Performance of the Truenat tuberculosis and rifampicin-resistance assays in the microscopy centre: a prospective multicentre diagnostic accuracy study

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29

Abstract

Background: Bringing reliable and accurate tuberculosis (TB) diagnosis closer to patients is a key priority for global TB control. Molbio Diagnostics have developed the Truenat point-of-care molecular assays for detection of TB and rifampicin (RIF) resistance.

Methods: We conducted a prospective multicentre study at 19 microscopy centres and seven reference laboratories in Peru, India, Ethiopia and Papua New Guinea to determine the diagnostic accuracy of the Truenat MTB, MTB Plus and MTB-RIF Dx assays in comparison with Xpert MTB/RIF ('Xpert') and Xpert MTB/RIF Ultra ('Ultra') using culture as the reference standard (NCT03712709).

Findings: Of 1,807 enrolled participants with TB signs/symptoms, 24% were culture positive for *Mycobacterium tuberculosis*, of which 15% were RIF-resistant by phenotypic drug sensitivity testing. In microscopy centres, the pooled sensitivity of Truenat MTB and Truenat MTB Plus was 73% [95% CI: 67, 78] and 80% [95% CI: 75, 84], respectively. Among smear-negative specimens, sensitivities were 36% [95% CI: 27, 47] and 47% [95% CI: 37, 58], respectively. Specificity of Truenat MTB and MTB Plus was 98% [95% CI: 97, 99] and 96% [95% CI: 95, 97], respectively. Sensitivity of Truenat MTB-RIF was 84% [95% CI: 62, 95], and specificity was 95% [95% CI: 90, 97].

Interpretation: Truenat assays have comparable accuracy with Xpert and can be performed in microscopy centres and primary health centres.

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Research in Context

Evidence before this study

Both the Cepheid Xpert MTB/RIF and Xpert Ultra MTB/RIF molecular assays, while showing excellent diagnostic performance, are currently limited to operations in district/sub-district hospital settings. In 2016, the World Health Organization (WHO) approved the use of loop-mediated isothermal amplification (TB-LAMP) as an alternative to smear microscopy, although with a low certainty of evidence based on a pooled sensitivity of 78−80% and specificity of 98%. We searched PubMed Central for reports of Truenat for the diagnosis of tuberculosis and RIF resistance using the terms ("Truenat" or "Trueprep") and ("tuberculosis" or "TB"). The search was done on 01 October 2020, with no search date or language restrictions. Two clinical studies were identified, both conducted at PD Hinduja Hospital in Mumbai, India (a site included in the study described here). These were limited in size but showed promising diagnostic accuracy of the Truenat MTB assay. Molbio subsequently modified the design of the Truenat MTB chip to enhance stability; the study presented here is the only such study on a design-locked assay. We did not find any evidence of analytical or clinical studies that assessed the Truenat MTB Plus assay or the Truenat MTB-RIF Dx assay.

Added value of this study

This is the first study to provide robust clinical evidence of the diagnostic performance of the design-locked version of Molbio's Truenat MTB, MTB plus and MTB-RIF Dx assays for detection of MTB and RIF resistance in a primary health care setting. Performance is comparable to Xpert and Ultra, which are currently not available at most level 1 healthcare settings.

Implications of all the available evidence

Based on review of these data, the Truenat MTB, MTB Plus and MTB RIF-Dx assays are now recommended by WHO as an initial test for detection of TB and RIF resistance. The recommendation by WHO provides countries with a sensitive diagnostic tool for TB and RIF resistance that can be used closer to the point of care than Xpert and Ultra. Further studies will be needed to assess the performance of the Truenat assays for diagnosis of TB in people living with HIV, in cases of extra-pulmonary TB, in paediatric TB and using non-sputum based specimens.

Introduction

Effective control of the tuberculosis (TB) epidemic requires rapid diagnosis and initiation of appropriate treatment. However, of the 10 million new TB cases in 2018, up to 3 million cases went undiagnosed.[1] The emergence of multi- and extensively drug-resistant TB (MDR-TB/XDR-TB) has further complicated TB control efforts. Conventional culture and drug susceptibility testing (DST) methods rely on the slow growth of *Mycobacterium tuberculosis* in solid or liquid media, which can take weeks to months to yield results[1] and can lead to prolonged periods of ineffective therapy and ongoing disease transmission. Furthermore, many countries with high TB burdens lack the resources to establish the stringent laboratory conditions needed for these growth-based methods and must rely upon sputum smear microscopy tests which, on average, detect only 45% of TB infections.[2]

In 2018, approximately half a million people were diagnosed with resistance to rifampicin (RIF), one of the most important first-line anti-TB drugs. However, only 51% of all bacteriologically confirmed TB cases diagnosed in 2018 were tested for RIF resistance.[1] Bringing rapid and accurate TB and drug resistance diagnostics closer to patients is a key priority for TB control, particularly to reach patients in low-resource settings and avoid existing high rates of pre-treatment loss to follow up.[3] This requires robust point-of-care diagnostic tests that are easily implementable at lower levels of the healthcare system. The increasing incidence of MDR-TB/XDR-TB also makes the development of rapid molecular tests for RIF resistance detection at the microscopy centre level a priority.

