIF resistance (RR), previously treated TB cases were also recruited as presumptive MDR-TB patients as per programmatic guideline[8]. Presumptive PTB patients with a positive sputum culture were classified as confirmed TB-positive for sensitivity, while those with a negative culture served as confirmed TB-negative for specificity.

Sample size: Based on manufacturer-generated in-house test performance data[6], we anticipated a sensitivity of 90 per cent with an absolute precision of ±5 per cent and a specificity of 95 per cent with an absolute precision of ±2.5 per cent, both at a 95 per cent confidence level for the PathoDetect™ assay (index test) in detecting MTB and resistance to RIF and INH. Accordingly, a sample size of 140 confirmed TB cases and 295 confirmed TB-negative cases was required for the analysis. Similarly, 140 confirmed drug-resistant TB cases (RIF and INH) and 295 drug-sensitive TB cases were required for the drug resistance analysis. The required sample sizes were achieved by including samples from all six study sites.

Study procedures: After obtaining informed consent, study participants were asked to provide an early morning sputum specimen (≥3 ml) at each hospital site.

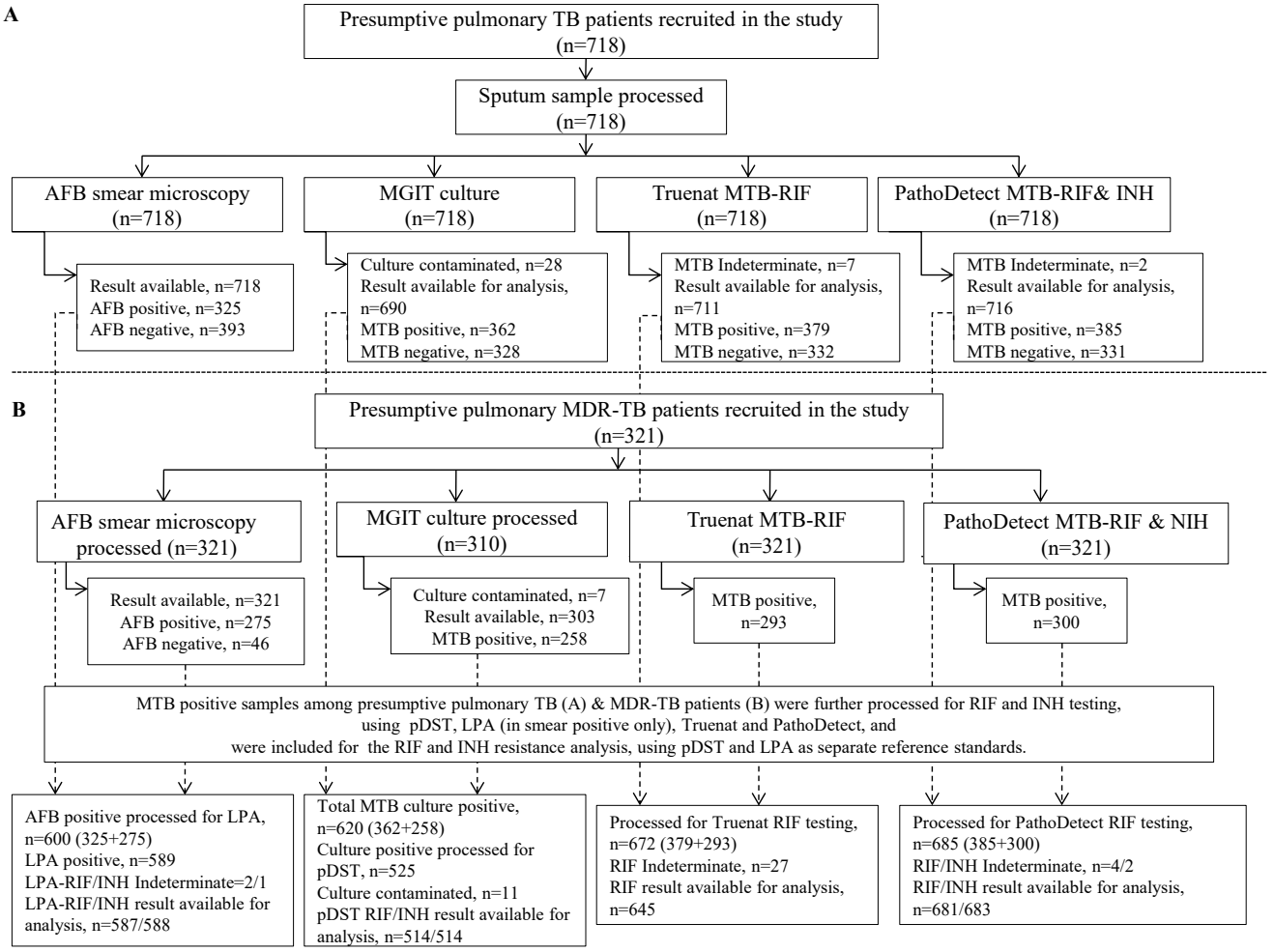

Figure. Study flowchart depicting participant enrollment and laboratory tests conducted on sputum samples from (**A**) presumptive pulmonary TB and (**B**) presumptive MDR-TB patients. MGIT, mycobacterial growth indicator tube; AFB, Acid fast bacilli; LPA, line probe assay; RIF, rifampicin; INH, isoniazid.

If a participant was unwilling to return the next day, a spot sample was collected instead. The specimens were transported to the site under a cold chain and processed on the same day if received before 3 PM; otherwise, they were refrigerated at 4°C and processed the following day. The specimens were homogenised using the N-acetyl-L-cysteine-NaOH (NALC-NAOH) method described in the ICMR-NIRT standard protocol[9] and divided into three portions. One portion was used for smear microscopy and MGIT culture, the second portion was processed for molecular methods (Truenat® and LPA), and the third portion of 200 µl was reserved for processing with the index test using the PathoDetect™ assay, as outlined in figure. Specimens that tested positive for acid-fast bacilli (AFB) by smear microscopy alone were processed for LPA.

