EDITORIALS

Tuberculosis Diagnosis — Time for a Game Change

Peter M. Small, M.D., and Madhukar Pai, M.D., Ph.D.

The effective treatment of tuberculosis is a lifesaving intervention. The global scale-up of tuberculosis therapy has averted 6 million deaths over the past 15 years, making it one of the greatest public health interventions of our lifetime.[1] Unfortunately, by the time most patients are treated, they have already infected many others.[2] This failure to interrupt transmission fuels the global epidemic so that every year there are more new cases of tuberculosis than in the previous year.[1]

National tuberculosis programs are particularly challenged by multidrug-resistant tuberculosis. Globally, fewer than 2% of the estimated cases of multidrug-resistant disease are reported to the World Health Organization (WHO) and managed according to international guidelines. The vast majority of the remaining cases are probably never properly diagnosed or treated, further propagating the epidemic of multidrug-resistant tuberculosis. The situation is further worsened by the epidemic of human immunodeficiency virus (HIV), especially in Africa.

For decades there has been little effort to improve techniques for diagnosing tuberculosis.[3,4] Consequently, tuberculosis tests are antiquated and inadequate. The most widely used test (smear microscopy) is 125 years old and routinely misses half of all cases. These inadequacies are particularly problematic since such tests are generally performed in underfunded and dysfunctional health care systems.[4,5] The problem is exacerbated by the widespread use of inaccurate and inappropriate diagnostic tools, such as serologic assays, in many countries.[6]

Fortunately, in the past few years, several improved tuberculosis tests have received WHO endorsement for widespread use.[6,7] In this issue of the *Journal*, Boehme and colleagues[8] describe a new automated nucleic acid–amplification test that may allow a relatively unskilled health care worker to diagnose tuberculosis and detect resistance to a key antibiotic within 90 minutes. This test and others that are likely to follow have the potential to revolutionize the diagnosis of tuberculosis. Thus, in the coming years, rapid diagnosis and targeted treatment will provide the greatest opportunity for stopping the tuberculosis epidemic.

In a large, well-conducted, multicountry study, Boehme et al. evaluated an automated tuberculosis assay (Xpert MTB/RIF) for the presence of *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF). With a single test, this assay identified 98% of patients with smear-positive and culture-positive tuberculosis (including more than 70% of patients with smear-negative and culture-positive disease) and correctly identified 98% of bacteria that were resistant to rifampin.[8]

The assay has several critical advantages over conventional nucleic acid–amplification tests, which have been licensed for nearly 20 years and yet have not had a substantial effect on tuberculosis control. The MTB/RIF assay is simple to perform with minimal training, is not prone to cross-contamination, requires minimal biosafety facilities, and has a high sensitivity in smear-negative tuberculosis (the last factor being particularly relevant in patients with HIV infection).[8]

However promising these findings, issues involving the MTB/RIF assay may limit its global utility. These issues include its high cost, limitations in testing only for rifampin resistance, a platform that detects a relatively small number of mutations, and inability to indicate which patients are "sputum smear–positive" for reporting purposes, infection-control intervention, and treatment monitoring.

On the plus side, the MTB/RIF assay promises to decentralize molecular diagnosis, since it potentially can be used at the point of treatment in a microscopy center or in a tuberculosis or HIV clinic. However, because Boehme et al. used the test at reference laboratories, their study offers only indirect proof of concept for use in such settings. Critical to a rapid scale-up of the test will be the results of additional studies to determine how it performs in such settings and whether its use improves outcomes for patients in a cost-effective manner.

If an improved rapid nucleic acid–amplification test is adopted globally, it could help avert more than 15 million tuberculosis-related deaths by 2050.[9] However, even the most promising diagnostic test will have only limited impact if it does not reach the patients who need it. As with any diagnostic test or intervention, its actual impact will depend on the system in which it is used. Health systems must be strengthened so that patients do not delay in seeking care and have prompt access to appropriate treatment once they receive a diagnosis. Health-system barriers to the use of improved technologies must be anticipated and addressed. Although the burden on health systems will be reduced by a simple dipsticklike, point-of-care assay, such tests are not likely to be available in the short term.[7]

To realize the potential of improved technologies, a diverse set of stakeholders need to support large-scale innovation and delivery. Scientists and industry need to develop radically improved tools, including drugs and vaccines, while offering reasonable pricing that reflects public health needs and economic realities in resource-limited countries. Operational and implementation researchers need to quickly identify and respond to the full spectrum of issues that form the critical path to improving the prevention and control of tuberculosis. Policymakers and regulators must turn scientific evidence into permissive policies and regulations that allow national programs to rapidly incorporate new tools. Funders must increase and reprogram resources to become conduits for innovation and not fund decades-old technologies for years into the future. Programs must maintain focus on the basics of tuberculosis control while quickly modifying delivery systems to take advantage of the benefits of improved tools. Lastly, patient advocates and activists should hold everyone accountable and ensure that communities drive demand for improved systems and tools.

