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

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Summary

Background **The Xpert MTB/RIF test (Cepheid, Sunnyvale, CA, USA) can detect tuberculosis and its multidrug-resistant form with very high sensitivity and specificity in controlled studies, but no performance data exist from district and subdistrict health facilities in tuberculosis-endemic countries. We aimed to assess operational feasibility, accuracy, and effectiveness of implementation in such settings.**

Methods **We assessed adults (≥18 years) with suspected tuberculosis or multidrug-resistant tuberculosis consecutively presenting with cough lasting at least 2 weeks to urban health centres in South Africa, Peru, and India, drug-resistance screening facilities in Azerbaijan and the Philippines, and an emergency room in Uganda. Patients were excluded from the main analyses if their second sputum sample was collected more than 1 week after the first sample, or if no valid reference standard or MTB/RIF test was available. We compared one-off direct MTB/RIF testing in nine microscopy laboratories adjacent to study sites with 2–3 sputum smears and 1–3 cultures, dependent on site, and drug-susceptibility testing. We assessed indicators of robustness including indeterminate rate and between-site performance, and compared time to detection, reporting, and treatment, and patient dropouts for the techniques used.**

Findings **We enrolled 6648 participants between Aug 11, 2009, and June 26, 2010. One-off MTB/RIF testing detected 933 (90·3%) of 1033 culture-confirmed cases of tuberculosis, compared with 699 (67·1%) of 1041 for microscopy. MTB/RIF test sensitivity was 76·9% in smear-negative, culture-positive patients (296 of 385 samples), and 99·0% specific (2846 of 2876 non-tuberculosis samples). MTB/RIF test sensitivity for rifampicin resistance was 94·4% (236 of 250) and specificity was 98·3% (796 of 810). Unlike microscopy, MTB/RIF test sensitivity was not significantly lower in patients with HIV co-infection. Median time to detection of tuberculosis for the MTB/RIF test was 0 days (IQR 0–1), compared with 1 day (0–1) for microscopy, 30 days (23–43) for solid culture, and 16 days (13–21) for liquid culture. Median time to detection of resistance was 20 days (10–26) for line-probe assay and 106 days (30–124) for conventional drug-susceptibility testing. Use of the MTB/RIF test reduced median time to treatment for smear-negative tuberculosis from 56 days (39–81) to 5 days (2–8). The indeterminate rate of MTB/RIF testing was 2·4% (126 of 5321 samples) compared with 4·6% (441 of 9690) for cultures.**

Interpretation **The MTB/RIF test can effectively be used in low-resource settings to simplify patients' access to early and accurate diagnosis, thereby potentially decreasing morbidity associated with diagnostic delay, dropout and mistreatment.**

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Introduction

Two of the three key infectious diseases of man, HIV and malaria, can be diagnosed in primary-care settings with straightforward rapid tests. No such technology has been available to accurately detect tuberculosis and its drug-resistant forms, and this absence has been a major obstacle to improvement of tuberculosis care and reduction of the global burden of disease. Microscopy alone, although inexpensive, misses many patients and detects only those with relatively advanced disease.[1–3] Presently, only 28% of expected incident cases of tuberculosis are detected and reported as smear positive.[4] Undetected cases of disease increase morbidity, mortality, and disease transmission.[5–7] In many countries, epidemic HIV infection has further reduced the sensitivity of microscopy and increased the necessity of rapid diagnosis of tuberculosis. The mortality of untreated or mistreated tuberculosis in people with advanced HIV is high.[8–10] Autopsy studies in various countries have shown that 30–60% of people with HIV infection may die with

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For the **study protocol** see http://www.finddiagnostics.org/programs/tb/find_activities/xpert_mtb-rif_clinical_studies.html

tuberculosis, often undiagnosed, moving the cure-rate target of 85% for tuberculosis out of reach unless available diagnostic technologies can be improved.[11,12]

Multidrug-resistant tuberculosis is an increasing concern globally and directly threatens disease-control efforts in many countries.[13] Only 30 000 of nearly 500 000 new cases of multidrug-resistant tuberculosis every year[13] are detected and reported,[4] and misdiagnosis causes thousands of deaths, nosocomial and community transmission, and amplification of drug resistance.[14–16]

In recognition of these issues, substantial efforts are being made to strengthen laboratory capacity to diagnose smear-negative and multidrug-resistant tuberculosis, including increased use of solid and liquid culture, conventional drug-susceptibility testing, and line-probe assays. Unfortunately, these tests require extensive laboratory infrastructure and cannot be done outside of reference facilities.

Recently, a real-time PCR assay for *Mycobacterium tuberculosis* that simultaneously detects rifampicin resistance was developed on the GeneXpert platform (Cepheid, Sunnyvale, CA, USA), which integrates sample processing and greatly simplifies testing.[17,18] This assay, Xpert MTB/RIF, showed excellent performance in a multicentre study[19] undertaken in reference laboratories. In the study,[19] one-off direct MTB/RIF testing detected 92·2% of cases of pulmonary tuberculosis, including 72·5% of those with smear-negative disease, which was equivalent to that reported for solid culture.

Diagnostic tests often do well in initial studies that are usually done in near-ideal settings in reference laboratories; however, performance is frequently reduced when assays are tested in settings of intended use. In our study, we aimed to establish whether the MTB/RIF test was robust enough to retain high accuracy when used in district and subdistrict health facilities in resource-poor countries, and to measure the operational feasibility and effectiveness of its implementation in such settings.

Methods

Study population

In our multicentre implementation study, we enrolled adults aged 18 years or older with at least 2 weeks of cough who presented consecutively to urban or periurban primary-care health centres in South Africa, Peru, and India, to drug-resistance screening facilities in Azerbaijan and the Philippines, and to an emergency room at a central hospital in Uganda, and provided at least two sputum samples. Patients were excluded from the main analyses if their second sputum sample was collected more than 1 week after the first sample, if no culture was done, or if there was no valid culture, no valid MTB/RIF test result, smear-positive with no positive cultures, only one positive culture with 20 or fewer colonies for solid culture or more than 28 days to positivity for liquid culture, a positive culture during follow-up only, only one a positive culture with missing speciation result, a positive culture with only non-tuberculous mycobacterial growth, or discrepant rifampicin results by conventional drug-susceptibility testing on two samples.