Reference standards: A direct smear was prepared for fluorescent smear microscopy for AFB (FM) detection[9], and the remaining specimen was processed using the NALC-NAOH method for liquid culture (MGIT 960) reference standard. Cultures positive for MTB complex were confirmed with MPT64 antigen detection. TB-positive samples were subjected to pDST testing for RIF and INH. According to the manufacturer's instructions, FM smear-positive samples were processed for LPA (Hain Life Science, Germany).

Comparator test: Following the manufacturer's instructions, the second portion of the homogenised sputum specimen was tested using the Truenat® MTB-RIF assay (Molbio Diagnostics Pvt. Ltd., Goa). The Truenat® MTB-RIF assay provides results as MTB

detected or not detected, RIF-resistance detected or not detected, and invalid or indeterminate.

Index test: The last portion of the homogenised sputum sample (200 µl) was blinded and processed for the PathoDetect™ MTB RIF & INH assay with an automated Compact XL/Q system as per manufacturer instructions. The process involved the following steps: nucleic acid extraction and qPCR preparation in Compact XL, amplification and detection on the Compact Q (qPCR reader), and result interpretation with Dx solver software[7]. The results recorded were as MTB Not Detected, MTB Detected with RIF and INH resistance detected, MTB Detected with RIF and INH Sensitive, MTB Detected with RIF Resistance and INH Sensitive, MTB Detected with RIF Sensitive and INH Resistance, indeterminate, or invalid.

Data analysis: Quantitative variables were represented as mean (standard deviation), while categorical variables were expressed as counts (percentages). The analysis included samples with valid results for culture, pDST, LPA, PathoDetect™, and Truenat®, excluding those with culture contamination, missing data, or indeterminate results after one retest. Diagnostic characteristics including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated along with their 95 per cent binomial exact (Clopper-Pearson) confidence intervals (CIs) using Stata statistical software, version 18 (StataCorp LP, College Station, TX, USA).