Xpert® MTB/RIF ('Xpert') has revolutionized the diagnosis of both TB and RIF resistance and the Xpert® MTB/RIF Ultra ('Ultra') was developed to achieve even higher sensitivity.[4,5] However, these tests, run on GeneXpert instruments (Cepheid, Sunnyvale, USA), require a

temperature-controlled environment and are susceptible to dust.[5] Molbio Diagnostics Pvt. Ltd. (Bangalore, India) developed three assays that utilize chip-based real-time micro PCR, two for detection of *M. tuberculosis:* the Truenat™ MTB (including the *nrdZ* single copy target) and MTB Plus (including *nrdZ* and multi-copy *IS6110* targets) assays; and one for the detection of RIF resistance: the MTB-RIF Dx reflex assay targeting the rpoB gene.[6,7] These assays can be run from the same DNA eluate[8–11], obtained from the automated bead-based Trueprep® DNA extraction device that uses a universal cartridge-based system to extract DNA from 0·5 mL of sputum in under 20 minutes. The DNA eluate is loaded onto the chip-based Truelab™ micro PCR device to detect the presence of *M. tuberculosis* DNA in approximately 40 minutes. If *M. tuberculosis* is detected, the Truenat MTB-RIF Dx reflex test can similarly be run in the Truelab machine using the same DNA eluate. Both the Trueprep and Truelab devices are portable, battery-operated and can function at up to 40°C ambient temperature and up to 80% relative humidity.[12,13] Here we report results from a multicentre diagnostic accuracy study of the Truenat MTB, MTB Plus and MTB-RIF Dx assays, in which we assessed performance at the microscopy centre level against culture and phenotypic DST as a reference standard and compared against performance of Xpert and Ultra.

Methods:

Study design and settings

This prospective, multicentre diagnostic accuracy study of the performance of the Truenat MTB assays was conducted in 19 clinical sites (each with a microscopy centre attached) and seven reference laboratories across four countries (NCT03712709) (Supplementary Table 1). The countries, Ethiopia, India, Papua New Guinea and Peru, were selected to provide broad representation of the global TB epidemic. The two primary objectives were; firstly, to estimate the diagnostic accuracy of the Truenat assays (MTB and MTB Plus) for *M. tuberculosis* detection among individuals undergoing evaluation for pulmonary TB at a peripheral healthcare facility (microscopy centre) using a culture reference standard; and secondly to estimate the diagnostic accuracy of the Truenat MTB-RIF Dx assay for RIF resistance detection among individuals undergoing evaluation for pulmonary and drug-resistant TB, using phenotypic DST as the reference standard. A secondary objective was to compare the diagnostic accuracy of the Truenat assays to that of Xpert and Ultra, using a reference standard of culture for TB diagnosis and phenotypic DST for detection of RIF resistance.

Participants

The study population comprised men and women above 18 years of age presenting to clinics with symptoms suggestive of pulmonary TB disease, who were willing to provide at least three sputum specimens (>2 mL) at enrolment. Supplementary Table 2 shows the inclusion and exclusion criteria for the study. Participants were recruited sequentially at each clinic or through neighbouring satellite clinics, and enrolled once informed consent was obtained, into one of two groups, a “Case Detection Group” for those without any prior treatment for TB in

the last 60 days, or a "Drug-Resistant Risk Group" for those deemed at risk of drug-resistant TB through prior failed treatment or other programmatic factors.

The study was conducted in accordance with the 1964 Helsinki declaration and its subsequent amendments and approved by the relevant institutional review boards and independent ethics committees. All participants provided informed consent, either written, or if illiterate, as a thumbprint on the consent form signed and dated by an impartial witness.

Procedures

Participants were enrolled at clinics (microscopy centres). All enrolled participants had their medical history reviewed. HIV testing was offered to all participants. Participants were asked to provide three sputum specimens for reference lab testing and an additional specimen for microscopy centre testing, each of at least 2 mL in volume, over two days (Figure 1). For the Case Detection Group, all specimens were collected before initiation of TB treatment. After collection at the clinics, sputum specimens 1, 2 and 3 were transported to the centralized reference laboratory for culture, Xpert/Ultra, Truenat and smear testing. Sputum specimen 4 remained at the attached microscopy centre and was tested using the Truenat assays.

Laboratory testing was performed by index and reference standard tests as per specimen flow (Figure 1; Supplementary Table 3). Quality-assured smear microscopy, liquid and solid culture and DST were performed at the reference laboratories. Two independent sputa per participant were used for each of smear microscopy, liquid (MGIT) and solid (LJ) culture, and phenotypic DST. Further information about specimen collection and processing can be found in the supplementary materials methodology section. GeneXpert systems were used for routine and study-specific Xpert testing according to manufacturer's instructions on direct sputum and decontaminated pellet.[14,15] All reference laboratories used Xpert as the comparator due to Ultra

availability issues at study initiation, except the reference laboratory in Peru, which had access to Ultra.

Truenat testing occurred either in the reference laboratory (Day 1 sputa) or the microscopy centre (Day 2 sputa) and was performed as per the manufacturer's recommendations.[16–18] To compare Truenat performance to that of Xpert and/or Ultra in the reference laboratory, two separate sputa collected on Day 1 were pooled and homogenized by disposable glass beads, then split, processed and tested on both direct (raw) sputum and decontaminated pellet. Quality control was conducted through daily negative control testing (sterile water across all lysis, extraction and PCR steps) and weekly swab testing of workspace, external equipment and internal PCR trays on Truenat MTB Plus chips.

Staff performing Truenat tests were blinded to results of other study tests through the use of specimen codes and staffing assignments. Data were captured through dedicated data-entry systems that were password protected.