Despite these challenges, it is clear that improvements in diagnostics are driving a virtuous cycle in care: the promise of improved tests drives their uptake, their uptake results in better health outcomes, improved outcomes attract more funding for health care systems, and better-funded systems are an incentive to the development of even better technologies. We are particularly optimistic about the potential role of governments, product developers, and companies in emerging economies with high tuberculosis burdens, such as China, India, Brazil, and South Africa. These countries now have the capacity to develop low-cost generic or novel assays adapted to local contexts and incorporate their scale-up in both national tuberculosis-control programs and private laboratories, supported by successful public–private partnerships. Emerging economies have the potential to become global leaders in innovative product development and delivery. If these countries successfully tackle their own tuberculosis problems, the elimination of tuberculosis by 2050 might become a reality.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

From the Global Health Program, Bill and Melinda Gates Foundation, and the Institute for Systems Biology, Seattle (P.M.S.); and the Department of Epidemiology and Biostatistics, McGill University, and Montreal Chest Institute, Montreal (M.P.).

This article (10.1056/NEJMe1008496) was published on September 1, 2010, at NEJM.org.

1. Lonnroth K, Castro KG, Chakaya JM, et al. Tuberculosis control and elimination 2010-50: cure, care, and social development. Lancet 2010;375:1814-29.
2. Dye C, Williams BG. The population dynamics and control of tuberculosis. Science 2010;328:856-61.
3. Perkins MD, Small PM. Partnering for better microbial diagnostics. Nat Biotechnol 2006;24:919-21.
4. Small PM. Strengthening laboratory services for today and tomorrow. Int J Tuberc Lung Dis 2008;12:1105-9.
5. Perkins MD, Cunningham J. Facing the crisis: improving the diagnosis of tuberculosis in the HIV era. J Infect Dis 2007; 196:Suppl 1:S15-S27.
6. Pai M, Minion J, Steingart K, Ramsay A. New and improved tuberculosis diagnostics: evidence, policy, practice, and impact. Curr Opin Pulm Med 2010;16:271-84.
7. Wallis RS, Pai M, Menzies D, et al. Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. Lancet 2010;375:1920-37.
8. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 2010;363:1005-15.
9. Abu-Raddad LJ, Sabatelli L, Achterberg JT, et al. Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. Proc Natl Acad Sci U S A 2009;106:13980-5.

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 SEPTEMBER 9, 2010 VOL. 363 NO. 11

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabeta, M.D., Doris Hillemann, Ph.D., Mark P. Nicol, Ph.D., Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahirli, M.D., Robert Blakemore, B.S., Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O'Brien, Ph.D., David H. Persing, M.D., Ph.D., Sabine Ruesch-Gerdes, M.D., Eduardo Gotuzzo, M.D., Camilla Rodrigues, M.D., David Alland, M.D., and Mark D. Perkins, M.D.

ABSTRACT

BACKGROUND

Global control of tuberculosis is hampered by slow, insensitive diagnostic methods, particularly for the detection of drug-resistant forms and in patients with human immunodeficiency virus infection. Early detection is essential to reduce the death rate and interrupt transmission, but the complexity and infrastructure needs of sensitive methods limit their accessibility and effect.

METHODS

We assessed the performance of Xpert MTB/RIF, an automated molecular test for *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF), with fully integrated sample processing in 1730 patients with suspected drug-sensitive or multidrug-resistant pulmonary tuberculosis. Eligible patients in Peru, Azerbaijan, South Africa, and India provided three sputum specimens each. Two specimens were processed with *N*-acetyl-L-cysteine and sodium hydroxide before microscopy, solid and liquid culture, and the MTB/RIF test, and one specimen was used for direct testing with microscopy and the MTB/RIF test.

RESULTS

Among culture-positive patients, a single, direct MTB/RIF test identified 551 of 561 patients with smear-positive tuberculosis (98.2%) and 124 of 171 with smear-negative tuberculosis (72.5%). The test was specific in 604 of 609 patients without tuberculosis (99.2%). Among patients with smear-negative, culture-positive tuberculosis, the addition of a second MTB/RIF test increased sensitivity by 12.6 percentage points and a third by 5.1 percentage points, to a total of 90.2%. As compared with phenotypic drug-susceptibility testing, MTB/RIF testing correctly identified 200 of 205 patients (97.6%) with rifampin-resistant bacteria and 504 of 514 (98.1%) with rifampin-sensitive bacteria. Sequencing resolved all but two cases in favor of the MTB/RIF assay.