We established the MTB/RIF test in the microscopy area of nine laboratories that were located within the same building at eight sites or a nearby building at one site (in one of two sites in Cape Town, South Africa). We chose study sites to represent diverse populations of patients and laboratory capacities. Sites in South Africa and Uganda served populations with a high prevalence of HIV, centres in Peru and India served populations with low prevalence of HIV and multidrug-resistant tuberculosis, and sites in Azerbaijan and the Philippines served populations with a high prevalence of multidrug-resistant tuberculosis.

The study was endorsed by national tuberculosis programmes of participating countries and approved by nine governing institutional review boards. The requirement to obtain individual informed consent was waived by all institutional review boards.

	Lima, Peru	Baku, Azerbaijan	Kampala, Uganda	Vellore, India	Manila, Philippines	Cape Town, South Africa
Routine smear microscopy and MTB/RIF test	In parallel	In parallel	In parallel	In parallel	In parallel	Weekly alternation
Number of sputum samples	2 (spot, morning)	3 (spot, spot, spot)	3 (spot, spot, morning)	2 (spot, morning)	3 (spot, morning, spot)	2 (spot, morning)
Direct MTB/RIF test	Sp 2 (morning)	Sp 1 (spot)	Sp 1 (spot)	Sp 2 (morning)	Sp 1 (spot)	Sp 1 (spot)
Routine smear microscopy	2 direct ZN (Sp 1, Sp2)	3 direct ZN (Sp 1, Sp2, Sp3)	2 direct ZN (Sp 1, Sp2)	2 direct ZN (Sp 1, Sp2)	3 direct ZN (Sp 1, Sp2, Sp3)	2 FM on pellet (Sp 1, Sp2)*
Culture method	1 MGIT (Sp 1)	1 MGIT, 1 LJ (Sp 2)	1 MGIT (Sp 2), 2 LJ (Sp 2, Sp3)	1 LJ (Sp 1)	1 MGIT (Sp 2), 2 Ogawa (Sp 2, Sp 3)	1 MGIT (Sp 2)
DST method	MGIT SIRE	MGIT SIRE	Indirect LPA, LJ proportion	LJ proportion	LJ proportion	Direct and indirect LPA

MTB=*Mycobacterium tuberculosis*. RIF=rifampicin. Sp1=sputum sample 1. Sp2=sputum sample 2. Sp3=sputum sample 3. ZN=light microscopy after Ziehl Neelsen staining of sputum smear. FM=conventional fluorescence microscopy after Auramine O staining. LPA=line-probe assay (direct: done from decontaminated sputum for smear-positive specimens; indirect: done from culture isolates for smear-negative specimens). MGIT=mycobacteria growth indicator tube. LJ=Löwenstein–Jensen. DST=drug-susceptibility testing. SIRE=streptomycin, isoniazid, rifampicin, ethambutol. *One smear was prepared from an NaOH-treated pellet (all patients) and one from a bleach-treated pellet (smear group only).

Table 1: **Laboratory procedures**

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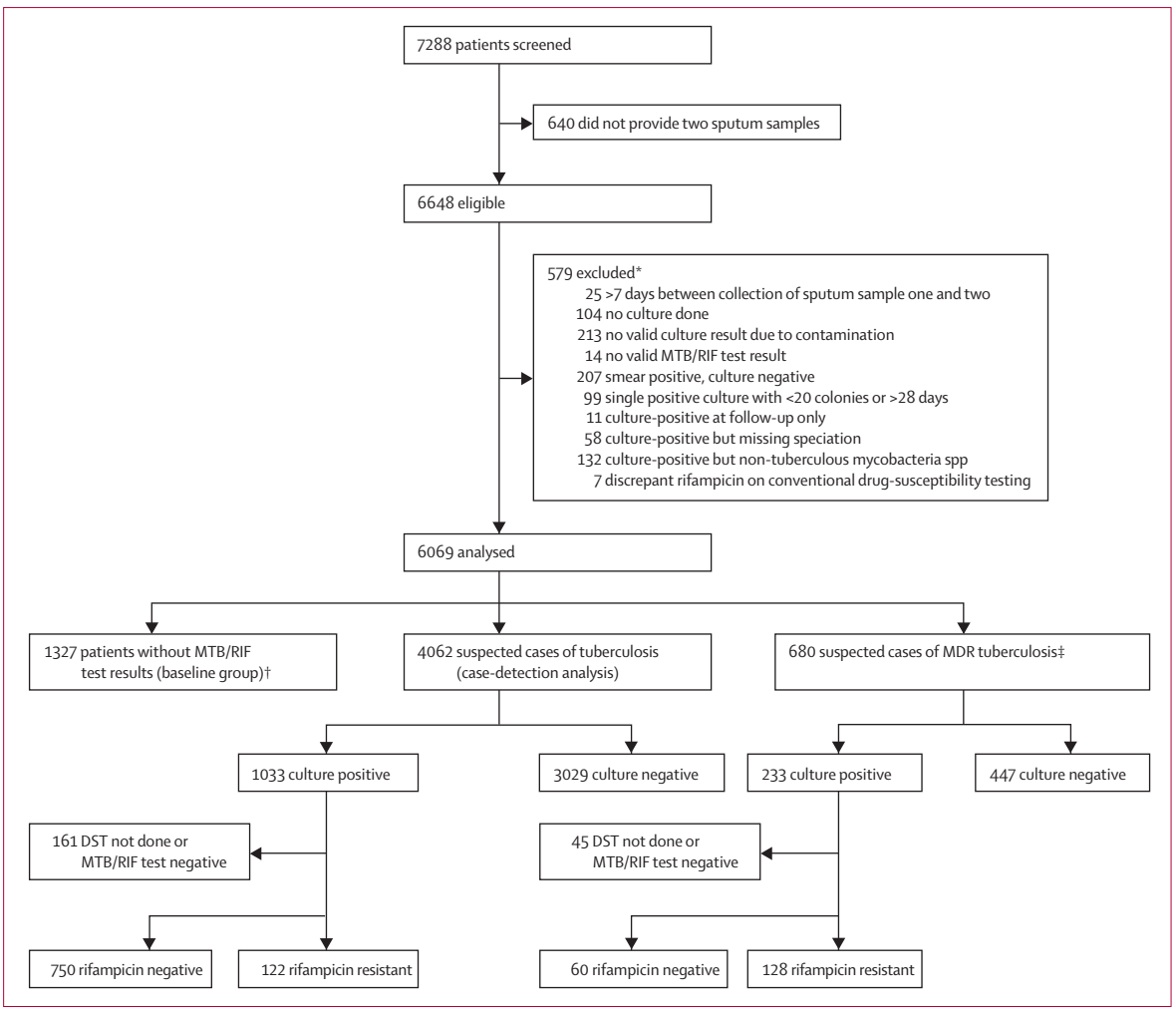

Figure 1: **Study profile**
MTB=*Mycobacterium tuberculosis*. RIF=rifampicin. MDR=multidrug resistant. DST=drug-susceptibility testing. *Some patients met several exclusion criteria and are listed more than once. †In South Africa only. ‡680 suspected cases of MDR tuberculosis were not included in the case-detection analysis to avoid patient-selection bias (patients were expected to have a higher tuberculosis prevalence and supposedly higher bacillary load); a subgroup analysis for these patients is shown in webappendix p 3.