Sample size calculation

A sample size of 1,666 participants was selected to allow analysis of 80 smear-negative culture-positive TB cases across sites (95% CI: 55, 77), based on an estimated 67% Truenat MTB Plus sensitivity, a TB prevalence of 20%, and a 30% prevalence of smear-negative, culture-positive TB cases. We estimated 2·8% RIF resistance among all culture-positive TB cases, and 12% prevalence of RIF resistance amongst TB retreatment cases. PD Hinduja Hospital, a DR-TB referral centre in Mumbai, India, was specifically selected to increase enrolment of participants into the Drug-Resistance Risk Group.

Analysis

Participants in the Case Detection Group were included in all analyses, whereas participants in the Drug-Resistant Risk Group were only included in analyses of rifampicin-resistance detection.

Case definitions for primary analyses were as follows:

- The reference standard for TB classification was based on TB culture and *M. tuberculosis* complex (MTBC) identification results: a specimen was defined as TB positive if at least one of the culture results was positive and confirmed MTBC; a specimen was defined as negative if no culture was positive for MTBC and at least two culture results were negative. A TB case was defined as one with any TB-positive specimen.
- For RIF detection, the analyses were based on phenotypic DST results.
- Smear-positive, culture negative specimens were excluded.

Analyses of the diagnostic accuracy of the Truenat index tests and comparator tests were conducted per case or per specimen in the Case Detection Group and reported as point estimates and 95% confidence intervals based on Wilson's score method. Subgroup analyses by site of testing (microscopy centre versus reference laboratory for Truenat), by smear status, TB history and HIV status were performed.

The proportion of non-determinate results, defined as any non-valid results, was assessed in both clinics and reference laboratories. These non-determinate results included both operator errors and equipment/software errors or failures, or invalid results or indeterminate results.

The study protocol and statistical analysis plan are available in the supplementary materials. All statistical analysis was performed using R version 3·5·1.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Participant demographics

Between March 2019 and February 2020 1,917 participants met the eligibility criteria for enrolment across the 19 study sites (Figure 2). After excluding 155 participants due to incomplete data (missing culture or index test results), a total of 1,762 participants remained for the analysis. Of the 1,762 participants, 1,660 (94%) were in the Case Detection Group for analysis of accuracy for MTB detection and 102 (6%) already on treatment regimens at the time of enrolment met the criteria of the Drug-Resistant Risk Group. A total of 331 participants only had a sputum sample collected at the reference lab and not at the microscopy centre, and 21 participants did not have any available culture result.

Demographic and clinical characteristics of the enrolled participant population are shown in Table 1. The median age of participants was 41 years (range 18 to 88 years), with women making up 43% of the total participant population. HIV results were only available for 51% (n=903) of the participants, for whom HIV prevalence was 5·3%, n=48, including 12 diagnosed with active TB. The prevalence of TB (based on the reference standard) across all sites was 24%, with 22% in the Case Detection Group and 66% in the Drug-Resistant Risk Group. Among the 358 culture-positive participants in the Case Detection Group, 32% tested negative by smear microscopy on both specimens. The prevalence of RIF resistance in culture-positive participants, based on phenotypic DST results, was 15% in total (13% among new cases and 24% among participants in the Drug-Resistant Risk Group). Most RIF-resistant cases (31%) were enrolled at PD Hinduja Hospital, a drug-resistant TB referral clinic.

Diagnostic accuracy of the Truenat MTB detection assays

For specimens tested in the microscopy centre, 1,356 participants in the Case Detection Group had valid Truenat results for both the MTB and MTB Plus assays and had valid culture results. Of these, 263 participants were culture positive with MTBC identification; 177 were smear-positive culture-positive and 86 were smear-negative culture-positive.

For testing at microscopy centres, sensitivity was 73% [95% CI 67, 78] for Truenat MTB and 80% [95% CI 75, 84] for Truenat MTB Plus (Table 2 and Supplementary Table 4). Specificity was 98% [95% CI 97, 99] and 96% [95% CI 95, 97] for Truenat MTB and MTB Plus, respectively. Sensitivity for smear-negative, culture positive participant specimens was 36% [95% CI 27, 47] for Truenat MTB and 47% [95% CI 36, 57] for Truenat MTB Plus (Table 2). Comparison of the diagnostic accuracy of Truenat MTB and MTB Plus assays on the same sputum specimens in the microscopy centre showed higher sensitivity for Truenat MTB Plus than Truenat MTB (sensitivity difference = +6·8% [95% CI: +3·5, +20]), with lower specificity (specificity difference = −1·4 [95% CI: −2·5, −0·3]). There was no appreciable difference in Truenat specificity between sputa run in the microscopy centres and the reference laboratories (Supplementary Table 5). Additional sub-analyses by TB history are reported in Supplementary Table 6.

Diagnostic accuracy of the Truenat MTB rifampicin resistance detection assay

DNA extracted from participant sputum with a positive result on either the Truenat MTB or MTB Plus assay was reflexed for subsequent testing on the Truenat MTB-RIF Dx assay. At the microscopy centre the Truenat MTB-RIF Dx assay had 84% [95% CI 62, 95] sensitivity and 95% [95% CI 90, 97] specificity for RIF resistance detection relative to RIF DST (Table 2). The MTB-RIF Dx assay conducted on sputum in the reference laboratories had a sensitivity of 85% [95% CI 73, 92] and specificity of 97% [95% CI 94, 98] (Table 2). There was no difference in performance of the Truenat MTB-RIF Dx assay run in the microscopy centres and the reference laboratories (Supplementary Table 5).

