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Early View

Original article

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A prospective multicentre diagnostic accuracy study for the Truenat tuberculosis assays

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Short title: Diagnostic accuracy of the point-of-care Truenat tuberculosis assays

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Take home message: Diagnostic performance of Molbio's point-of-care Truenat assays in primary health care centres is comparable to that of Xpert MTB/RIF placed in reference laboratories. WHO now recommends Truenat as an initial test for detection of TB and RIF resistance.

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 diagnostic accuracy study at 19 primary health care centres and seven reference laboratories in Peru, India, Ethiopia and Papua New Guinea to estimate the diagnostic accuracy of the point-of-care Truenat MTB, MTB Plus and MTB-RIF Dx assays for pulmonary TB using culture and phenotypic drug susceptibility testing as the reference standard, compared to Xpert MTB/RIF or Ultra.

Results: Of 1,807 enrolled participants with TB signs/symptoms, 24% were culture positive for *Mycobacterium tuberculosis*, of which 15% were RIF-resistant. 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. Sensitivity of Truenat MTB-RIF was 84% [95% CI: 62, 95]. Truenat assays showed high specificity. Head-to-head comparison in the central reference laboratories suggested that the Truenat assays have similar performance to Xpert MTB/RIF.

Conclusion: We found performance of Molbio's Truenat MTB, MTB plus and MTB-RIF Dx assays to be comparable to that of the Xpert MTB/RIF assay. Performing the Truenat tests in primary health care centres with very limited infrastructure was feasible. These data supported the development of a WHO policy recommendation of the Molbio assays.

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Introduction

Effective control of the tuberculosis (TB) epidemic requires rapid diagnosis and initiation of appropriate treatment. However, of the estimated 10 million new TB cases in 2019, 2.9 million cases went undiagnosed [1]. Only 61% of bacteriologically confirmed TB cases were tested for rifampicin (RIF) resistance [1]. 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].

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.

Xpert® MTB/RIF and Xpert® MTB/RIF Ultra ('Ultra')) have revolutionized the diagnosis of both TB and RIF resistance [4, 5], with Xpert MTB/RIF demonstrating pooled sensitivity of 85% (82-88%) and specificity of 98% (94-97%), and Ultra providing slightly higher sensitivity of 88% (85-91%) and slightly lower specificity 96% (94-97%) in a recent systematic review [6]. However, these tests, run on GeneXpert instruments (Cepheid, Sunnyvale, USA), require a temperature-controlled environment, a stable power supply and are susceptible to dust [5, 7–10], limiting operation to district/sub-district hospital settings. A novel point-of-care, cost-effective assay with higher performance and/or a robust, battery

operated assay with minimal operational requirements could provide a viable alternative to Xpert and drive greater access for TB testing. 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 *nrdB* 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 [11, 12]. These assays can be run from the same DNA eluate [13–16], 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 [17, 18].

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 primary health care centre level against culture and phenotypic DST as a reference standard and compared against performance of Xpert MTB/RIF, Ultra and the Truenat assays conducted at centralised reference laboratories.

Methods:

Study design

This prospective, multicentre diagnostic accuracy study of the performance of the Truenat tuberculosis assays was conducted in 19 clinical sites (with attached microscopy centres) and seven reference laboratories across Ethiopia, India, Papua New Guinea and Peru (NCT03712709) (Supplementary Table 1). The study population comprised adult men and women presenting to clinics with symptoms suggestive of pulmonary TB disease (Supplementary Table 2). Participants were recruited sequentially at each clinic or through neighbouring satellite clinics, and enrolled once informed consent was obtained, into one either, a "Case Detection Group" or a "Drug-Resistant Risk Group" (Supplement).

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 enrolled at primary health care centre clinics were asked to provide three sputum specimens for reference laboratory testing and an additional specimen for microscopy centre testing (Figure 1, Supplement). Sputum specimens 1, 2 and 3 were transported to the centralized reference laboratory for culture, Xpert MTB/RIF or Ultra, Truenat and smear testing. Sputum specimen 4 remained at the attached microscopy centre for Truenat assay testing.

Laboratory testing was performed by index and reference standard tests (Figure 1; Supplementary Table 3). Quality-assured smear microscopy (predominantly Ziehl-Neelsen staining, where PD Hinduja used auramine-O fluorescence staining and Peru used both methods), liquid (MGIT) and solid (LJ) culture, BACTEC MGIT 960 phenotypic DST and speciation [19] were performed at the reference laboratories using two independent sputa per participant. All reference laboratories used Xpert MTB/RIF as the comparator due to Ultra availability issues at study initiation, except the reference laboratory in Peru, which only used Ultra.

Truenat testing was done either in the reference laboratory (Day 1 sputa) or the microscopy centre (Day 2 sputa) and was performed as per the manufacturer's recommendations [20–22]. Truenat test results were not shared with clinical staff and did not influence patient treatment options.

Statistics and Analysis

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). 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. 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 study protocol and statistical analysis plan are available in the supplementary materials. All statistical analysis was performed using R version 3.5.1.

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 laboratory setting and not at the primary health care centre, and amongst those 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 culture as 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). PD Hinduja Hospital, a drug-resistant TB referral clinic, contributed 51% (32/63) of all RIF-resistant cases diagnosed as part of the study, and 31% (32/102) of all enrolled participants at PD Hinduja Hospital were RIF-resistant.

Diagnostic accuracy of the Truenat MTB detection assays

For specimens tested in the primary health care centres, 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 primary health care 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 primary health care 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 accuracy for any Truenat assay run at the primary health care centres or reference laboratories (Supplementary Table 5). While sensitivity of the Truenat MTB assay was marginally lower in the primary health care centres (difference = -5.4% [-10, -1.2]) the small sample size, known heterogeneity across sputa collected on different days, and lack of difference for the Truenat MTB Plus or MTB-RIF Dx assays suggest caution in interpretation. 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 primary health care 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 primary health care centres and the reference laboratories (Supplementary Table 5).

Diagnostic accuracy of Truenat assays compared with Xpert MTB/RIF and Ultra

To compare the performance of Truenat with Xpert MTB/RIF 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 MTB/RIF. Among 1,542 participants in the Case Detection Group with valid culture, Truenat, and Xpert MTB/RIF or Ultra results, performance of Truenat MTB and MTB Plus was largely comparable to that of Xpert MTB/RIF (Figure 3a). In sites where Xpert MTB/RIF was run on 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 MTB/RIF; 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 MTB/RIF. 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 MTB/RIF, irrespective of smear status (Supplementary Table 6). In

Peru, sensitivity was higher in Ultra than Truenat MTB (difference = -23% [95% CI -15, -32]) and MTB Plus (difference = -16% [95% CI -10, -25]) (Supplementary Table 7). Ultra and Truenat MTB specificities were comparable.

For the 252 individuals with valid Truenat TB detection and Xpert MTB/RIF results, the sensitivities of Truenat MTB-RIF Dx and Xpert MTB/RIF 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 MTB/RIF (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 MTB/RIF or Ultra (Supplementary Tables 6 and 7).

Non-determinate results for Truenat, Xpert MTB/RIF 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 MTB/RIF 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 [23]. The specificity of the assays in the primary health care centre was equivalent to that seen in the reference laboratory, despite the open nature of the assay.