Procedures

Our study was divided into two phases. In the validation phase, MTB/RIF test results were not reported or used for management of patients. This phase allowed the collection of baseline data and confirmed that the site could accurately undertake the MTB/RIF test. In the implementation phase, MTB/RIF test results informed tuberculosis treatment decisions. Before sites could move to the implementation phase they were required to meet predefined performance targets, which were reviewed and approved by the institutional review boards. Table 1 shows the laboratory procedures used in every country. In both phases, participants provided 2–3 sputum samples as per local routine. One sample underwent smear microscopy and direct MTB/RIF testing, the second underwent smear microscopy, culture, and drug-susceptibility testing. The third sample was only collected at sites that routinely required three microscopy results for management of patients.

In South Africa, the routine use of bleach-pretreatment for fluorescent microscopy meant that MTB/RIF testing on the same sputum sample was not possible. Therefore, in South Africa we used a study design with weekly alternation between a baseline group and implementation group. In the baseline group, routine smear microscopy from a bleach-treated pellet was done, which was replaced by the MTB/RIF test (used for management of patients) in the implementation group. In both groups, a second specimen was obtained for smear microscopy from a sodium hydroxide (NaOH)-treated pellet, culture, and drug-susceptibility testing.

The MTB/RIF test was done on raw sputum samples with an automated readout provided to the user as described elsewhere.[18] GeneXpert four-module devices were placed on an open bench in the microscopy area. On the basis of biosafety data,[17] the MTB/RIF test sample preparation step was done applying the same local

	Lima, Peru	Baku, Azerbaijan	Cape Town, South Africa	Kampala, Uganda	Vellore, India	Manila, Philippines	Total
Characteristics of tuberculosis laboratories implementing the MTB/RIF test							
Number of laboratories	Three	One	Two	One	One	One	Nine
Level of health system	Two health centres; one district hospital	MDR tuberculosis screening facility	One health centre; one provincial hospital	Emergency unit of referral hospital	Health centre	MDR tuberculosis screening facility	..
Methods in routine use (during the study)	Health centres: ZN; district hospital: ZN, Ogawa	ZN, LJ, MGIT SIRE	Health centre: FM; provincial hospital: FM	ZN	ZN	ZN, Ogawa, LJ	..
Mean MTB/RIF test operating temperature (range)	24°C (19–32°C)	21°C–AC (12–34°C)	22°C–AC (16–29°C)	25°C (20–32°C)	25°C–AC (19–42°C)	23°C–AC (19–25°C)	..
Median MTB/RIF test workload per day (range, IQR)	Health centre: 3 (1–16, 2–4); district hospital: 5 (1–15, 2–7)	8 (1–20, 3–12)	Health centre: 5 (1–15, 3–8); provincial hospital: 6 (1–24, 3–14)	2 (1–6, 1–3)	6 (1–20, 3–8)	5 (1–20, 3–7)	4 (1–24, 2–7)
Characteristics of study population							
Estimated incidence of tuberculosis (new cases per 100 000)	113[22]	110[22]	Health centre: 1622;[23] provincial hospital: 600[24]	293[22]	145[25]	129[26]	..
Estimated MDR tuberculosis rate (new cases, retreatment cases)	5·3%, 23·6%[27]	22·3%, 55·8%[28]	3·3%, 7·7%[29]	1·1%, 11·7%[30]	2·4%, 17·4%[28]	3·8%, 20·9%[31]	..
Estimated HIV co-infection rate in patients with tuberculosis	<3%[32]	5·6%[33]	76·1%[19]	31·9%[30]	7·0%[25]	<1%[22]	..
Demographic characteristics of enrolled patients*							
Number	1185	749	2522	372	902	918	6648
Enrolled in validation phase (controls)	1185/1185 (100%)	443/749 (59%)	1327/2522 (53%)	282/372 (76%)	896/902 (99%)	601/918 (65%)	4734/6648 (71%)
Enrolled in implementation phase	0/1185	306/749 (41%)	1194/2522 (47%)	90/372 (24%)	0/902	317/918 (35%)	1907/6648 (29%)
Median age (range, IQR)	37 (18–91, 26–53)	36 (18–74, 30–44)	36 (18–101, 29–46)	32 (18–79, 26–38)	45 (18–90, 32–58)	47 (18–95, 34–58)	38 (18–101, 29–50)
Women	578/1185 (49%)	1/749 (<1%)	1247/2522 (49%)	170/372 (46%)	274/902 (30%)	335/918 (36%)	2605/6648 (39%)
HIV status							
Positive	5/1185 (<1%)	1/749 (<1%)	947/2522 (38%)	254/372 (68%)	40/902 (4%)	8/918 (<1%)	1255/6648 (19%)
Negative	289/1185 (24%)	609/749 (81%)	855/2522 (34%)	118/372 (32%)	4/902 (<1%)	9/918 (1%)	1884/6648 (28%)
Unknown	891/1185 (75%)	139/749 (19%)	720/2522 (29%)	0/372	858/902 (95%)	901/918 (98%)	3509/6648 (53%)
Diagnosis group at enrolment†							
Group 1 (suspicion of drug-sensitive tuberculosis)							
Patients	1092/1185 (92%)	644/749 (86%)	2372/2522 (94%)	363/372 (98%)	888/902 (98%)	503/918 (55%)	5862/6648 (88%)
Prevalence of tuberculosis‡	177/1031 (17%)	229/578 (40%)	473/1968 (24%)	146/307 (48%)	101/837 (12%)	148/415 (36%)	1274/5136 (25%)
Prevalence of rifampicin resistance§	15/165 (9%)	46/224 (21%)	24/462 (5%)	4/130 (3%)	7/101 (7%)	48/134 (36%)	144/1216 (12%)
Group 2 (suspicion of MDR tuberculosis)							
Patients	93/1185 (8%)	105/749 (14%)	150/2522 (6%)	9/372 (2%)	14/902 (2%)	415/918 (45%)	786/6648 (12%)
Prevalence of tuberculosis	32/83 (39%)	17/99 (17%)	20/122 (16%)	1/8 (13%)	7/14 (50%)	168/328 (51%)	245/654 (37%)
Prevalence of rifampicin resistance	8/27 (30%)	11/16 (69%)	5/20 (25%)	0/1	4/7 (57%)	113/142 (80%)	141/213 (66%)