Diagnostic accuracy of Truenat assays compared with Xpert and Ultra

To compare the performance of Truenat with Xpert and Ultra, specimens received in the reference laboratory were split and tested side by side on Truenat and Xpert assays; in Peru Ultra was used instead of Xpert. Among 1,542 participants in the Case Detection Group with valid culture, Truenat, and Xpert or Ultra results, performance of Truenat MTB and MTB Plus was largely comparable to that of Xpert (Figure 3a). In raw sputa, the sensitivities were 82% [95% CI 77, 86] for Truenat MTB, 88% [95% CI 83, 91] for Truenat MTB Plus and 86% [95% CI 81, 90] for Xpert; respective specificities were 97% [95% CI 96, 98] for Truenat MTB, 95% [95% CI 94, 97] for Truenat MTB Plus, and 97% [95% CI 97, 98] for Xpert. In Peru, the only site where Ultra testing was performed, the sensitivities were 72% [95% CI 63, 80] for Truenat MTB, 79% [95% CI 70, 86] for Truenat MTB Plus, and 95% [95% CI 88, 98] for Ultra; respective specificities were 99% [95% CI 98, 100] for Truenat MTB, 98% [95% CI 95, 99] for Truenat MTB Plus, and 97% [95% CI 95, 98] for Ultra (Figure 3a and 3c). There was no significant difference in performance of the Truenat assays compared to Xpert, irrespective of smear status. In Peru, sensitivity was higher in Ultra than Truenat MTB and MTB Plus (Supplementary Tables 6 and 7). Ultra and Truenat MTB specificities were comparable.

For the 252 individuals with valid Truenat TB detection and Xpert results, the sensitivities of Truenat MTB-RIF Dx and Xpert assays for RIF resistance detection were 83% [95% CI: 70, 92] and 88% [95% CI: 75, 95], respectively; and specificity was 97% [95% CI: 93, 98] for Truenat MTB-RIF Dx and 97% [95% CI: 94, 99] for Xpert (Figure 3b). In Peru (the only site where Ultra was used) specimens from 70 participants were reflexed to Truenat MTB-RIF Dx testing, and sensitivity was 100% [95% CI: 65, 100] and specificity 97% [95% CI: 89, 99] for both Truenat MTB-RIF Dx and Ultra tests (Figure 3d). There was no difference in performance of Truenat MTB-RIF Dx against either Xpert or Ultra (Supplementary Tables 6 and 7).

In a sub-analysis of all patients with and without a history of TB disease, the specificity of all Truenat assays was lower in participants with a history of TB disease, as seen for Xpert and Ultra (Supplementary Table 8).

Non-determinate results for Truenat, Xpert and Ultra assays

The proportion of initial Trueprep non-determinate results was 2·4% (113/4731) (Supplementary Table 9). A single round of repeat testing, where possible, resolved results for 88% (98/111) of the specimens that failed on the initial test. Initial test non-determinate proportions for the Truenat MTB and MTB Plus chip were 6·2% (293/4720) and 9·2% (434/4720), respectively. Of the tests that failed, 21% (62/293) and 37% (159/432) remained non-determinate upon repeat testing. Comparatively, the non-determinate rate of Xpert was 2·6% (65/2522), with no failures observed for Ultra (0/786).

The non-determinate rate for the Truenat MTB-RIF Dx assay initial test was 23% (232/1042), of which 73% (157/216) did not resolve where repeat testing was possible. The non-determinate rate increased with low bacterial load in the specimen: the proportion of non-determinate Truenat MTB-RIF Dx results was 6·7% (58/886) if reflexed from a Truenat MTB-positive result vs. 72% (26/36) if reflexed from a specimen that was Truenat MTB-negative but Truenat MTB Plus-positive (Supplementary Table 10).

Discussion

This multicentre diagnostic accuracy study indicates that the rapid molecular Truenat assays have overall comparable performance characteristics to Xpert and could be considered as initial tests for the diagnosis of TB and detection of RIF resistance in primary health care facilities.[19] The specificity of the assays in the microscopy centre was equivalent to that seen in the reference laboratory, with no significant difference in sensitivity for each assay between tests done in the microscopy centres and the reference laboratories.

For TB detection, sensitivity of the Truenat MTB and MTB Plus assays on smear-positive culture-positive specimens at the microscopy centre was 91% and 96%, and amongst smear-negative culture-positive participants sensitivity was 36% and 47%, respectively. The low sensitivity of the Truenat assays in smear-negative participants was unexpected, although not different compared to Xpert. In Peru, the higher sensitivity of Ultra may be related to the inclusion of the *IS1081* target in Ultra, which is missing in the Truenat assays, although interpretation of these results should consider the limited sample size in Peru.

The low incidence of non-determinate Truenat MTB and MTB Plus results provides reassurance that the assays can be performed in primary health care settings. These findings are largely in line with those for Xpert non-determinate results and reflect results seen in early Xpert evaluation studies[20,21], although unlike Xpert, the Truenat assays were conducted in primary health care facilities. However, the proportion of non-determinate results for Truenat MTB-RIF Dx was high: 20% of all initial tests, with 73% of these remaining unresolved upon re-testing. The finding that the Truenat MTB-RIF Dx assay non-determinate rate varied heavily depending on the specimen bacillary load suggests that the increased sensitivity of Truenat MTB Plus to detect MTB is likely higher than that of the Truenat MTB-RIF Dx chip to detect RIF resistance, thereby producing a high number of indeterminate RIF resistance results.