CONCLUSIONS

The MTB/RIF test provided sensitive detection of tuberculosis and rifampin resistance directly from untreated sputum in less than 2 hours with minimal hands-on time. (Funded by the Foundation for Innovative New Diagnostics.)

From the Foundation for Innovative New Diagnostics, Geneva (C.C.B., P.N., M.D.P.); Forschungszentrum Borstel, Borstel, Germany (D.H., S.R.-G.); the Department of Clinical Laboratory Sciences, University of Cape Town, and National Health Laboratory Service, Cape Town (M.P.N., A.M.), and the Unit for Clinical and Biomedical TB Research, South African Medical Research Council, Durban (J.A., R.R.) — all in South Africa; P.D. Hinduja National Hospital and Medical Research Centre (Hinduja), Mumbai, India (S.S., C.R.); Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru (F.K., E.G.); Special Treatment Institution, Baku, Azerbaijan (R.T.); the Division of Infectious Diseases, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark (R.B., D.A.); Cepheid, Sunnyvale, CA (M.J., D.H.P.); and the Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC (S.M.O.). Address reprint requests to Dr. Boehme at the Foundation for Innovative New Diagnostics, Ave. de Budé 16, 1202 Geneva, Switzerland, or at catharina.boehme@finddiagnostics.org.

This article (10.1056/NEJMoa0907847) was published on September 1, 2010, at NEJM.org.

N Engl J Med 2010;363:1005-15.

Only a small fraction of the estimated 500,000 patients who have multidrug-resistant tuberculosis and 1.37 million patients who have coinfection with tuberculosis and the human immunodeficiency virus (HIV) worldwide each year have access to sufficiently sensitive case detection or drug-susceptibility testing.[1] Diagnostic delay, aggravated by the disproportionate frequency of smear-negative disease in HIV-associated tuberculosis, is common.[2-5] The failure to quickly recognize and treat affected patients leads to increased mortality, secondary resistance (including extensively drug-resistant tuberculosis), and ongoing transmission.[6,7] The complexity of mycobacterial culture and current nucleic acid–amplification technologies for the detection of tuberculosis and multidrug-resistant tuberculosis[8] and the need for the associated infrastructure restrict the use of such tests to reference laboratories.

To respond to the urgent need for simple and rapid diagnostic tools at the point of treatment in high-burden countries,[9] a fully automated molecular test for tuberculosis case detection and drug-resistance testing was developed through collaboration in a public–private partnership. Xpert MTB/RIF, an automated molecular test for *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF), uses heminested real-time polymerase-chain-reaction (PCR) assay to amplify an MTB-specific sequence of the *rpoB* gene, which is probed with molecular beacons for mutations within the rifampin-resistance determining region.[10,11] Testing is carried out on the MTB/RIF test platform (GeneXpert, Cepheid), which integrates sample processing and PCR in a disposable plastic cartridge containing all reagents required for bacterial lysis, nucleic acid extraction, amplification, and amplicon detection.[12] The only manual step is the addition of a bactericidal buffer to sputum before transferring a defined volume to the cartridge. The MTB/RIF cartridge is then inserted into the GeneXpert device, which provides results within 2 hours.

In accordance with recommendations on design and conduct of diagnostic accuracy assessments,[13] we undertook a multicenter, prospective evaluation of the MTB/RIF test to determine its sensitivity and specificity in the intended target population as compared with the best available reference standard.

METHODS

STUDY POPULATION

From July 2008 through March 2009, we conducted this study at five trial sites in Lima, Peru; Baku, Azerbaijan; Cape Town and Durban, South Africa; and Mumbai, India. We enrolled consecutive adults with symptoms suggestive of pulmonary tuberculosis or multidrug-resistant tuberculosis who were able to provide three sputum samples of at least 1.5 ml. Patients in the group at risk for pulmonary tuberculosis were eligible only if they had not received a tuberculosis medication within the past 60 days, whereas the group at risk for multidrug-resistant disease included patients who had undergone previous treatment, those with nonconverting pulmonary tuberculosis who were receiving therapy, and symptomatic contacts of patients with known multidrug-resistant disease. All patients were enrolled from populations that were selected for diversity in the prevalence of tuberculosis, HIV coinfection, and multidrug resistance. (For details regarding the sites, see the Supplementary Appendix, available with the full text of this article at NEJM.org.)

The study protocol was reviewed and approved by eight institutional review boards or technical committees at the ministerial level. The study was conducted in accordance with the protocol (available at NEJM.org). Written informed consent was obtained from all patients. Study participation did not alter the standard of care.