For TB detection, the low sensitivity of the Truenat MTB and MTB Plus assays in smear-negative participants was unexpected. However, the head-to-head comparison to Xpert MTB/RIF showed similarly low sensitivity for Xpert MTB/RIF, suggesting that sub-optimal performance was due to a challenging patient spectrum, rather than poor assay performance. 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 known heterogeneity in performance frequently seen across different Xpert MTB/RIF and Ultra accuracy studies may also reflect population or patient spectrum specific differences [6].

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 [24, 25], 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.

The high rate of non-determinate results seen at specific sites and by specific operators highlights the importance of appropriate on-site training, robust quality assurance/quality control programmes and effective remote monitoring. For the Truenat assays, Molbio Diagnostics' integrated online/SIM connectivity systems can facilitate remote monitoring. In addition, it is not uncommon for non-determinate results to be higher than normal when a new system is introduced, with improvements seen as operators gain experience with the systems. A recent study found technicians reporting comfort with assay operations after a median of 10 tests, with an associate reduction in invalid test results [26]. In terms of patient-important outcomes, quicker turnaround from testing to treatment can be expected when testing is conducted at primary health care centres. Overall the Truenat tuberculosis assays have been estimated to be cost-effective in India compared to microscopy and Xpert [27].

Strengths of this study include the rigorous methodology employed, the use of a robust reference standard, large sample size, and the direct head-to-head comparison with Xpert MTB/RIF and Ultra. The study provides an important assessment of molecular TB test diagnostic accuracy in diverse populations representative of the global TB epidemic. However, the difficulty of diagnosing TB in real-world populations contributed to some of the limitations of the study. For example, the number of both HIV-infected participants and RIF-resistant TB cases was small, particularly so for samples tested in the primary health care centres, resulting in imprecise estimates of sensitivity in these groups. A recent analytical study using well-characterised *M.tb* strains showed that Truenat MTB-RIF Dx detected RIF resistance mutations representing 98.6% accuracy when weighted for global prevalence. Nevertheless, more work is needed to evaluate RIF-resistance coverage in clinical settings across different geographies and patient populations [28]. Given the clear benefit of rapid

diagnosis of TB in people living with HIV, further studies will be required to evaluate the accuracy of the Truenat assays in these vulnerable populations in the primary health care setting, particularly given the lower than anticipated performance of the Truenat assays in smear-negative culture-positive TB cases. In addition, availability issues meant that only the sites in Peru used Ultra assays, resulting in a small sample size and wider confidence intervals for the assessment of Truenat performance versus Ultra. Further, while the heterogeneity of sputa from the same participant was controlled for by pooling sputa on Day 1, use of the pooled sputa in the reference laboratory assessments could have artificially increased detection of *M. tuberculosis* in culture and Xpert versus Truenat assessments in the primary health care centre. Also, the microbiological reference standard is not perfect and may contribute to false-negative results through lengthy specimen transport or overly harsh decontamination of specimens, whereas additional diagnoses could have been made through clinical diagnosis [29]. However, culture can be standardised and is recommended by WHO as a reference standard for evaluation of novel sputum-based diagnostics [30]. Finally, the controlled environment of this study may have contributed to evaluation conditions atypical of routine clinical operating procedures, and more pragmatic studies could aid to confirm these study results.

Overall, this prospective clinical study demonstrates overall good performance of the Truenat assays in providing rapid diagnosis of TB and RIF resistance in intended settings of use. These results indicate that the Truenat MTB, MTB Plus and MTB-RIF Dx assays have similar accuracy to that of Xpert MTB/RIF and can be performed at the primary health care 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 [23].

References

1. World Health Organization. Global Tuberculosis Report 2020. 2020.

2. Dye C, Watt CJ, Bleed DM, Hosseini SM, Raviglione MC. Evolution of Tuberculosis Control and Prospects for Reducing Tuberculosis Incidence, Prevalence, and Deaths Globally. *JAMA* 2005; 293: 2767–2775.

3. MacPherson P, Houben RM, Glynn JR, Corbett EL, Kranzer K. Pre-treatment loss to follow-up in tuberculosis patients in low- and lower-middle-income countries and high-burden countries: a systematic review and meta-analysis. *Bull. World Health Organ.* 2014; 92: 126–138.

4. Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, Banada PP, Deshpande S, Shenai S, Gall A, Glass J, Krieswirth B, Schumacher SG, Nabeta P, Tukvadze N, Rodrigues C, Skrahina A, Tagliani E, Cirillo DM, Davidow A, Denkinger CM, Persing D, Kwiatkowski R, Jones M, Alland D. The New Xpert MTB/RIF Ultra: Improving Detection of Mycobacterium tuberculosisand Resistance to Rifampin in an Assay Suitable for Point-of-Care Testing. Nacy CA, editor. *MBio* 2017; 8: e00812-17–12.

5. Albert H, Nathavitharana RR, Isaacs C, Pai M, Denkinger CM, Boehme CC. Development, roll-out and impact of Xpert MTB/RIF for tuberculosis: what lessons have we learnt and how can we do better? *Eur Respir J* 2016; 48: 516–525.

6. Horne DJ, Kohli M, Zifodya JS, Schiller I, Dendukuri N, Tollefson D, Schumacher SG, Ochodo EA, Pai M, Steingart KR. Xpert MTB/RIF and Xpert MTB/RIF Ultra for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Infectious Diseases Group, editor. *Cochrane Database of Systematic Reviews* [Internet] 2019

[cited 2020 Dec 21]; Available from: http://doi.wiley.com/10.1002/14651858.CD009593.pub4.

7. Joshi B, Lestari T, Graham SM, Baral SC, Verma SC, Ghimire G, Bhatta B, Dumre SP, Utarini A. The implementation of Xpert MTB/RIF assay for diagnosis of tuberculosis in Nepal: A mixed-methods analysis. Hasnain SE, editor. *PLoS ONE* 2018; 13: e0201731.

8. Ndlovu Z, Fajardo E, Mbofana E, Maparo T, Garone D, Metcalf C, Bygrave H, Kao K, Zinyowera S. Multidisease testing for HIV and TB using the GeneXpert platform: A feasibility study in rural Zimbabwe. Yotebieng M, editor. *PLoS ONE* 2018; 13: e0193577.

9. Creswell J, Codlin AJ, Andre E, Micek MA, Bedru A, Carter EJ, Yadav R-P, Mosneaga A, Rai B, Banu S, Brouwer M, Blok L, Sahu S, Ditiu L. Results from early programmatic implementation of Xpert MTB/RIF testing in nine countries. *BMC Infect Dis* 2014; 14: 2.

10. Raizada N, Sachdeva KS, Nair SA, Kulsange S, Gupta RS, Thakur R, Parmar M, Gray C, Ramachandran R, Vadera B, Ekka S, Dhawan S, Babre A, Ghedia M, Alavadi U, Dewan P, Khetrapal M, Khanna A, Boehme C, Paramsivan CN. Enhancing TB Case Detection: Experience in Offering Upfront Xpert MTB/RIF Testing to Pediatric Presumptive TB and DR TB Cases for Early Rapid Diagnosis of Drug Sensitive and Drug Resistant TB. *PLoS ONE* 2014; 9: e105346.

11. Nikam C, Jagannath M, Narayanan MM, Ramanabhiraman V, Kazi M, Shetty A, Rodrigues C. Rapid diagnosis of Mycobacterium tuberculosis with Truenat MTB: a near-care approach. *PLoS ONE* 2013; 8: e51121.