Participants with presumptive PTB were included to evaluate the diagnostic characteristics of PathoDetect™ in detecting MTB, using MGIT culture as the reference standard. Confirmed TB cases among presumptive PTB and presumptive MDR-TB were included in the drug resistance analysis for RIF and INH, using pDST and LPA as separate reference standards. We also compared the performance of PathoDetect™ with Truenat® using Cohen's kappa statistic. Additionally, we conducted a subgroup analysis to determine the diagnostic characteristics of PathoDetect™ for RIF and INH resistance detection in presumptive PTB and MDR-TB separately. The recent guidelines for conducting studies to evaluate the accuracy of sputum-based tests to detect TB were followed[10].

Results & Discussion

Participants' demographics and test characteristics: The study enrolled 1,039 participants, including 718 presumptive PTB and 321 presumptive MDR-TB patients. Among presumptive PTB, the mean (SD) age was 40.7 (16.4) yr, with 450 (62.7%) males. MDR-TB patients had a mean (SD) age of 36.4 (15.1) yr, with 201 (62.6%) males (Supplementary Table I).

Among presumptive PTB patients, 50.4 per cent (362/718) of samples were culture-positive for MTB, with 9 per cent (26/289) resistant to RIF and 11.1 per cent (32/289) to INH by pDST. Smear positivity was 45.3 per cent (325/718), while MTB detection rates were 52.8 per cent (379/718) for Truenat® and 53.6 per cent (385/718) for PathoDetect™. Of 317 smear-positive LPA-positive samples, LPA identified 21 (6.6%) as RIF-resistant and 38 (12%) as INH-resistant. Truenat® detected RIF resistance in 9.5 per cent (36/379) TB cases, while PathoDetect™ identified 8.6 per cent (33/385) RIF-resistant and 13.2 per cent (51/385) INH-resistant cases (Supplementary Table II). Among presumptive MDR-TB patients, 83.2 per cent (258/310) of samples were culture-positive, with RIF resistance detected in 56.3 per cent (133/236) and INH resistance in 86.4 per cent (204/236) by pDST. Samples from presumptive PTB patients (n=718) were analyzed to assess the performance of PathoDetect™ for MTB detection using MGIT culture as the reference standard. Additionally, TB-positive samples from both groups (514 out of 620 TB positive cases) were evaluated for PathoDetect™'s ability to detect RIF resistance compared to Truenat®, using pDST and LPA as reference standards.

MGIT culture could not be performed on 11 samples from presumptive MDR-TB patients, while 95 TB-positive samples could not be processed for pDST. Furthermore, due to culture contamination, pDST results were unavailable for 11 TB-positive samples. PathoDetect™ showed fewer invalid (0.3%) and indeterminate results (1%) than Truenat® (1% invalid, 4.1% indeterminate) across tested groups (Supplementary Table II).

Diagnostic characteristics of the PathoDetect™ for MTB detection: In the presumptive PTB group, PathoDetect™ demonstrated high test sensitivity of 98.1 per cent [95% confidence interval (CI): 96.1-99.2] and specificity of 94.2 per cent (95% CI: 91-96.5), reflecting strong discriminatory ability for MTB detection, using MGIT culture as the reference. Importantly, it showed high PPV of 94.9 per cent (95% CI: 92.2 - 96.9) and NPV of 97.8 per cent (95% CI: 95.5-99.1) indicating excellent diagnostic performance in the study population (Table I). Truenat® MTB-RIF

Table I. Diagnostic characteristics of PathoDetect™ and Truenat® with culture (MGIT) as the reference standard for detecting tuberculosis in the presumptive pulmonary TB group

PathoDetect™ MTB (Index test)	MTB by MGIT Culture			Truenat® MTB (Comparator)	MTB by MGIT Culture		
	Positive	Negative	Total		Positive	Negative	Total
Positive	355	19	374	Positive	345	18	363
Negative	7	307	314	Negative	16	304	320
Total	362	326	*688	Total	361	322	#683
% Sensitivity (95% CI)	98.1 (96.1-99.2)			%Sensitivity (95% CI)	95.6 (92.9-97.4)		
% Specificity (95% CI)	94.2 (91-96.5)			% Specificity (95% CI)	94.4 (91.3-96.7)		
% PPV (95% CI)	94.9 (92.2-96.9)			% PPV (95% CI)	95 (92.3-97)		
% NPV (95% CI)	97.8 (95.5-99.1)			% NPV (95% CI)	95 (92-97.1)		

*Results of 30 samples (28 culture contaminated & 2 indeterminate in PathoDetect™ MTB test) not included in the analysis.