346 The high rate of non-determinate results seen at specific sites and by specific operators
347 highlights the importance of appropriate on-site training, robust quality assurance/quality
348 control programmes and effective remote monitoring. For the Truenat assays, Molbio
349 Diagnostics' integrated online/SIM connectivity systems can facilitate remote monitoring. In
350 addition, it is not uncommon for non-determinate results to be higher than normal when a new
351 system is introduced, with improvements seen as operators gain experience with the systems.
352 In terms of patient-important outcomes, quicker turnaround from testing to treatment can be
353 expected when testing is conducted at microscopy centres.

354 Strengths of this study include the rigorous methodology employed, the use of a robust
355 reference standard, large sample size, and the direct head-to-head comparison with Xpert and
356 Ultra. The study provides an important assessment of molecular TB test diagnostic accuracy in
357 diverse populations representative of the global TB epidemic. However, the difficulty of
358 diagnosing TB in real-world populations contributed to some of the limitations of the study.
359 For example, the number of both HIV-infected participants and RIF-resistant TB cases was
360 small, resulting in imprecise estimates of sensitivity in these groups; further studies will be
361 required to evaluate the accuracy of the Truenat assays in these populations in the primary
362 health care setting. In addition, availability issues meant that only the sites in Peru used Ultra
363 assays, resulting in a small sample size and wider confidence intervals for the assessment of
364 Truenat performance versus Ultra. Finally, while the heterogeneity of sputa from the same
365 participant was controlled for by pooling sputa on Day 1, use of the pooled sputa in the
366 reference laboratory assessments could have artificially increased detection of *M. tuberculosis*
367 in culture and Xpert versus Truenat assessments in the microscopy centre.

368 Overall, this prospective clinical study demonstrates the good overall performance of the
369 Truenat assays in providing rapid and accurate diagnosis of TB and RIF resistance in intended
370 settings of use. These results indicate that the Truenat MTB, MTB Plus and MTB-RIF Dx

assays have similar accuracy to that of Xpert and Ultra and can be performed at the microscopy centre level, although data were limited for the MTB-RIF Dx assay. Findings from the Truenat assays have been reviewed by WHO and meet the minimal criteria for recommendation for use as an initial test for detection of TB and RIF resistance rather than smear microscopy, culture and phenotypic DST.[19]

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Data sharing statement

Individual, de-identified participant data will be shared, including data dictionaries. Other documents that have been made available include the study protocol and statistical analysis plan. Templates of the informed consent forms may be shared upon request. The data will be available immediately following publication with no end date. The data will be shared with anyone who wishes to access the data. The data will be available for any purpose of analyses. For data, please contact the corresponding author.

Study team members

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Contributors

APN, AMa, PN, MJ, CMD, SGS designed the study; APN, NSG, SC, CB, RG, SS, PdC, ST, MS, MR, CMD and SGS oversaw the study. NSG, CUG, AMe, PP, BC, EL, CM, ET, EG coordinated the individual study sites. Statistical analysis was undertaken by AMa. The manuscript drafts were developed by TU, APN, CMD and SGS with input from the authors. All authors contributed to interpretation of data and editing of the article and approved the final version of the manuscript.

Declaration of interests

APN, AM, CM, MR, PN and SGS are employed by the Foundation for Innovative New Diagnostics (FIND). FIND is a not-for-profit foundation that supports the evaluation of

publicly prioritized tuberculosis assays and the implementation of WHO-approved (guidance and prequalification) assays using donor grants. FIND has product evaluation agreements with several private sector companies that design diagnostics for tuberculosis and other diseases. These agreements strictly define FIND's independence and neutrality with regard to these private sector companies

Tables and figures

Table 1. Demographic and clinical characteristics of enrolled participant population

	All	India				Peru	Ethiopia	Papua New Guinea
		Hinduja	Guwahati	Chennai	Ahmedabad			
N	1762	144	256	319	290	394	196	163
Age (years), median [min - max]	41 [18 - 88]	39 [18 - 86]	42 [18 - 82]	48 [19 - 83]	47 [19 - 85]	38 [19 - 88]	37 [18 - 81]	34 [18 - 78]
Female sex (%), (n/N)	43% (762/1762)	50% (71/144)	36% (91/256)	43% (136/319)	36% (103/290)	50% (196/394)	51% 99/196	40% (66/163)
HIV-infected (%)*	5·32% (48/903)	1·61% (1/62)	0% (0/5)	0% 0/313)	0·61% (1/165)	2·68% (7/261)	61% (28/46)	22% (11/51)
Culture positive (%), (n/N)	24% (425/1762)	71% (102/144)	23% (59/256)	13% (40/319)	19% (55/290)	24% (96/394)	12% (24/196)	30% (49/163)
Smear-negative, culture-positive (%), (n/N)	30% (128/425)	22% (22/102)	25% (15/59)	38% (15/40)	18% (10/55)	44% (42/96)	33% (8/24)	33% (16/49)
DST RIF-resistant among culture positive (%), (n/N)	15% (63/425)	31% (32/102)	19% (11/59)	2·5% (1/40)	5·5% (3/55)	11% (11/96)	4·2% (1/24)	8·2% (4/49)
DR Risk Group (%), (n/N)	5·8% (102/1762)	67% (96/144)	0% (0/256)	0% (0/319)	1·0% (3/290)	0·8% (3/394)	0% (0/196)	0% (0/163)

DR-TB Risk Group, Drug-Resistant Risk Group; DST, drug-susceptibility testing; RIF, rifampicin.
*Proportion of HIV infection was reported based on available test results.