12. Nikam C, Kazi M, Nair C, Jaggannath M, M M, R V, Shetty A, Rodrigues C. Evaluation of the Indian TrueNAT micro RT-PCR device with GeneXpert for case detection of pulmonary tuberculosis. *Int J Mycobacteriol* 2014; 3: 205–210.

13. Beall SG, Cantera J, Diaz MH, Winchell JM, Lillis L, White H, Kalnoky M, Gallarda J, Boyle DS. Performance and workflow assessment of six nucleic acid extraction technologies for use in resource limited settings. Kalendar R, editor. *PLoS ONE* 2019; 14: e0215753-21.

14. Vijayalakshmi J, Surekha A, Devi AR, Devi SU. Truenat - A Novel Diagnostic Tool for Rapid Detection of Mycobacterium Tuberculosis and Rifampicin Resistance in Pulmonary Samples. 2019; : 9.

15. Mangayarkarasi V, P S, R S, Jayaprakash J. Ergonomic Diagnostic Tool based on Chip Mini RT-PCR for Diagnosis of Pulmonary and Extra Pulmonary Tuberculosis. *J Pure Appl Microbiol* 2019; 13: 1185–1190.

16. Ranjana H, Sadhna S. Truelab Micro PCR In Diagnosis of Extrapulmonary Tuberculosis: Our Experience. 2016.

17. Molbio Diagnostics Pvt. Ltd. Truelab™ Duo user manual 2020 [Internet]. Available from: http://www.molbiodiagnostics.com/uploads/product_download/20191226.115102~TRUELAB-Duo-Manual.pdf.

18. Molbio Diagnostics Pvt. Ltd. Trueprep®AUTO v2 user manual 2020 [Internet]. Available from: http://www.molbiodiagnostics.com/uploads/product_download/20200521.170649~Trueprep_AUTO--v2-device-manual-V-03.pdf.

19. World Health Organization. Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis. 2018.

20. Molbio Diagnostics Pvt. Ltd. Truenat™ MTB package insert 2020 [Internet]. Available from: http://www.molbiodiagnostics.com/uploads/product_download/20200127.112354~Truenat-MTB-packinsert.pdf.

21. Molbio Diagnostics Pvt. Ltd. Truenat™ MTB Plus package insert 2020 [Internet]. Available from: http://www.molbiodiagnostics.com/uploads/product_download/20190927.152146~Truenat-MTB-Plus-packinsert.pdf.

22. Molbio Diagnostics Pvt. Ltd. Truenat™ MTB-RIF Dx 2020 [Internet]. Available from: http://www.molbiodiagnostics.com/uploads/product_download/20200110.140848~Truenat-MTB-RIF-Dx.pdf.

23. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva; 2020.

24. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A, Jones M, O'Brien SM, Persing DH, Ruesch-Gerdes S, Gotuzzo E, Rodrigues C, Alland D, Perkins MD. Rapid Molecular Detection of Tuberculosis and Rifampin Resistance. *New England Journal of Medicine* 2010; 363: 1005–1015.

25. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahirli R, Gler MT, Blakemore R, Worodria W, Gray C, Huang L, Caceres T, Mehdiyev R, Raymond L,

Whitelaw A, Sagadevan K, Alexander H, Albert H, Cobelens F, Cox H, Alland D, Perkins MD. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *Lancet* 2011; 377: 1495–1505.

26. Jeyashree K, Shanmugasundaram D, Rade K, Gangakhedkar RR, Murhekar MV. Impact and operational feasibility of TrueNat^{TM} MTB/Rif under India's RNTCP. *Public Health Action* 2020; 10: 87–91.

27. Lee DJ, Kumarasamy N, Resch SC, Sivaramakrishnan GN, Mayer KH, Tripathy S, Paltiel AD, Freedberg KA, Reddy KP. Rapid, point-of-care diagnosis of tuberculosis with novel Truenat assay: Cost-effectiveness analysis for India's public sector. Subbian S, editor. *PLoS ONE* 2019; 14: e0218890-17.

28. Georghiou SB, Gomathi NS, Rajendran P, Nagalakshmi V, Prabakaran L, Prem Kumar MM, Macé A, Tripathy S, Ruhwald M, Schumacher SG, Penn-Nicholson A. Accuracy of the Truenat MTB-RIF Dx assay for detection of rifampicin resistance-associated mutations. *Tuberculosis* 2021; 127: 102064.

29. Catanzaro A, Perry S, Clarridge JE, Dunbar S, Goodnight-White S, LoBue PA, Peter C, Pfyffer GE, Sierra MF, Weber R, Woods G, Mathews G, Jonas V, Smith K, Della-Latta P. The Role of Clinical Suspicion in Evaluating a New Diagnostic Test for Active Tuberculosis: Results of a Multicenter Prospective Trial. *JAMA* 2000; 283: 639.

30. Schumacher SG, Wells WA, Nicol MP, Steingart KR, Theron G, Dorman SE, Pai M, Churchyard G, Scott L, Stevens W, Nabeta P, Alland D, Weyer K, Denkinger CM, Gilpin C. Guidance for Studies Evaluating the Accuracy of Sputum-Based Tests to Diagnose Tuberculosis. *The Journal of Infectious Diseases* 2019; 220: S99–S107.

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.

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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, AMa, 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:

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 primary health care centre (microscopy centre) and the reference laboratory

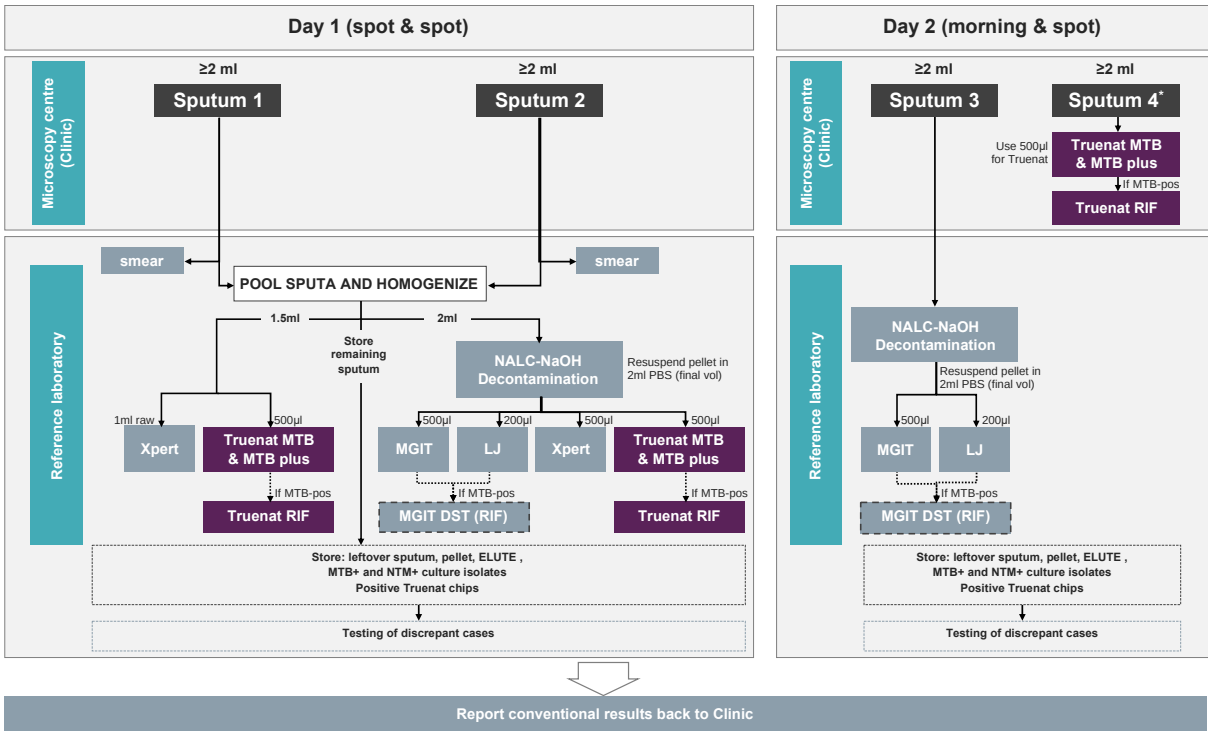
	N	TP	FP	FN	TN	Sensitivity % (95% CI)		Sensitivity % Smear Positive (95% CI) - N		Sensitivity % Smear Negative (95% CI) - N		Specificity % (95% CI)	
Microscopy centre sputum													
Truenat MTB	1356	192	25	71	1068	73.0	[67.3,78.0]	91.0	[85.8,94.4] - N:177	36.0	[26.7,46.6] - N:86	97.7	[96.7,98.5]
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 laboratory are shown Supplementary Table 5.