Data are n/N (%), unless otherwise stated. MTB=*Mycobacterium tuberculosis*. RIF=rifampicin. MDR=multidrug resistant. ZN=light microscopy after Ziehl Neelsen staining of sputum smear. LJ=Löwenstein–Jensen. MGIT SIRE=mycobacteria growth indicator tube streptomycin, isoniazid, rifampicin, ethambutol. FM=conventional fluorescence microscopy after Auramine O staining. AC=air conditioning. *For 0·1% of enrolled patients, whether they were part of the validation or implementation phase was not reported. †Estimation based on epidemiological studies or surveys. ‡For calculations of prevalence of tuberculosis and rifampicin resistance, the exclusion criteria described in the methods section have been applied. §Calculations of rifampicin resistance prevalence were done only on the basis of patients who had rifampicin sensitivity testing.

Table 2: **Characteristics of patients and study sites**

See Online for webappendix

biosafety conditions as for the preparation of microscopy smears: a biosafety cabinet was used at five of the nine sites. Temperature logs were placed at each facility to record the operating and reagent storage temperatures. Laboratory staff chosen as MTB/RIF test operators had little experience with laboratory methods other than smear microscopy, had never undertaken molecular testing, and had basic or no computer skills (see webappendix p 1). Masking, which was not necessary in South Africa due to study design, was accomplished at

the other sites by having different staff do smear microscopy and MTB/RIF testing.

The reference standard, quality-assured culture and drug-susceptibility testing, was done at reference laboratories located within 1 h of MTB/RIF test sites. Samples undergoing Löwenstein–Jensen or liquid culture (Bactec MGIT; BD Microbiology Systems, Cockeysville, MD, USA) were processed with standard *N*-acetyl-L-cysteine–NaOH (2%) decontamination. For Ogawa culture, sputum specimens were decontaminated with the modified Petroff method.[20] All positive cultures underwent MPT64-based (Capilia tuberculosis assay; Tauns, Numazu, Japan) species confirmation[21] and, if positive for *M tuberculosis*, conventional drug-susceptibility testing with Löwenstein–Jensen proportion or mycobacteria growth indicator tube (MGIT). In South Africa, the line-probe assay MTBDRplus (Hain Lifescience, Nehren, Germany) was done on NaOH-treated pellets for smear-positive sputum and on culture isolates for smear-negative sputum. Conventional drug-susceptibility testing was then used for specimens testing positive for drug-resistance-associated mutations. In Uganda, line-probe assay and, for 10% of culture positive patients (every tenth patient), Löwenstein–Jensen proportion was performed on MGIT isolates (except when only positive on Löwenstein–Jensen). HIV results were obtained from clinical records.

Clinicians categorised participants into two groups: patients who had suspected tuberculosis and presented for case detection and patients with suspected multidrug-resistant tuberculosis who presented for resistance detection (patients who received tuberculosis treatment within the past year or had contact with multidrug-resistant tuberculosis). For analysis, patients with suspected tuberculosis were divided into four categories: smear-positive and culture-positive pulmonary tuberculosis; smear-negative and culture-positive pulmonary tuberculosis; smear-negative, culture-negative and not treated (non-tuberculosis); and smear-negative and culture-negative but treated for tuberculosis on the basis of clinical and radiological findings (clinical tuberculosis). A patient was regarded as having smear-positive tuberculosis on the basis of at least two scanty smears (1–9 bacilli per 100 fields [1000× for light microscopy and 400× for fluorescence microscopy]) or one or more smears of grade 1+ or higher (10–99 bacilli per 100 fields). A culture-positive case was defined as the isolation of *M tuberculosis* in at least one culture. Patients who were culture-positive (suspected tuberculosis and multidrug-resistant tuberculosis) were categorised as sensitive or resistant to rifampicin.

Statistical analysis

We calculated sensitivity and specificity of the MTB/RIF test for each patient category stratified by HIV and smear microscopy status, and used the results of all microscopy and culture examinations to classify patients into the four groups. To prevent selection bias, patients with suspected multidrug-resistant tuberculosis were only included in the analysis of MTB/RIF test rifampicin-detection endpoints.

We quantitatively assessed operational feasibility of introduction of the MTB/RIF test by examining indicators of robustness such as indeterminate rate, frequency of DNA contamination events, and variation of performance in time and between sites. We used a hands-on and question-based proficiency test and user-appraisal questionnaire to qualitatively establish the minimal training needs and ease of use. The Foundation for Innovative New Diagnostics (FIND; Geneva, Switzerland) study team did the training.

We assessed effectiveness of every method by examining the time to detection of tuberculosis and rifampicin resistance and the time to reporting of results to the clinics. Additionally, we compared the time to treatment initiation from first sputum collection and the dropout rate (patients with confirmed tuberculosis who had not started treatment) between validation and implementation phases.

Within sites we measured association between variables with the Pearson's χ^2 test and between sites we used the Cochran-Mantel-Haenszel statistic. We did within-patient analysis with McNemar's test. We did a subgroup analysis for excluded patients. All analyses were done with SAS version 9.2, and $p<0\cdot05$ was regarded as significant.

Role of the funding source

The FIND cosponsored the study and led study design, training, study coordination and monitoring, data analysis, and writing of the report. The other sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

	Culture positive		Culture negative	
	Smear positive	Smear negative	Clinical tuberculosis	Non-tuberculosis
Suspected cases of tuberculosis				
HIV positive	86/648 (13%)	124/385 (32%)	392/2876 (14%)	19/153 (12%)
HIV negative	206/648 (32%)	129/385 (34%)	753/2876 (26%)	36/153 (24%)
HIV status unknown	356/648 (55%)	132/385 (34%)	1731/2876 (60%)	98/153 (64%)
Suspected cases of multidrug-resistant tuberculosis				
HIV positive	0/195	3/38 (8%)	1/33 (3%)	54/414 (13%)
HIV negative	19/195 (10%)	9/38 (24%)	8/33 (24%)	127/414 (31%)
HIV status unknown	176/195 (90%)	26/38 (68%)	24/33 (73%)	233/414 (56%)

Table 3: **HIV statuses in patients with suspected cases of tuberculosis and multidrug-resistant tuberculosis**