#Results of 35 samples (28 culture contaminated & 7 indeterminate in Truenat® MTB test) not included in the analysis.

Table II. Strength of agreement between PathoDetect™ and Truenat® for the detection of tuberculosis in the presumptive pulmonary TB group.

PathoDetect™ MTB (Index test)	Truenat® MTB (Comparator)			
	Positive	Negative	Total	
Positive	363	20	383	Cohen's Kappa=0.89 Almost perfect agreement
Negative	16	310	326	
Total	379	330	$709	

$Results of 9 samples (2 indeterminate in PathoDetect™ & 7 indeterminate in Truenat®) not included in the analysis.

showed comparable performance with sensitivity of 95.6 per cent (95% CI: 92.9-97.4) and specificity of 94.4 per cent (95% CI: 91.3-96.7), with an almost perfect agreement (k = 0.89; Table II).

Multiple studies have reported varying diagnostic accuracy of Truenat® for MTB detection[11-14]. A recent meta-analysis reported pooled sensitivity and specificity of Truenat® at 88 per cent (95% CI: 82-92) and 79 per cent (95% CI: 57-92) against MGIT culture[11]. PathoDetect™, the index test in this study, demonstrated higher sensitivity (98.1%) and comparable specificity (94.2%) against culture. Truenat® also showed improved performance here (sensitivity 95.6%, specificity 94.4%) relative to the previous studies. The high sensitivity and PPV observed in this study may be attributed to the higher disease prevalence and the advanced disease status of the study population, which was recruited from tertiary care facilities.

Diagnostic characteristics of the PathoDetect™ for RIF and INH resistance detection: In the combined cohort of 514 confirmed TB cases, PathoDetect™ showed a sensitivity of 86.5 per cent (95% CI: 80.2-91.5) and specificity of 91.6% (95% CI: 88.2- 94.3) for RIF resistance detection, with a PPV of 82.3 per cent (95% CI: 75.6-87.8) and NPV of 93.8 per cent (95% CI: 90.7-96.1; Table III). For INH resistance, sensitivity was 88.9 per cent (95% CI: 84.1-92.6) and specificity 87 per cent (95% CI: 82.4-90.8), with a PPV of 85.6 per cent (95% CI: 80.5-89.8) and NPV of 90 per cent (95% CI: 85.7-93.4) using pDST as the reference. Truenat® performance for RIF resistance was similar, with sensitivity of 88.9 per cent (95% CI: 82.6-93.5) and specificity of 89.9 per cent (95% CI: 86.2-92.9) and comparable PPV and NPV values. Substantial agreement (k=0.75) was observed between PathoDetect™ and Truenat® for RIF resistance detection. These findings align with previous studies indicating robust test discrimination and diagnostic reliability[15-16].

Using LPA as the molecular reference, PathoDetect™ demonstrated better performance than Truenat® for RIF resistance detection, with higher sensitivity (93.4%; 95% CI: 88.8-96.6 *vs*. 88.8%; 95% CI: 83.1-93.1) and specificity (98.2%; 95% CI: 96.4-99.3 *vs*. 93.9%; 95% CI: 91.0-96.1), alongside superior PPV (96.1%; 95% CI: 92.1-98.4 *vs*. 86.8%; 95% CI: 80.8-91.4) and NPV of (97%; 95% CI: 94.8-98.4 *vs*. 94.9%; 95% CI: 92.2-96.9). For INH resistance, PathoDetect™ showed strong diagnostic performance with a PPV of 95.1 per cent (95% CI: 91.4-97.4) and NPV of 94.3 per cent (95% CI: 91.1-96.6; Table III). A detailed 2x2 contingency table is provided as supplementary table III.