FIGURES:

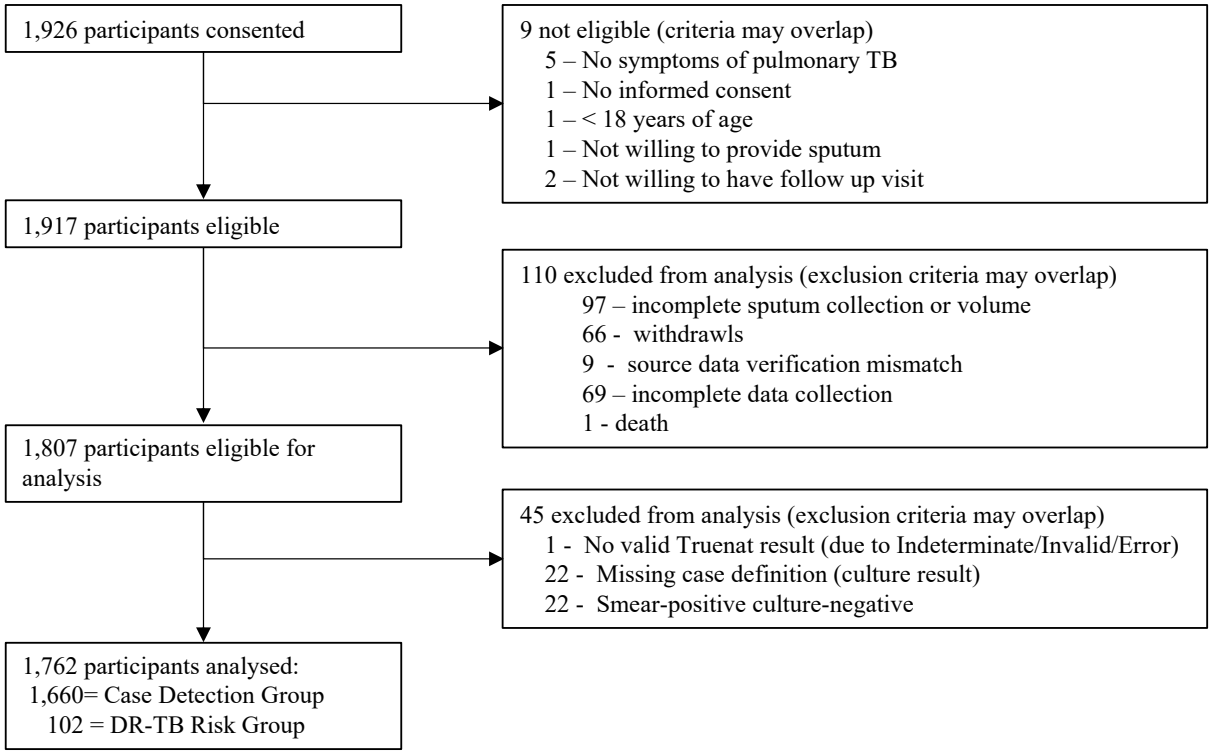
Figure 1: Specimen flow at enrolment



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. As Truenat assays are not indicated for decontaminated sputum sediments and do not contribute to our study objective, test results are not presented within this report, but are available upon request.

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



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

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

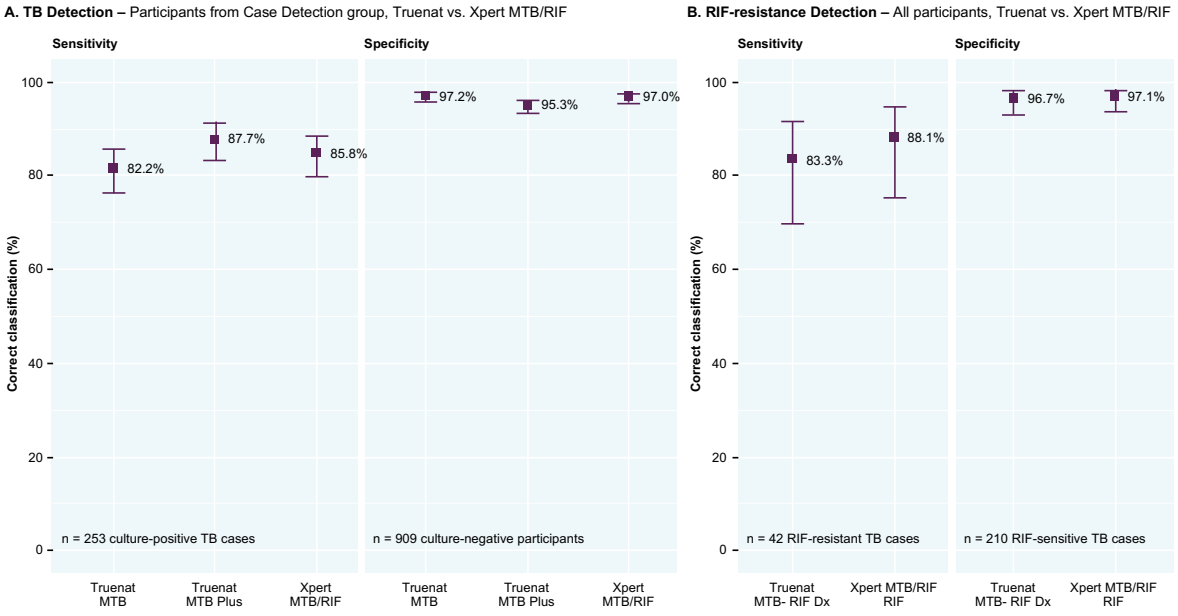
Figure 3. Performance of the Truenat, Xpert MTB/RIF and Ultra assays conducted at the reference laboratories