Results

From Aug 11, 2009, until June 26, 2010, we enrolled 6648 eligible adults (figure 1, tables 2 and 3). One-off MTB/RIF testing correctly detected tuberculosis in more

	Sensitivity			Specificity (non-tuberculosis)	Positive predictive value	Negative predictive value
	All culture positive	Sputum positive, culture positive	Sputum negative, culture positive			
Lima, Peru	171/177 (96·6%, 92·8–98·4)	134/135 (99·3%, 95·9–99·9)	37/42 (88·1%, 75·0–94·8)	825/828 (99·6%, 98·9–99·9)	98·0%	99·3%
Baku, Azerbaijan	203/229 (88·6%, 83·9–92·1)	135/138 (97·8%, 93·8–99·3)	68/91 (74·7%, 64·9–82·5)	303/307 (98·7%, 96·7–99·5)	97·6%	93·5%
Cape Town, South Africa	201/233 (86·3%, 81·3–90·1)	80/80 (100·0%, 95·4–100·0)	121/153 (79·1%, 72·0–84·8)	669/671 (99·7%, 98·9–99·9)	99·0%	95·6%
Kampala, Uganda	121/145 (83·4%, 76·6–88·6)	91/93 (97·8%, 92·5–99·4)	30/52 (57·7%, 44·2–70·1)	144/144 (100·0%, 97·4–100·0)	100·0%	87·7%
Vellore, India	101/101 (100·0%, 96·3–100·0)	70/70 (100·0%, 94·8–100·0)	31/31 (100·0%, 89·0–100·0)	671/687 (97·7%, 96·3–98·6)	85·8%	100·0%
Manila, Philippines	136/148 (91·9%, 86·4–95·3)	127/132 (96·2%, 91·4–98·4)	9/16 (56·3%, 33·2–76·9)	234/239 (97·9%, 95·2–99·1)	95·7%	95·9%
Total	933/1033 (90·3%, 88·4–92·0)	637/648 (98·3%, 97·0–99·0)	296/385 (76·9%, 72·4–80·8)	2846/2876 (99·0%, 98·5–99·3)	96·8%	96·8%

Data are number of positive results/number of samples tested (%, 95% CI). MTB=*Mycobacterium tuberculosis*. RIF=rifampicin.

Table 4: **Sensitivity, specificity, and predictive values of a one-off direct MTB/RIF test**

	HIV positive	HIV negative	HIV negative or unknown	p value*
Sensitivity in culture-positive samples				
Smear microscopy	86/193 (44·6%, 37·7–51·6)	234/341 (68·6%, 63·5–73·3)	613/848 (72·3%, 69·2–75·2)	<0·0001
MTB/RIF test	173/210 (82·4%, 76·7–86·9)	304/335 (90·7%, 87·2–93·4)	760/823 (92·3%, 90·3–94·0)	0·0849
Sputum positive	84/86 (97·7%, 91·9–99·4)	204/206 (99·0%, 96·5–99·7)	553/562 (98·4%, 97·0–99·2)	0·2167
Sputum negative	89/124 (71·8%, 63·3–78·9)	100/129 (77·5%, 69·6–83·9)	207/261 (79·3%, 74·0–83·8)	0·8976
Specificity in non-tuberculosis samples				
Smear microscopy	660/660 (100·0%, 99·4–100·0)	1054/1060 (99·4%, 98·8–99·7)	3040/3058 (99·4%, 99·1–99·6)	0·2545
MTB/RIF test	389/392 (99·2%, 97·8–99·7)	748/753 (99·3%, 98·5–99·7)	2457/2484 (98·9%, 98·4–99·3)	0·2246

Data are number of positive results/number tested (%, 95% CI). On the basis of the p values, the performance of the MTB/RIF test in this study did not differ significantly in patients who were HIV positive compared with those who were HIV negative or who were not tested for HIV infection, while the sensitivity of smear microscopy was significantly reduced in patients who were HIV positive. MTB=*Mycobacterium tuberculosis*. RIF=rifampicin. *Determined by use of the Cochran-Mantel-Haenszel method comparing patients who are HIV positive with those whose statuses are HIV negative or unknown.

Table 5: **Sensitivity and specificity of smear microscopy (two to three microscopy examinations as per routine practice) and a one-off direct MTB/RIF test, stratified by HIV status of patients**

than 90% of patients with positive cultures, with 99% specificity for non-tuberculosis (table 4). Performance was much the same during validation and implementation phases (webappendix p 2). A one-off MTB/RIF test identified significantly (p<0·0001) more cases of tuberculosis than did 2–3 smear microscopy examinations per patient, which detected 699 of 1041 culture-positive patients (sensitivity of 67·1%) and 3700 of 3718 patients without tuberculosis (specificity of 99·5%). Although HIV co-infection significantly decreased the sensitivity of smear microscopy (p<0·0001), the sensitivity of MTB/RIF was not significantly affected by HIV co-infection status (p=0·0849; table 5). MTB/RIF test sensitivity and specificity were much the same between basic health centres and sites with increased capacity both between countries (p=0·895 and p=0·097, respectively; webappendix p 2), and within countries with more than one site (webappendix p 2).

Sensitivity of MTB/RIF testing for smear-negative tuberculosis varied between countries (p<0·0001). It was lower at sites that used a reference standard of solid and liquid cultures (Azerbaijan, Uganda, and the Philippines) and slightly higher at sites that tested morning sputum samples rather than spot sputum collections (Peru and India).

MTB/RIF testing correctly identified 242 of 250 cases of rifampicin-resistant tuberculosis (sensitivity of 96·8%) and 779 of 810 rifampicin-sensitive cases (specificity of 96·2%). However, because of concern over false-positive results, especially for settings with a low-prevalence of multidrug-resistant disease, we changed the software cutoff defining drug resistance during the study on May 12, 2010. With modified software definitions, our post-hoc analysis showed that sensitivity decreased to 94·4% and specificity increased to 98·3% (table 6). 17 (6·8%) of 250 cases of rifampicin-resistant tuberculosis were sensitive to isoniazid.

24 (16%) of 153 patients with clinically diagnosed tuberculosis, but negative culture had positive results on MTB/RIF testing. 20 (83%) of these 24 patients had clinical and radiological follow-up, and all 20 improved on tuberculosis treatment. For the 118 (91%) of 129 patients who tested negative on MTB/RIF but were treated for tuberculosis on the basis of a clinical diagnosis and had clinical and radiological follow-up, only 67 (57%) showed improvement (p<0·0001).