Table 2. Performance of Truenat assays for TB and for RIF resistance detection at the microscopy centre and the reference laboratory

	N	TP	FP	FN	TN	Sensitivity % (95% CI)		Sensitivity % Smear Positive (95% CI) - N		Sensitivity % Smear Negative (95% CI) - N		Specificity % (95% CI)	
Microscopy centre sputum													
Truenat MTB	1356	192	25	71	1068	73·0	[67·3,78·0]	91·0	[85·8,94·4] - N:177	36·0	[26·7,46·6] - N:86	97·7	[96·7,98·5]
Truenat MTB Plus	1356	210	40	53	1053	79·8	[74·6,84·2]	96·0	[92·1,98·1] - N:177	46·5	[36·4,57·0] - N:86	96·3	[95·1,97·3]
Truenat MTB RIF-Dx	190	16	9	3	162	84·2	[62·4,94·5]	87·5	[64·0,96·5] - N:16	66·7	[20·8,93·8] - N:3	94·7	[90·3,97·2]
Reference lab sputum													
Truenat MTB	1541	275	27	71	1168	79·5	[74·9,83·4]	95·8	[92·4,97·7] - N:236	44·5	[35·6,53·9] - N:110	97·7	[96·7,98·4]
Truenat MTB Plus	1541	295	51	51	1144	85·3	[81·1,88·6]	98·3	[95·7,99·3] - N:236	57·3	[47·9,66·1] - N:110	95·7	[94·4,96·7]
Truenat MTB RIF-Dx	332	44	9	8	271	84·6	[72·5,92·0]	86·7	[73·8,93·7] - N:45	71·4	[35·9,91·8] - N:7	96·8	[94·0,98·3]

CI, confidence interval; FN, false negative; FP, false positive; TN, true negative; TP, true positive.

Note: Analysis of Truenat performance is shown on specimens tested at the microscopy centre and at the reference laboratory separately, with valid results available for both the Truenat MTB assay and the Truenat MTB Plus assay; denominators differ as two sites (PD Hinduja hospital and Papua New Guinea) only had reference lab facilities available. Comparative performance of each assay performed on samples processed in the microscopy centre or the reference lab are shown Supplementary Table 5.

Figure 1. Specimen flow at enrolment

DST, drug susceptibility testing; LJ, Löwenstein Jensen; MGIT, mycobacterial growth indicator tube; RIF, rifampicin.

Note: Sputum 4 was not collected at PD Hinduja Hospital or in Papua New Guinea. All sites performed Xpert MTB/RIF except Peru, which performed Xpert MTB/RIF Ultra.

507

508 **Figure 2: STARD figure showing the number of participant enrolled excluded and with data analysed**

509 CRF, case report form; DR Risk Group, Drug-Resistant Risk Group; TB, tuberculosis.

510 Note: Truenat non-determinate results are excluded from the accuracy analyses but are reported separately.

511

Figure 3. Performance of the Truenat, Xpert and Ultra assays conducted at the reference laboratories

a) Performance of Truenat and Xpert for TB detection

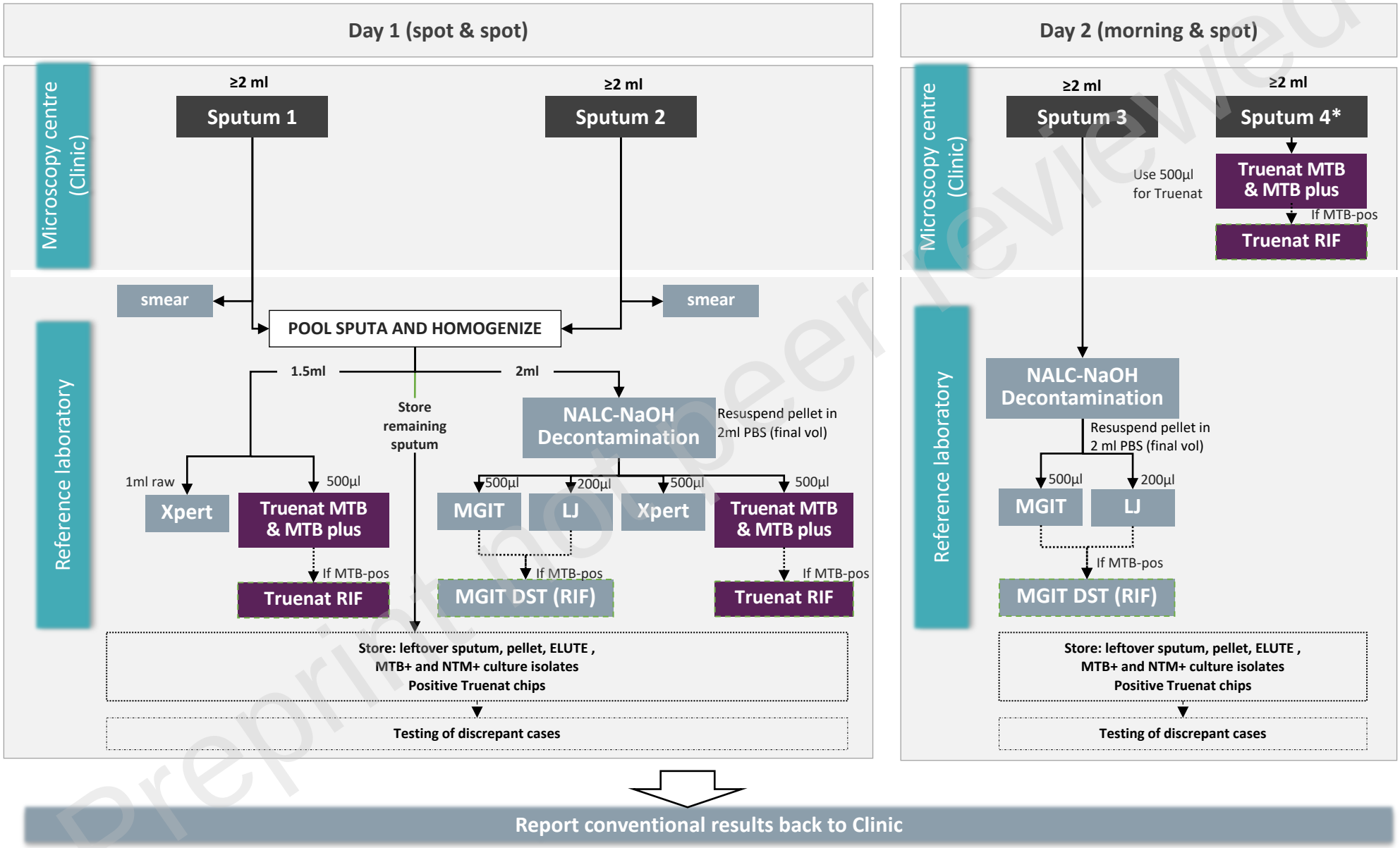
b) Performance of Truenat and Xpert for rifampicin resistance detection