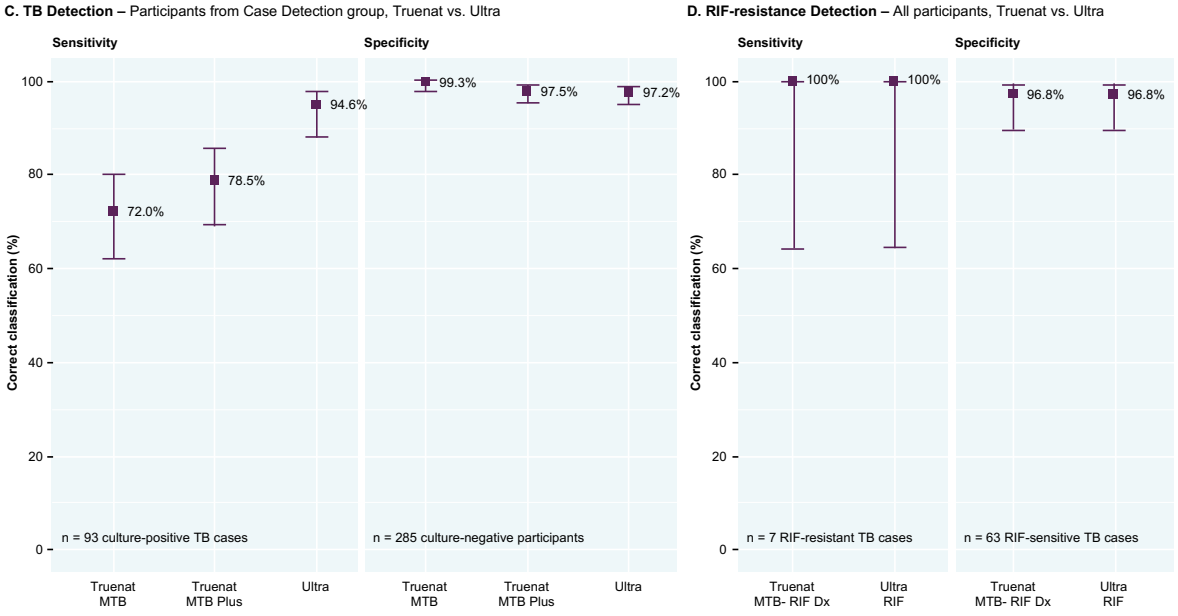
a) Performance of Truenat and Xpert MTB/RIF for TB detection

b) Performance of Truenat and Xpert MTB/RIF for rifampicin resistance detection

c) Performance of Truenat and Ultra for TB detection

d) Performance of Truenat and Ultra for rifampicin resistance detection





Supplementary materials

Supplementary methods

Objectives:
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 primary health care 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 MTB/RIF and Ultra, using a reference standard of culture for TB diagnosis and phenotypic DST for detection of RIF resistance.

Procedures
Participants were enrolled at clinics at each primary health care centre. All enrolled participants had their medical history reviewed. HIV testing was offered to all participants. Participants enrolled 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. For the Case Detection Group, all specimens were collected before initiation of TB treatment.

On Day 1, each participant was asked to submit two spot sputa of at least 2ml. Participants were given a labelled sputum cup and instructions for use, and asked to collect an additional sputum specimen (S3) the next morning (Day 2) before going to the clinic. At the clinic, participants were asked to provide a final spot sputum (S4). In the event that a participant failed to return on Day 2, S3 and S4 were permitted to be collected a maximum of 7 days after enrolment, provided that no TB treatment had been initiated (Case Detection Group).

Day 1: S1 and S2 – Two spot sputa were collected approximately 30−60 minutes apart. A smear of each sputum specimen was prepared. Thereafter, sputa totalling 4 mL or more were pooled and homogenized by glass beads and vortexing in the reference labs. This enabled comparable testing across the different index and reference tests in the centralized reference laboratory. Homogenized sputa were further split: 1·5 mL was used for analysis on raw/direct sputa, and at least 2 mL used for NALC-NaOH decontamination. Briefly, DNA was extracted independently from raw sputum and decontaminated pellet by the Trueprep Auto device and tested on both the Truenat MTB and the MTB Plus chips, both of which were read by the Truelab real-time PCR analyser. All DNA extracts testing positive by the MTB assay were subsequently tested by the Truenat MTB-RIF Dx assay (reflex), which was also read by Truelab analyser. Xpert assays were performed on the same raw and decontaminated specimens. GeneXpert systems were used for routine and study-specific Xpert testing according to manufacturer's instructions on direct sputum and decontaminated pellet [1, 2]. Mycobacteria growth indicator tube (MGIT) and Löwenstein–Jensen (LJ) culture were performed only on the decontaminated specimen. Each positive culture was identified for *M. tuberculosis* complex using MPT64 identification test. MGIT SIRE was used to determine the phenotypic DST for rifampicin (RIF).

Day 2: S3 – Morning sputum was returned to the clinic in a labelled sputum cup. S3 was sent to the reference laboratory and a second round of MGIT and LJ culture was performed on the decontaminated sediment.

Day 2: S4 – At the time that S3 was returned to the clinic, the participant was asked to provide spot sputum S4. The intended objective of this additional sputum specimen was to test the Truenat assay in the setting of use (i.e. primary health care centres with associated microscopy centres). In sites where the primary health care centre and the reference lab are the same, the Truenat assays were only performed once alongside Xpert (on Day 1). As PD Hinduja Hospital in India and the Port Moresby General Hospital in Papua New Guinea are centralized laboratory facilities, Sputum 4 was not collected as no dedicated microscopy centre as part of a primary health care centre was available. Spot sputum S4 was processed in the primary health care centre: the entire volume of sputum was liquefied and lysed using Trueprep Auto kit reagents, and 500 µL raw sputum was used for DNA extraction by Trueprep Auto and MTB detection by the Truenat assays. Any *M. tuberculosis*-positive specimens were subsequently tested by the reflex Truenat MTB-RIF Dx assay.

All positive Truenat chips were stored (refrigerated) at the sites to allow for sequencing from DNA amplicons if required for discordance resolution, as pre-defined in the protocol. Additionally, any leftover sputum, pellet, and culture isolates positive for non- tuberculous mycobacteria or *M. tuberculosis* were stored (frozen).

For all sample testing days, 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 either the index test of the reference standard 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

For the primary analyses, 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.

Supplementary results:

Quality control

Positive results from testing swabs and negative controls were rare, indicating appropriate daily cleaning and handling of materials. All positive results were resolved after cleaning and did not persist or inhibit subsequent specimen testing. Days where swabs or negative controls tested positive never coincided with days where participant specimens tested false-positive, suggesting that the risk of carry-over contamination was low in the context of this study.

Performance in participants with a history of TB

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 MTB/RIF and Ultra (Supplementary Table 8).

Discordance analysis

Overall, 126 participants had at least one false-positive result and 131 participants had at least one false-negative result by either Truenat MTB or MTB Plus assays on at least one of the six tests done per participant. Of the 126 participants with false-positive results, 40 were also false-positive by either Xpert MTB/RIF or Ultra. None of the false-positive results coincided with positive test results on negative controls or swabs.

Supplementary references

1. Cepheid. Xpert® MTB/RIF package insert 2020 [Internet]. Available from: https://www.cepheid.com/Package%20Insert%20Files/Xpert-MTB-RIF-ENGLISH-Package-Insert-301-1404-Rev-F.pdf.

2. Cepheid. Xpert® MTB/RIF Ultra 2020 [Internet]. Available from: https://www.cepheid.com/en/tests/Critical-Infectious-Diseases/Xpert-MTB-RIF-Ultra.