Median time to detection of tuberculosis for the MTB/RIF test was 0 days (IQR 0–1), compared with 1 day (0–1) for smear microscopy, 30 days (23–43) for solid culture,

	Sensitivity in rifampicin-resistant cases	Specificity in rifampicin-sensitive cases	Positive predictive value	Negative predictive value
Lima, Peru	22/23 (95·7%, 79·0–99·2)	161/162 (99·4%, 96·6–99·9)	95·6%	99·4%
Baku, Azerbaijan	47/50 (94·0%, 83·8–97·9)	160/161 (99·4%, 96·6–99·9)	98·0%	98·1%
Cape Town, South Africa	9/10 (90·0%, 59·6–98·2)	175/178 (98·3%, 95·2–99·4)	77·1%	99·3%
Kampala, Uganda	1/3 (33·3%, 6·1–79·2)	112/113 (99·1%, 95·2–99·8)	54·2%	97·9%
Vellore, India	8/10 (80·0%, 49·0–94·3)	91/93 (97·8%, 92·5–99·4)	80·5%	97·7%
Manila, Philippines	149/154 (96·8%, 92·6–98·6)	97/103 (94·2%, 87·9–97·3)	95·5%	95·9%
Total	236/250 (94·4%, 90·8–96·6)	796/810 (98·3%, 97·1–99·0)	93·2%	98·6%

Data are number of positive results/number tested (%, 95% CI). The reference standard was phenotypic susceptibility testing in Peru, Azerbaijan, Uganda, and the Philippines and genotypic testing by line-probe assay followed by phenotypic drug-susceptibility testing for resistant cases in South Africa and Uganda. MTB=*Mycobacterium tuberculosis*. RIF=rifampicin.

Table 6: **MTB/RIF test sensitivity and specificity for detection of rifampicin resistance after change to software cutoff**

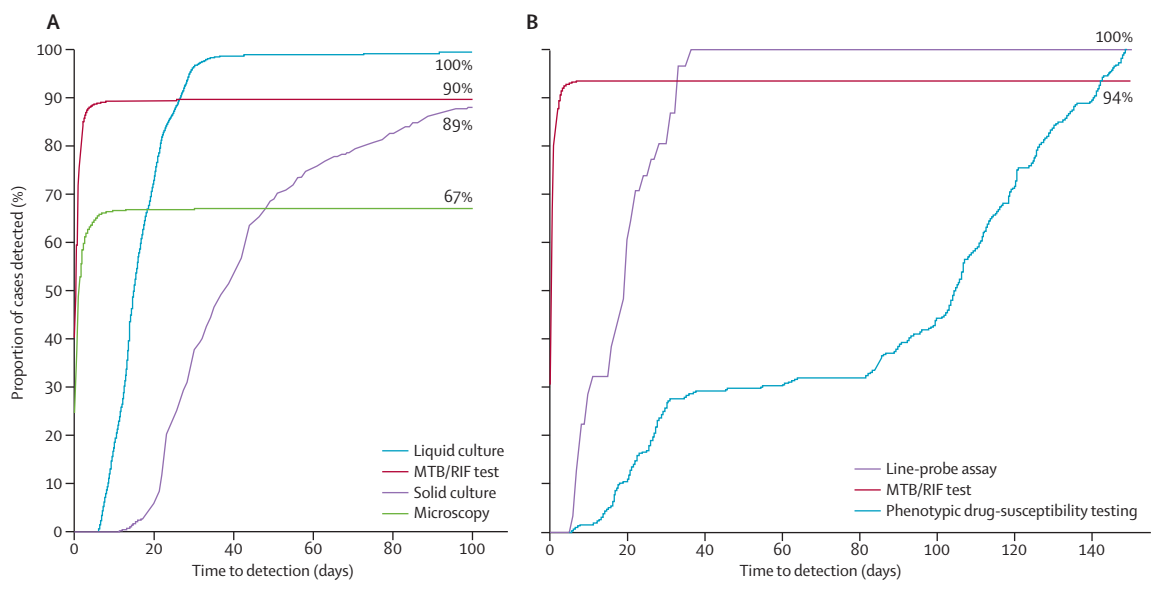

Figure 2: **Proportion of tuberculosis cases detected by each method in culture-positive patients**
Percentages are the maximum proportion of cases detected by every method. (A) Tuberculosis case detection. (B) Detection of rifampicin resistance. Time to detection was defined as time between date of sputum sample collection and date of positive result. MTB=*Mycobacterium tuberculosis*. RIF=rifampcicin.

and 16 days (13–21) for liquid culture (figure 2). Median time to detection of rifampicin resistance was 1 day (0–1) for the MTB/RIF test, 20 days (10–26) for line-probe assay (done directly from sputum pellet for smear-positive specimens and from culture isolates for smear-negative specimens) and 106 days (30–124) for phenotypic drug-susceptibility testing (figure 2). Although MTB/RIF testing and microscopy were done near the clinics and results were rapidly received by clinicians (median 1 day [IQR 0–2] for MTB/RIF testing and 2 days [2–3] for microscopy), there were significant delays in receiving results from cultures (median 58 days [42–62]), line-probe assays (40 days [27–53]), and conventional drug-susceptibility testing (63 days [38–102]). Some results were lost or unreported (figure 3).

Time between sputum collection and treatment initiation was very dependent on the testing method (figure 4). In the baseline group in South Africa and the validation phase at other sites (ie, when MTB/RIF test results were not used to direct therapy), patients with smear-negative, culture-positive tuberculosis started treatment after a median of 56 days (IQR 39–81). Once MTB/RIF test results were used to direct therapy, the median time-to-treatment for smear-negative tuberculosis reduced to 5 days (2–8). Rates of untreated smear-negative, culture-positive tuberculosis reduced from 39·3% (95% CI 32·6–46·6) at baseline to 14·7% (9·9–21·2) after implementation of the MTB/RIF test.