Table III. Diagnostic characteristics of PathoDetect™ and Truenat® for detection of rifampicin and isoniazid resistance against phenotypic drug-susceptibility test and line probe assay as the reference standard.

	Reference standard: pDST			
	Rifampicin assay		Isoniazid assay	
	PathoDetect™	Truenat®	PathoDetect™	Truenat®
% Sensitivity (95% CI)	86.5 (80.2-91.5)	88.9 (82.6-93.5)	88.9 (84.1-92.6)	-
% Specificity (95% CI)	91.6 (88.2-94.3)	89.9 (86.2-92.9)	87 (82.4-90.8)	-
% PPV (95% CI)	82.3 (75.6-87.8)	79 (71.9-85)	85.6 (80.5-89.8)	-
% NPV (95% CI)	93.8 (90.7-96.1)	95 (92-97.1)	90 (85.7-93.4)	-
	Reference standard: LPA			
% Sensitivity (95% CI)	93.4 (88.8-96.6)	88.8 (83.1-93.1)	93.3 (89.6-96)	-
% Specificity (95% CI)	98.2 (96.4-99.3)	93.9 (91-96.1)	95.8 (93-97.8)	-
% PPV (95% CI)	96.1 (92.1-98.4)	86.8 (80.8-91.4)	95.1 (91.4-97.4)	-
% NPV (95% CI)	97 (94.8-98.4)	94.9 (92.2-96.9)	94.3 (91.1-96.6)	-

pDST, phenotypic drug-susceptibility test; LPA, line probe assay; positive predictive value; negative predictive value

During the subgroup analysis within the presumptive PTB group, where the prevalence of drug resistance was comparatively lower, both tests showed reduced sensitivity and PPV. Against pDST, PathoDetect™ demonstrated a sensitivity of 45.8 per cent (95% CI: 25.6-67.2) and specificity of 91.8 per cent (95% CI: 87.6-94.9), with a PPV of 35.5 per cent (95% CI: 19.2-54.6) and NPV of 94.5 per cent (95% CI: 90.8-97). Truenat® showed a sensitivity of 65.2 per cent (95% CI: 42.7-83.6), specificity of 92.4 per cent (95% CI: 88.3-95.5), PPV of 45.5 per cent (95% CI: 28.1-63.6) and NPV of 96.5 per cent (95% CI: 93.2-98.5). When using LPA-RIF as reference, sensitivity and PPV improved slightly: PathoDetect™ showed a sensitivity of 57.1 per cent (95% CI: 34-78.2) and a PPV of 70.6 per cent (95% CI: 44-89.7), while Truenat® demonstrated a sensitivity of 63.2 per cent (95% CI: 38.4-83.7) and PPV of 57.1 per cent (95% CI: 34-78.2), with both tests maintaining high specificity and NPV. The agreement between the assays was moderate (k = 0.44). PathoDetect™ also showed lower sensitivity for INH resistance in this group compared to presumptive MDR-TB cohort using both pDST (54.8% *vs*. 94.1%) and LPA (71.1% *vs*. 97%) references (Supplementary Table IV).

The limitation of this study is that the validation was conducted in TB reference laboratories enrolling participants from high-burden setting, with an MTB prevalence of 50.4 per cent by culture and resistance rates of 30.9 per cent for RIF and 45.9 per cent for INH by pDST. Therefore, the findings may not be generalizable to low-prevalence settings. Positive and negative predictive values for detecting MTB and drug resistance using PathoDetect™ assay across different prevalence levels/settings are provided in supplementary Table V. Further studies are needed to validate these findings, particularly in lower TB prevalence settings.

Currently, the diagnostic algorithm under the national TB programme emphasises the use of upfront NAAT to detect RIF resistance in microbiologically confirmed TB patients. An additional sample is collected for referral to the nearest Intermediate Reference Laboratory (IRL) or Culture & Drug Susceptibility Testing (DST) laboratory for LPA testing[2]. PathoDetect™'s comparable performance to Truenat®, which is currently used in the programme, offers a viable alternative for the detection of MTB and RIF resistance. Moreover, PathoDetect provides the added advantage of detecting INH resistance upfront, thereby facilitating early and accurate treatment initiation.