Supplementary Table 1. Participating sites

India	Site 01	Mumbai: Hinduja
India	Site 02	Guwahati: Kamrup
	Site 03	Guwahati: Railway
	Site 04	Guwahati: Sonapur
	Ref Lab	Guwahati: Intermediate Reference Laboratory, Guwahati Medical College
India	Site 05	Chennai: Ayanavaram
	Site 06	Chennai: Villiwakkam
	Site 07	Chennai: Thanthai Perivar
	Ref Lab	Chennai: National Institute of Research in Tuberculosis
India	Site 08	Ahmedabad: Madhupura
	Site 09	Ahmedabad: CHC Chhala
	Site 10	Ahmedabad: PHC Kuha
	Ref Lab	Ahmedabad: Intermediate Reference Laboratory, State TB and Demonstration Center, Civil Hospital Campus
Peru	Site 11	Lima: CS Huascar II
	Site 12	Lima: CS Huascar XV
	Site 13	Lima: CS Jose Carlos Mariategui
	Site 14	Lima: CS Fraternidad
	Site 19	Lima: CS El Porvenir
	Ref Lab	Lima: Universidad Peruana Cayetano Heredia
Ethiopia	Site 15	Addis Ababa: Hiwot Amba
	Site 16	Addis Ababa: St. Gebrel
	Site 17	Addis Ababa: Woreda 01
	Ref Lab	Addis Ababa: Ethiopian Public Health Institute
Papua New Guinea	Site 18	Port Moresby: Central Public Health Laboratory, Port Moresby General Hospital

Supplementary Table 2. Participant inclusion and exclusion criteria for the Case Detection and Drug-Resistant Risk TB Groups

Case Detection Group	Drug-Resistant Risk Group
Inclusion criteria	
Age 18 years or above Provision of informed consent Willingness to provide at least 3 sputum specimens (>2 mL) at enrolment	
• Willingness to have a study follow-up visit approximately 42 to 70 days after enrolment • Clinical suspicion of pulmonary TB (including cough ≥2 weeks and at least 1 other symptom typical of TB)	• Non-converting pulmonary TB cases (category I and category II failures) • Retreatment cases* (those having failed a regimen, relapses or returned after loss to follow-up) • Close contacts of drug-resistant TB patients who have been diagnosed with active TB* • Participants at high risk for MDR-TB as determined by local programme*
Exclusion criteria	
• Receipt of any dose of TB treatment within 60 days prior to enrolment • Participants for whom, at the time of enrolment, the follow-up visit was poorly feasible (e.g. individuals planning to relocate)	• Receipt of any MDR-TB treatment within 60 days prior to enrolment

MDR-TB, multi-drug resistant tuberculosis; TB, tuberculosis.

*Pulmonary TB cases on TB treatment were eligible if they were suspected to be treatment failures irrespective of how long TB treatment had been ongoing. All culture-negative study participants on TB treatment were excluded from the analysis, even if they were smear-positive.

Supplementary Table 3. Reference standard test and index test procedures

Test	Notes*
Smear	All sites used light microscopy (Ziehl Neelsen), except for PD Hinduja Hospital which used fluorescence microscopy (auramine-O), and sites in Peru, which used both methods. Testing and reporting was undertaken as per WHO/IUATLD guidelines (1).
Xpert MTB/RIF	2:1 sample reagent added to raw sputum. In case of invalid, error or no result, testing was repeated if enough specimen was available.
Ultra	2:1 sample reagent added to raw sputum and pellet (2). In case of invalid, error or no result, testing was repeated if enough specimen was available.
Liquid culture	Mycobacteria Growth Indicator Tube 960 culture; BD Microbiology Systems
Solid culture	Löwenstein Jensen. Testing and reporting done as per GLI mycobacteriology laboratory manual and local guidelines.
MGIT DST	BD MGIT AST SIRE Test kit
MTB identification	MPT-64, SD Bioline, BD, or Capilia TB-Neo, TAUNS

IUATLD, International Union Against Tuberculosis and Lung Disease; WHO, World Health Organization.
*Testing done as per manufacturer's instructions unless otherwise specified.

Supplementary Table 4. Performance of Truenat assays for TB and for RIF resistance detection at the primary health care centre (microscopy centre) and the reference laboratory, for participants with result for either Truenat MTB, MTB Plus or MTB-RIF Dx assays.

All participants	N	TP	FP	FN	TN	Sensitivity % (95% CI)	Sensitivity % Smear Pos (95% CI) - N	Sensitivity % Smear Neg (95% CI) - N	Specificity % (95% CI)
Microscopy Centre sputum									
Truenat MTB	1402	192	25	71	1114	73.0 [67.3,78.0]	91.0 [85.8,94.4] - N:177	36.0 [26.7,46.6] - N:86	97.8 [96.8,98.5]
Truenat MTB Plus	1369	212	41	54	1062	79.7 [74.5,84.1]	96.1 [92.2,98.1] - N:179	46.0 [35.9,56.4] - N:87	96.3 [95.0,97.2]
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	1603	278	28	74	1223	79.0 [74.4,82.9]	95.8 [92.4,97.7] - N:238	43.9 [35.1,53.0] - N:114	97.8 [96.8,98.5]
Truenat MTB Plus	1552	297	52	51	1152	85.3 [81.2,88.7]	98.3 [95.7,99.3] - N:236	58.0 [48.8,66.8] - N:112	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]

FN, false negative; FP, false positive; TN, true negative; TP, true positive.

Note: Analysis of Truenat performance is shown on specimens collected at the microscopy centre and at the reference laboratory separately, with valid results available for either the Truenat MTB assay or the Truenat MTB Plus assay; denominators differ based on the number of valid results for each assays, and as two sites (PD Hinduja hospital and Papua New Guinea) only had reference lab facilities available.

Supplementary Table 5. Performance of the Truenat assays performed in primary health care centres (microscopy centres) and reference laboratories

	N	TP	FP	FN	TN	Sensitivity % (95% CI)	Sensitivity % Smear Pos (95% CI) - N	Sensitivity % Smear Neg (95% CI) - N	Specificity % (95% CI)
Truenat MTB									
Ref Lab sputum	1376	203	25	56	1092	78.4 [73.0,83.0]	97.1 [93.5,98.8] - N:175	39.3 [29.5,50.0] - N:84	97.8 [96.7,98.5]
Microscopy Centre sputum	1376	189	24	70	1093	73.0 [67.3,78.0]	90.9 [85.7,94.3] - N:175	35.7 [26.3,46.4] - N:84	97.9 [96.8,98.6]
Difference (Microscopy Centre - Ref lab)						-5.4 [-10.0,-1.2]	-6.2 [-11.3,-2.3]	-3.6 [-13.8,+6.5]	+0.1 [-0.9,+1.1]
Truenat MTB Plus									
Ref Lab sputum	1311	215	43	44	1009	83.0 [78.0,87.1]	98.3 [95.1,99.4] - N:176	50.6 [40.1,61.1] - N:83	95.9 [94.5,97.0]
Microscopy Centre sputum	1311	208	39	51	1013	80.3 [75.0,84.7]	96.6 [92.8,98.4] - N:176	45.8 [35.5,56.5] - N:83	96.3 [95.0,97.3]
Difference (Microscopy Centre - Ref lab)						-2.7 [-7.1,+1.5]	-1.7 [-5.5,+1.6]	-4.8 [-16.3,+6.6]	+0.4 [-1.0,+1.8]
Truenat RIF									
Ref Lab sputum	175	14	7	3	151	82.4 [59.0,93.8]	81.2 [57.0,93.4] - N:16	100 [20.6,100.0] - N:1	95.6 [91.1,97.8]
Microscopy Centre sputum	175	15	9	2	149	88.2 [65.7,96.7]	87.5 [64.0,96.5] - N:16	100 [20.6,100.0] - N:1	94.3 [89.5,97.0]
Difference (Microscopy Centre - Ref lab)						+5.8 [-13.6,+27.0]	+6.3 [-14.3,+28.3]	0 [-79.3,+79.3]	-1.3 [-4.9,+1.8]

Note: Differences in sensitivity and specificity were calculated as performance of each Truenat assay conducted in the microscopy centre (Day 2) minus that conducted in the reference lab (Day 1), relative to *M. tuberculosis* culture (for TB detection) or RIF DST (for RIF resistance detection).