GeneXpert provides an indeterminate result if unexpected results occur with any of the internal control measures. The MTB/RIF test was indeterminate in 126 (2%) of 5321 samples tested. 112 repeat tests were successful when adequate sputum remained, with the

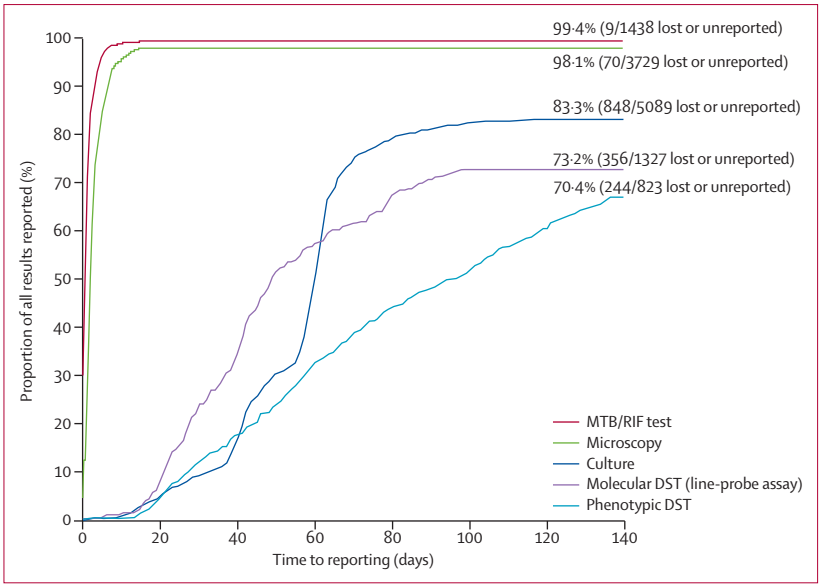

Figure 3: **Proportion of results reported to the clinics for each method from date of first sputum sample**
Percentages are the maximum proportion of results received by the clinic within 30 days of recorded date of smear microscopy, MTB/RIF test, or culture, or within 150 days of sputum collection for drug-susceptibility testing (DST). TB=*Mycobacterium tuberculosis*. RIF=rifampicin.

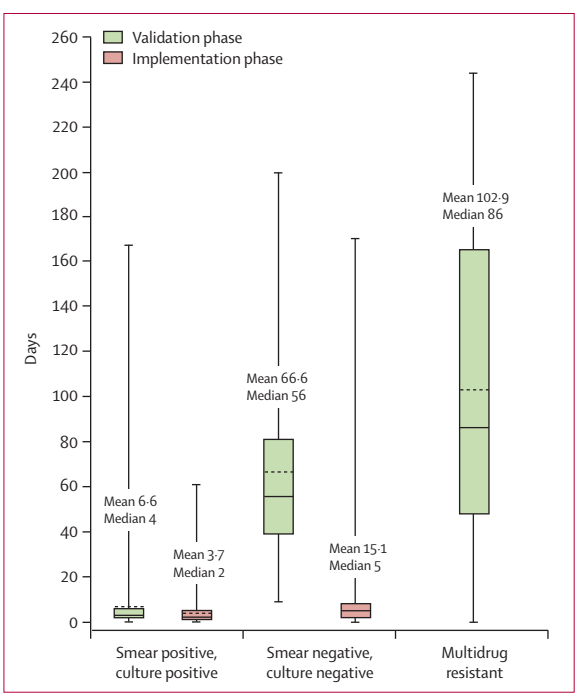

Figure 4: **Time to treatment during validation phase (treatment based on conventional methods only) and implementation phase (treatment based on MTB/RIF test and conventional methods) for patients with smear-positive, culture-positive tuberculosis, smear-negative, culture-positive tuberculosis, or multidrug-resistant tuberculosis**
Box plots show median time to treatment (black line), mean (dashed black line), 25th and 75th percentiles, and minimum and maximum reported time to treatment (whiskers). Time to treatment was calculated from the date of first sputum collection to the date of treatment initiation. For the time to multidrug-resistant treatment, treatment decisions during this study were only made on the basis of routine drug-susceptibility testing methods. MTB=*Mycobacterium tuberculosis*. RIF=rifampicin.

indeterminate rate reduced to less than 1% (14/5321 samples). In 1449 samples that were positive on MTB/RIF testing, 17 (1%) had indeterminate results for rifampicin resistance. These tests were not repeated. By comparison, the contamination rate was 441 (5%) of 9690 cultures, including repeated cultures from re-decontaminated pellets from all countries apart from South Africa and the Philippines.

Operators without previous molecular biology experience or computer skills passed proficiency testing after 1–3 days of training on MTB/RIF tests, including three hands-on runs. A 1 day online training was successfully used at two sites (Peru and Azerbaijan). Monthly variation in MTB/RIF test performance did not differ between sites ($p_{sensitivity}$=0·52 on Cochran-Mantel-Haenszel test stratified by smear status and $p_{specificity}$=0·46 on χ^2).

In one of the high HIV-prevalence sites, microscopy was introduced at the same time as MTB/RIF testing. Although MTB/RIF sensitivity for culture-positive tuberculosis at this site was much the same as in other centres (85·9%; 116 of 135 cases), the sensitivity of microscopy with one smear per patient was only 17·8% (21 of 118 smears) compared with 46·6% (55 of 118 smears) with a second smear at the reference laboratory. These findings support the laboratory managers' perception, expressed in user appraisal questionnaires, that MTB/RIF test performance might be less dependent on user skills, motivation, or workload than is microscopy.

We did not detect any DNA contamination events during monthly negative control runs, and test specificity was high across sites. The four-module GeneXpert device was used for 1–24 tests a day with only two incidents needing product support (one network-card failure requiring device replacement and one module replacement). At four sites, the recorded operating temperatures exceeded the maximum recommended operating temperature (15–30°C) during more than 10% of runs. Test performance and frequency of indeterminate results did not show seasonal variation in these sites. In one case, the operating temperature exceeded 40°C and an error message appeared as described in the manual. Several sites had daily temperatures higher than the 2–28°C recommended for cartridge storage temperature; cartridges were stored centrally and distributed twice every month. All sites had power cuts, but used uninterruptible power supplies to support the device during short power cuts and one site used an inverter and serial car batteries during a longer power outage.

Discussion

The MTB/RIF test assay was designed specifically for use close to point-of-treatment in endemic disease settings, and is the first of a new generation of diagnostic tests that have the potential to bring highly sensitive nucleic acid amplification testing to peripheral sections of the health system (panel). In our large multicentre study, MTB/RIF testing in subdistrict microscopy facilities by

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routine staff with minimal training retained the accuracy seen in previous controlled studies that were undertaken in reference centres.[18,19,34–36] Previous studies of the MTB/RIF test that assessed either sputum samples or concentrated, decontaminated sputum pellets, have consistently reported test sensitivity of 72–75% in cases of smear-negative tuberculosis and 98–100% in cases of smear-positive tuberculosis.[18,19,34–36] One small retrospective study of 28 frozen pellets reported a sensitivity of 57% for cases of smear-negative tuberculosis.[35] In our study, a one-off direct MTB/RIF test detected tuberculosis in more than 90% of patients who were culture positive, including nearly 77% of those with negative smears. The robustness of these data suggests that the test can be used in various resource-scarce settings for case detection and for rapid decentralised screening of multidrug-resistant tuberculosis. The ability to rapidly detect smear-negative tuberculosis in peripheral settings, including among patients with HIV, is a breakthrough in tuberculosis care and control.