c) Performance of Truenat and Ultra for TB detection

d) Performance of Truenat and Ultra for rifampicin resistance detection

Figure legend: a) Diagnostic accuracy for TB detection, compared with Xpert. Only participants in the Case Detection Group are included. b) Performance for RIF resistance detection compared with Xpert. Participants in both the Case Detection and the Drug-Resistant Risk Group were included. c) Diagnostic accuracy for TB detection, compared with Ultra. Only participants in the Case-Detection Group are included. d) Diagnostic accuracy for RIF resistance detection compared with Ultra. Participants in both the Case Detection and the Drug-Resistant Risk Group were included. For c and d, analysis was done exclusively on data from participants enrolled in Peru where only Ultra testing was conducted. Squares represent point estimates and bars represent 95% confidence intervals

Figure 1: Specimen flow at enrolment



DST, drug susceptibility testing; LJ, Löwenstein Jensen; MGIT, mycobacterial growth indicator tube; RIF, rifampicin.
Sputum 4 not required in PNG or PD Hinduja Hospital; All sites used Xpert MTB/RIF, except Peru which used Xpert MTB/RIF Ultra

Figure 2: STARD figure showing the number of participant enrolled excluded and with data analysed

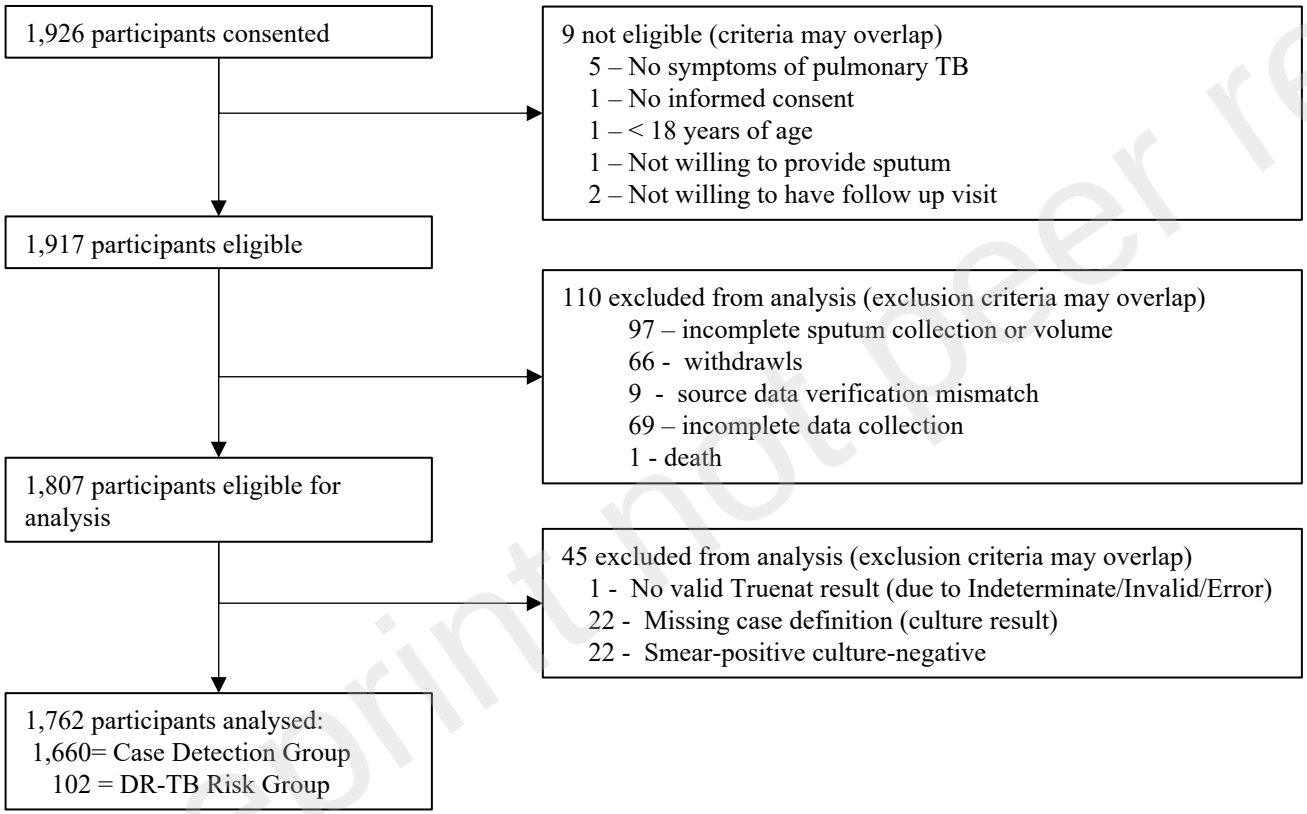


Figure 3: Performance of the Truenat, Xpert and Ultra assays conducted at the reference laboratories
A) Performance of Truenat and Xpert for TB detection
B) Performance of Truenat and Xpert for rifampicin resistance detection

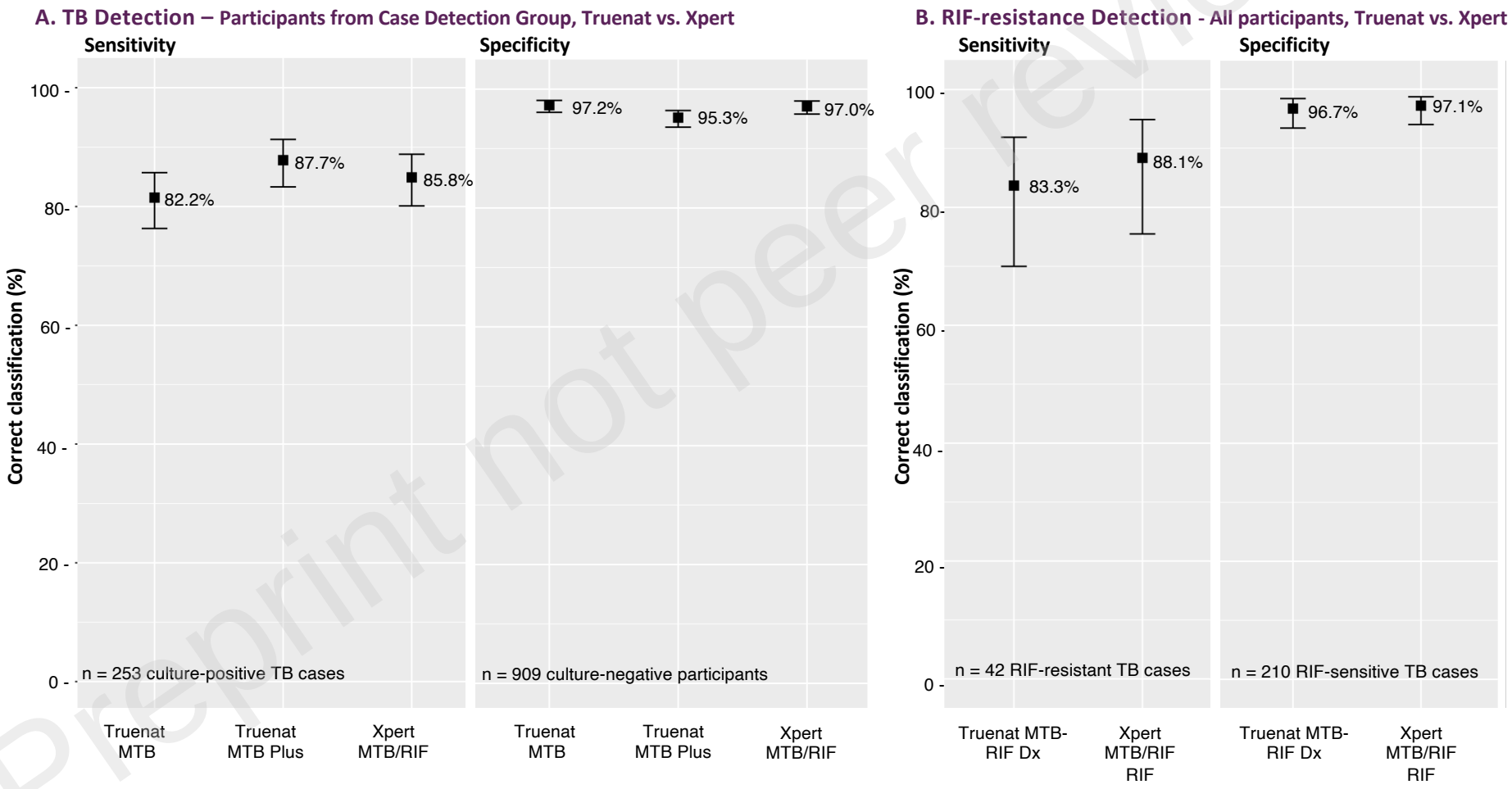


Figure 3 (cont): Performance of the Truenat, Xpert and Ultra assays conducted at the reference laboratories
C) Performance of Truenat and Ultra for TB detection
D) Performance of Truenat and Ultra for rifampicin resistance detection