Supplementary Table 6. Performance of the Truenat assays for TB and RIF resistance detection compared to Xpert MTB/RIF

		N	TP	FP	FN	TN	Sensitivity % (95% CI)	Sensitivity % Smear Pos (95% CI) - N	Sensitivity % Smear Neg (95% CI) - N	Specificity % (95% CI)
Case Detection Group only	**Truenat MTB**									
	Xpert	1162	217	27	36	882	85.8 [80.9,89.5]	98.9 [96.1,99.7] - N:185	50.0 [38.4,61.6] - N:68	97.0 [95.7,98.0]
	Truenat MTB	1162	208	25	45	884	82.2 [77.0,86.4]	96.2 [92.4,98.2] - N:185	44.1 [33.0,55.9] - N:68	97.2 [96.0,98.1]
	Difference (Truenat MTB- Xpert)						-3.6 [-7.8,+0.3]	-2.7 [-6.4,+0.1]	-5.9 [-18.6,+6.7]	0.2 [-0.8,+1.3]
	Truenat MTB Plus									
	Xpert	1162	217	27	36	882	85.8 [80.9,89.5]	98.9 [96.1,99.7] - N:185	50.0 [38.4,61.6] - N:68	97.0 [95.7,98.0]
	Truenat Plus MTB	1162	222	43	31	866	87.7 [83.1,91.2]	98.9 [96.1,99.7] - N:185	57.4 [45.5,68.4] - N:68	95.3 [93.7,96.5]
	Difference (Truenat Plus MTB - Xpert)						1.9 [-1.3,+5.6]	0 [-2.5,+2.5]	7.4 [-4.2,+19.2]	-1.7 [-3.2,-0.5]
Case Detection and DR-Risk Group	**RIF detection**									
	Xpert Rif	252	37	6	5	204	88.1 [75.0,94.8]	89.7 [76.4,95.9] - N:39	66.7 [20.8,93.8] - N:3	97.1 [93.9,98.7]
	Truenat Rif	252	35	7	7	203	83.3 [69.4,91.7]	84.6 [70.3,92.8] - N:39	66.7 [20.8,93.8] - N:3	96.7 [93.3,98.4]
	Difference (Truenat Rif - Xpert Rif)						-4.8 [-15.8,+4.0]	-5.1 [-16.9,+4.3]	0 [-56.1,+56.1]	-0.4 [-2.6,+1.3]

Note: Differences in sensitivity and specificity were calculated as performance of each Truenat assay conducted in the reference laboratory (Day 1) for Truenat assays minus Xpert MTB/RIF, on the same homogenized specimen, relative to *M. tuberculosis* culture (for TB detection) or RIF DST (for RIF resistance detection). As Peru did not run the Xpert MTB/RIF assay, this site did not contribute to the analysis shown here.

Supplementary Table 7. Performance of the Truenat assays for TB and RIF resistance detection compared to Ultra (Peru only)

		N	TP	FP	FN	TN	Sensitivity % (95% CI)	Sensitivity % Smear Pos (95% CI) - N	Sensitivity % Smear Neg (95% CI) - N	Specificity % (95% CI)
Case Detection Group only	**Truenat MTB**									
	Ultra	378	88	8	5	277	94.6 [88.0,97.7]	100.0 [93.0,100.0] - N:51	88.1 [75.0,94.8] - N:42	97.2 [94.6,98.6]
	Truenat MTB	378	67	2	26	283	72.0 [62.2,80.2]	94.1 [84.1,98.0] - N:51	45.2 [31.2,60.1] - N:42	99.3 [97.5,99.8]
	Difference (Truenat MTB - Ultra)						-22.6 [-32.1,-15.3]	-5.9 [-15.9,+1.5]	-42.9 [-57.8,-29.1]	2.1 [0.7,+4.5]
	Truenat MTB Plus									
	Ultra	378	88	8	5	277	94.6 [88.0,97.7]	100.0 [93.0,100.0] - N:51	88.1 [75.0,94.8] - N:42	97.2 [94.6,98.6]
	Truenat Plus MTB	378	73	7	20	278	78.5 [69.1,85.6]	96.1 [86.8,98.9] - N:51	57.1 [42.2,70.9] - N:42	97.5 [95.0,98.8]
	Difference (Truenat MTB Plus - Ultra)						-16.1 [-24.9,-10.0]	-3.9 [-13.2,+3.4]	-31 [-46.0,-19.1]	0.3 [-1.8,+2.6]
Case Detection and DR-Risk Group	**RIF detection**									
	Ultra Rif	70	7	2	0	61	100 [64.6,100.0]	100 [61.0,100.0] - N:6	100 [20.6,100.0] - N:1	96.8 [89.1,99.1]
	Truenat Rif	70	7	2	0	61	100 [64.6,100.0]	100 [61.0,100.0] - N:6	100 [20.6,100.0] - N:1	96.8 [89.1,99.1]
	Difference (Truenat Rif - - Ultra Rif)						0 [-35.4,+35.4]	0 [-39.0,+39.0]	0 [-79.3,+79.3]	0 [-5.7,+5.7]

Note: Differences in sensitivity and specificity were calculated as performance of each Truenat assay conducted in the reference laboratory (Day 1) for Truenat assays minus Ultra, on the same homogenized specimen, relative to *M. tuberculosis* culture (for TB detection) or RIF DST (for RIF resistance detection). As Peru was the only site to run the Ultra assay, no other site contributed to the analysis shown here.