Conclusion

This is the first multicenter validation study evaluating the diagnostic characteristics of the PathoDetect™ MTB RIF &INH assay for detecting MTB and resistance to RIF and INH in sputum samples. PathoDetect™ showed comparable performance to Truenat® for pulmonary TB detection and RIF resistance, with the added advantage of detecting INH resistance. It demonstrated high accuracy for the detection of MTB, as well as RIF and INH resistance, in the study population. As a high-throughput assay with reduced manual steps and lyophilised reagents

that allow room-temperature storage, it is well suited for resource-limited settings. PathoDetect™ presents an opportunity to scale up molecular diagnostics within the national TB diagnostic system, thereby improving TB detection. While the findings are promising, continued efforts are essential to generate additional evidence that supports informed decision-making and guides policy updates during implementation.

***Declaration*:** The following members were part of the "ICMR-Expert Committee on TB Diagnostics": Dr. V. M. Katoch, Former Secy, DHR & DG, ICMR (Chairman); Dr. D. Behera, Former Dean, PGIMER, Chandigarh (Co-Chair); Dr. Rohit Sarin, Principal Consultant NITRD & Technical Advisor NTEP, Gov of India; Dr. Sarman Singh, Director AIIMS Bhopal; Dr. R.M Pandey, Ex-HOD, Biostatistics, AIIMS & Dr. A.S. Paintal, Chair of ICMR; Dr. Camilla Rodriques, Sr. Consultant, Hinduja Hospital, Mumbai; Dr. R P Joshi, DDG (TB), NTEP, Delhi; Dr. Ravi. P. Singh, Sr. Scientist E1, CSIR, Pune; Mr. Rajeev Roy, Sr. FA, ICMR; Dr. Prabha Dasikan, Director BMHRC, Bhopal; Dr. Mandira Varma-Basil, Professor, V. P. Chest Institute, Delhi. The PathoDetect™ MTB RIF & INH assay kit was provided by the manufacturer of the kit 'Mylab Discovery Solutions Pvt. Limited (Pune, Maharashtra)' for validation of the kit. The company also provided manpower at each site for performing index test only. Memorandum of Agreement (MoA) was signed between the manufacturer and ICMR. The MoA has been submitted to the journal.

***Acknowledgment*:** Authors acknowledge the support of ICMR-Expert Committee on TB Diagnostics, the Scientific Advisory and Ethics Committees of the institutes in guiding the study to completion, and the staff of all the reference laboratories for their cooperation, participation, and technical assistance in the conduct of the study.

***Financial support & sponsorship*:** India TB Research Consortium (ITRC) under Indian Council of Medical Research, New Delhi was the coordinating center for the study. No funds were provided separately for the study. The consumables from TB reference laboratories at respective participating Institutes were used for reference and comparator test for the study. Mylab Discovery Solutions Pvt. Limited (Pune, Maharashtra) provided the test kit and manpower for performing index test only.

***Conflicts of Interest*:** None.

***Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation*:** The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