This is the first study in which MTB/RIF test results have been made available to clinic staff to inform patient management, and hence the first to describe the effect on time to detection and treatment. The short turnaround time resulted in substantially faster initiation of appropriate tuberculosis therapy, particularly for patients with smear-negative disease, and lower dropout rates. Many patients with tuberculosis drop out during the diagnostic process through failing to submit specimens for microscopy when prescribed,[37] submitting an initial specimen but not returning,[38] or not receiving or acting on positive test results.[39–41] Rapid testing, even if less sensitive than slower methods, can result in more patients being correctly treated. Overall, patient dropout with one-off MTB/RIF testing could possibly be reduced even further in routine conditions, as our analysis excluded 640 (9%) of 7288 enrolled patients who did not provide a second sample (figure 1). Although treatment decisions for multidrug-resistant tuberculosis were not informed by MTB/RIF test results, delays in result reporting for rapid, but centralised drug-susceptibility testing (line-probe assay and MGIT drug-susceptibility testing) were substantially shortened by decentralised MTB/RIF testing, and would probably translate into reduced time-to-appropriate-treatment.

Although the sensitivity and specificity of MTB/RIF test for detection of rifampicin resistance in this study was high (94·4% sensitivity and 98·3% specificity), accuracy was higher in previous publications (99–100% sensitivity and 100% specificity after discordant resolution by genotyping).[18,19,34–36] Assay development partners are working to further improve MTB/RIF test accuracy of detection of multidrug-resistant tuberculosis. The low positive-predictive value of MTB/RIF for rifampicin resistance detection that we noted in patients with a low pretest probability of multidrug-resistant tuberculosis might justify the need for confirmatory testing with conventional methods in such settings.

Panel: Research in context

Systematic review

We searched the PubMed database for studies about the Xpert MTB/RIF test published in English up to March 18, 2011, with the search terms "Xpert" or "GeneXpert" and "tuberculosis". We did not identify any systematic reviews. We identified five studies reporting on performance of the MTB/RIF test for detection of tuberculosis in respiratory specimens.

Interpretation

All studies that we identified were done in research or referral laboratories and were small,[18,34–36] apart from one large multicentre assessment.[19] Most included testing of previously collected archived samples. In these studies, the reported sensitivity of the MTB/RIF test for detection of smear-positive tuberculosis (98–100%) and smear-negative tuberculosis (72–75%) were consistent, apart from one small study that documented a sensitivity of 57% for smear-negative tuberculosis in 28 previously frozen sputum pellets. With regard to detection of rifampicin resistance, sensitivity and specificity were very high in all previous studies (99–100% sensitivity and 100% specificity after resolution of discordant cases by genotyping), although numbers of rifampicin-resistant cases were small in all studies apart from multicentre assessment. Our study confirms the sensitivity of the MTB/RIF test for smear-positive and smear-negative tuberculosis, when undertaken in routine microscopy centres, and showed reduced, but good, performance for detection of rifampicin resistance. Furthermore, we suggest the MTB/RIF test can provide a substantially reduced time to detection and treatment for smear-negative tuberculosis.

Several issues might restrict the applicability of the MTB/RIF test at small health centres. The device requires stable electricity supply, although some centres successfully tested battery operation. Device deployment above 30°C is presently not recommended by the manufacturer and cartridges are confirmed as stable at 2–28°C (efforts are ongoing to increase the operating and storage temperatures). There were few device breakdowns in this study as the devices used were new, and there are no data for their extended use in dusty and humid conditions. The GeneXpert device needs calibration yearly, which requires either access to an MTB/RIF test distributor or internal capacity to replace modules as per manufacturer instructions.

In the study, MTB/RIF test cartridges were handled with the same level of biosafety as microscopy. As the MTB/RIF tuberculosis assay was designed to keep biohazards to a minimum, the risk should be substantially lower than that noted in microscopy. As published elsewhere,[17] the only specimen processing required is the addition of a sample reagent that is bactericidal and results in a 10^7 reduction in viable

mycobacteria in the first 15 min. Additionally, unlike smear microscopy, the manual pipetting steps and the automated portion of the assay do not generate viable mycobacterial aerosols.[17] Together, these results suggest that the MTB/RIF test can be done without special biosafety precautions.

Our study findings have several limitations. The use of different study designs and diagnostic algorithms across sites made a direct comparison of findings challenging. Our study did not allow us to determine the effect of rapid and early detection on the number of patients treated and on treatment outcomes, as longterm follow-up was not undertaken and as the parallel use of culture, not otherwise routinely available, may have affected physicians' choices. Additionally, the study did not include any testing of close contacts to measure effect on transmission. Participating sites were urban or periurban and supply chain management, reagent storage, and calibration are likely to be more problematic in rural areas.

Overall, our findings suggest that decentralised MTB/RIF test implementation is feasible and could lead to an improvement in tuberculosis care and control. Any improvement will require increased detection of tuberculosis and multidrug-resistant-tuberculosis to coincide with scale-up of first-line, and more importantly, second-line treatment.[42] Whether early and appropriate treatment after MTB/RIF testing can reduce tuberculosis-associated morbidity and mortality, and its effect on transmission, needs to be established.

Contributors

CCB and MPN designed the study. CG, CCB, PN, FC, MDP, and MPN analysed the final data and developed the first manuscript draft. All authors contributed to data collection, interpretation of data, and revision of the article.

Conflicts of interest

CCB, PN, CG, HAlb, and MDP are employed by the Foundation for Innovative New Diagnostics (FIND, Geneva, Switzerland), a non-profit organisation that collaborates with industry partners, including Cepheid (Sunnyvale, CA, USA), on the development, assessment, and demonstration of new diagnostic tests. HAle was a consultant for FIND. DA has been a consultant to Cepheid and received royalties personally; DA and RB's institution received royalties under a licensing agreement between University of Medicine and Dentistry of New Jersey (Newark, NJ, USA) and Cepheid. DA's royalties generated by the Xpert assay have been voluntarily (but irrevocably) capped at US$5000 per year (personal income) and $50 000 per year (laboratory income) to mitigate potential conflicts of interest. No commercial partner was involved in the study.

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