Supplementary Table 8. Specificity of the Truenat assays compared with Xpert MTB/RIF and Ultra among participants with and without a prior history of TB

		All samples	Specificity % - TB History (95% CI)		Specificity % - No TB History (95% CI)	
Compared to Xpert	Case Detection Group only	**Truenat MTB**				
		Xpert MTB/RIF	93.3	[86.2,96.9]	97.3	[95.9,98.2]
		Truenat MTB	94.4	[87.6,97.6]	97.4	[96.1,98.3]
		Difference (Truenat MTB - Xpert)	+1.1	[-4.1,+6.8]	+0.1	[-1.0,+1.3]
		Truenat MTB Plus	--	--	--	--
		Xpert MTB/RIF	91.5	[83.4,95.8]	97.3	[95.9,98.3]
		Truenat Plus MTB	90.2	[81.9,95.0]	95.6	[93.9,96.9]
		Difference (Truenat Plus MTB - Xpert)	-1.3	[-8.9,+6.2]	-1.7	[-3.2,-0.4]
	Case Detection and DR-Risk Group	**RIF detection**				
		Xpert MTB/RIF Rif	93.8	[71.7,98.9]	97.9	[94.7,99.2]
		Truenat Rif	93.8	[71.7,98.9]	97.4	[94.0,98.9]
		Difference (Truenat Rif - Xpert Rif)	0	[-19.4,+19.4]	-0.5	[-2.9,+1.5]
Compared to Ultra	Case Detection Group only	**Truenat MTB**				
		Ultra	92.9	[85.3,96.7]	99.0	[96.5,99.7]
		Truenat MTB	97.6	[91.7,99.3]	100	[98.2,100.0]
		Difference (Truenat MTB - Ultra)	+4.7	[+0.2,+11.6]	+1.0	[-0.9,+3.5]
		Truenat MTB Plus	--	--	--	--
		Ultra	92.9	[85.3,96.7]	99.0	[96.5,99.7]
		Truenat Plus MTB	92.9	[85.3,96.7]	99.5	[97.3,99.9]
		Difference (Truenat Plus MTB - Ultra)	0	[-6.2,+6.2]	+0.5	[-1.8,+3.1]
	Case Detection and DR-Risk Group	**RIF detection**				
		Ultra Rif	100	[67.6,100.0]	96.4	[87.7,99.0]
		Truenat Rif	100	[67.6,100.0]	96.4	[87.7,99.0]
		Difference (Truenat Rif - Ultra)	0	[-32.4,+32.4]	0	[-6.5,+6.5]

Note: Differences in sensitivity and specificity were calculated as performance of each Truenat assay minus Xpert MTB/RIF or minus Ultra for the reference lab sputum relative to MTB culture (for TB detection). Only participants in the Case Detection Group were included in the TB detection analyses for Truenat MTB and Truenat MTB Plus. Truenat performance comparisons were drawn against Xpert MTB/RIF at all sites except Peru, where Ultra was performed as the comparator.

Supplementary Table 9. Proportion of non-determinate assay results for Trueprep extraction, Truenat assays and Xpert MTB/RIF and Ultra assays

Total non-determinates	Initial Test		Repeat Test	
	(%)	n/N	(%)	n/N
Trueprep	2.4%	113/4732	11.7%	13/111
Truenat MTB	6.2%	293/4720	21.2%	62/293
Truenat MTB Plus	9.2%	434/4720	36.8%	159/432
Truenat MTB RIF-Dx*	22.5%	232/1042	72.7%	157/216
Xpert MTB/RIF	2.6%	65/2522	7.9%	5/63
Xpert Ultra	0.0%	0/786	-	-

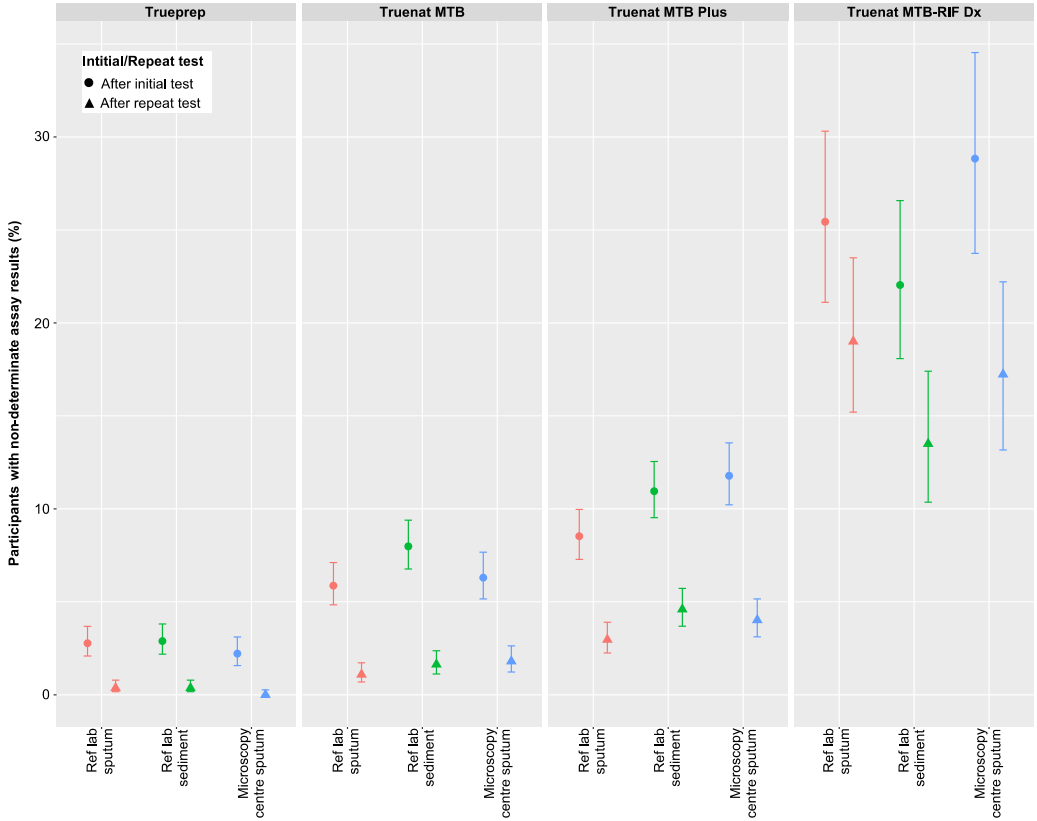
*Truenat MTB-RIF Dx was run on any specimen that tested positive for *M. tuberculosis* by either the Truenat MTB assay or the Truenat MTB Plus assay.

Note: Data represents all assays run, not the number of participants with any non-determinate result. Non-determinate results represent a combination of operator and equipment errors or failures, invalid results and indeterminate results, for all participant specimens tested as part of this study. The non-determinate results for the Truelab micro PCR machine represent results for all different Truenat assay performed at each site. Not all specimens that failed on the initial test were still available for repeat testing. The results presented here do not capture errors in DNA loading or chip loading as site incident logs did not report high levels of such errors.

Supplementary Table 10. The proportion of non-determinate Truenat MTB-RIF Dx results when reflexed from either the Truenat MTB or MTB Plus TB detection result

	Truenat RIF-Dx Non-determinates	
	% (95% CI)	n/N
If reflexed from Truenat MTB-pos and MTB Plus-pos	3.9% (2.7, 5.4)	32/830
If reflexed from Truenat MTB-neg and MTB Plus-pos	67% (60, 74)	120/179
If reflexed from Truenat MTB-pos and MTB Plus-neg	72% (56, 84)	26/36
If reflexed only from Truenat MTB-pos	6.7% (5.2, 8.6)	58/866
If reflexed only from Truenat MTB-Plus-pos	15% (13, 17)	152/1009

Supplementary Figure 1. Proportion of participants with non-determinate Truenat assay results at initial testing and after repeat testing, stratified by sputum sample



Note: Data represents the proportion of enrolled participants with a non-determinate Truenat assay result when run as an initial test and, if required, when repeated. Non-determinate results represent a combination of operator and equipment errors or failures, invalid results and indeterminate results, for all participant specimens tested as part of this study. Not all specimens that failed on the initial test were still available for repeat testing. Samples were only reflexed to the Truenat MTB-RIF Dx assay if a positive test for MTB detection was reported on either the Truenat MTB or Truenat MTB Plus chip.