References

1. World Health Organization. *Global Tuberculosis Report 2024*. Available from: *https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024*, accessed on April 29, 2025.
2. Central Tuberculosis Division. National tuberculosis Elimination Programme. Ministry of Health and Family Welfare, Government of India. *India TB Report 2024. Government of India*. Available from: *https://tbcindia.mohfw.gov.in/wp-content/uploads/2024/10/TB-Report_for-Web_08_10-2024-1.pdf*, accessed on April 29, 2025.
3. Lipsky BA, Gates J, Tenover FC, Plorde JJ. Factors affecting the clinical value of microscopy for acid-fast bacilli. *Rev Infect Dis* 1984; *6* : 214-22.
4. Central Tuberculosis Division. National tuberculosis Elimination Programme. Ministry of Health and Family Welfare, Government of India. *India TB Report 2023*. *Government of India*. Available from: *https://tbcindia.mohfw.gov.in/wp-content/uploads/2023/05/5646719104TB_AR_2023_04-04-2023_LRP_final.pdf,* accessed on April 29, 2025.
5. World Health Organization. Institutional Repository for Information Sharing. *WHO consolidated guidelines on tuberculosis: module 3: diagnosis: rapid diagnostics for tuberculosis detection, 2021 update.* Available from: *https://iris.who.int/handle/10665/342331,* accessed on April 29, 2025.
6. World Health Organization. Line probe assays for detection of drug-resistant tuberculosis: interpretation and reporting manual for laboratory staff and clinicians. Geneva: WHO; 2022.
7. Dakhave M, Rale T, Suryawanshi H, Patil N, Suryawanshi A, Kumar R, *et al*. Advanced integrative molecular platform for high-throughput screening of drug-resistant tuberculosis. *Diagn Microbiol Infect Dis* 2024; *109* : 116373.
8. Central Tuberculosis Division, National Tuberculosis Elimination Programme. Ministry of Health and Family Welfare, Government of India. *National Guidelines for Management of Drug-Resistant TB. Government of India*. Available from: *https://tbcindia.mohfw.gov.in/wp-content/uploads/2025/01/National-Guidelines-for-Management-of-DR-TB_Final.pdf,* accessed on April 29, 2025.
9. Department of Bacteriology, Tuberculosis Research Centre, ICMR-NIRT. *Standard Operating Procedure for Mycobacteriology laboratory. 2010*. Available from: *https://nirt.res.in/pdf/bact/SOP.pdf*, accessed on April 29, 2025.
10. Schumacher SG, Wells WA, Nicol MP, Steingart KR, Theron G, Dorman SE, *et al*. Guidance for studies evaluating the accuracy of sputum-based tests to diagnose tuberculosis. *J Infect Dis* 2019; *220* : S99-S107.
11. Mahmoudi S, Sadegh Moghaddasi AH. Evaluation of Truenat® assays for the diagnosis of pulmonary and extrapulmonary tuberculosis: a systematic review and meta-analysis. *Expert Rev Anti Infect Ther* 2024; *22* : 659-668.
12. Gomathi NS, Singh M, Singh UB, Myneedu VP, Chauhan DS, Sarin R, *et al*. Multicentric validation of indigenous molecular test Truenat™ MTB for detection of Mycobacterium tuberculosis in sputum samples from presumptive pulmonary tuberculosis patients in comparison with reference standards. *Indian J Med Res* 2020; *152* : 378-85.

13. Meaza A, Tesfaye E, Mohamed Z, Zerihun B, Seid G, Eshetu K, *et al*. Diagnostic accuracy of Truenat tuberculosis and rifampicin-resistance assays in addis Ababa, Ethiopia. *PLoS One* 2021; *16* : e0261084.
14. Ssengooba W, Katamba A, Sserubiri J, Semugenze D, Nyombi A, Byaruhanga R, *et al*. Performance evaluation of Truenat MTB and Truenat MTB-RIF DX assays in comparison to gene XPERT MTB/RIF ultra for the diagnosis of pulmonary tuberculosis in Uganda. *BMC Infect Dis* 2024; *24* : 190.
15. Penn-Nicholson A, Gomathi SN, Ugarte-Gil C, Meaza A, Lavu E, Patel P, *et al*. A prospective multicentre diagnostic accuracy study for the Truenat tuberculosis assays. *Eur Respir J* 2021; *58* : 2100526.
16. Gomathi NS, Singh M, Myneedu VP, Chauhan DS, Tripathy S, Sarin R, *et al*. Validation of an indigenous assay for rapid molecular detection of rifampicin resistance in presumptive multidrug-resistant pulmonary tuberculosis patients. *Indian J Med Res* 2020; *152* : 482-9.

For correspondence: Dr Manjula Singh, Division of Delivery Research, Indian Council of Medical Research, Delhi110 029, India
e-mail: drmanjulasb@outlook.com; drmanjulasb@gmail.com