STUDY DESIGN AND OVERSIGHT

This study was designed and supervised by the sponsor, the Foundation for Innovative New Diagnostics (FIND). Additional development support was provided by the National Institutes of Health, Cepheid, and the Bill and Melinda Gates Foundation, none of which were involved in the design or conduct of the study. Data were collected by investigators at each study site, and statistical analyses were performed by a statistician who was not involved in data collection. FIND authors wrote the first draft of the manuscript. All authors vouch for the accuracy and completeness of the data reported.

LABORATORY METHODS

Patients meeting the clinical eligibility criteria were asked to provide three sputum specimens over a

2-day period (two spot samples and one obtained in the morning) (Fig. 1). In a random fashion, two of the three samples were processed with *N*-acetyl-L-cysteine and sodium hydroxide (NALC–NaOH),[14] followed by centrifugation, and then were resuspended in 1.5 ml of phosphate buffer and subjected to microscopy with Ziehl–Neelsen staining, and cultivation on solid medium (egg-based Löwenstein–Jensen[15] or 7H11,[16] with the latter medium used only in Durban) and liquid medium (BACTEC MGIT [mycobacteria growth indicator tube] 960 culture; BD Microbiology Systems), and the MTB/RIF test. The third sputum sample was tested directly by Ziehl–Neelsen microscopy and the MTB/RIF test without NALC–NaOH decontamination.

The first positive culture from each specimen underwent confirmation of *M. tuberculosis* species by MPT64 antigen detection (Capilia TB, Tauns Laboratories)[17] and indirect drug-susceptibility testing with the proportion method on Löwenstein–Jensen medium (for sites in Lima, Durban, and Baku) or MGIT SIRE[18] (for sites in Cape Town and Mumbai). For three sites, conventional nucleic acid–amplification testing was carried out on DNA that was extracted from the NALC–NaOH centrifugation pellet of the first sputum sample with the use of Cobas Amplicor MTB (Roche) (in Cape Town and Mumbai) or ProbeTec ET MTB Complex Direct Detection Assay (BD) (in Baku), according to the manufacturer's instructions. At three sites, drug-resistant genotyping was carried out by line-probe assay with the use of the Genotype MTBDRplus assay (Hain Lifescience) performed from culture isolates (in Baku) or from the NALC–NaOH pellet of the second sputum sample (in Cape Town and Durban), according to the manufacturer's instructions, except that smear-negative specimens were also tested.

All participating laboratories were quality-assured reference laboratories. Study laboratories for four sites were located within 5 km of the enrollment clinic and tested samples within 2 days after collection. Sputum samples from Baku were shipped to the German National Reference Laboratory in Borstel for testing 1 to 5 days after collection.

Repeat tuberculosis analyses (smear, culture, MTB/RIF test, radiography, and clinical workup) were performed in patients who had smear- and culture-negative samples if the MTB/RIF test or other nucleic acid–amplification test was positive or if the patient was selected by the central database as a random control for follow-up. The final diagnosis for patients undergoing repeat analyses was established on the basis of conventional laboratory results and clinical information by clinical review committees composed of three local tuberculosis clinicians. HIV results were obtained by review of clinical records and were available for only a subgroup of patients. Bias was minimized through blinding, since technicians performing molecular and reference tests were not aware of the results of other tests. The interpretation of data from MTB/RIF tests was software-based and independent of the user. Clinical teams and review committees did not have access to nucleic acid–amplification test results. All study coordinators received lists of patients for follow-up but not the reasons for follow-up.

CATEGORIES FOR ANALYSIS

Patients were divided into four categories for analysis: those with smear- and culture-positive pulmonary tuberculosis; those with smear-negative, culture-positive pulmonary tuberculosis; those with no bacteriologic evidence of tuberculosis who had improvement without treatment (no tuberculosis); and those who were smear- and culture-negative for pulmonary tuberculosis who nonetheless were treated for tuberculosis on the basis of clinical and radiologic findings (clinical tuberculosis). A smear-positive case was defined as at least two smears of scanty grade (1 to 10 acid-fast bacilli per 100 fields) or one or more smears of 1+ or more (10 to 99 bacilli per 100 fields). A culture-positive case was defined as positive results on at least one of four culture vials. Because a clear final diagnosis was required, patients with an indeterminate diagnosis were excluded from the main analysis if there was a negative culture result while the patient was receiving tuberculosis treatment (for patients with suspected multidrug resistance), contamination of at least three of four cultures, growth of nontuberculous mycobacteria only, indeterminate phenotypic rifampin susceptibility, a negative culture with a positive sputum smear, or suspected cross-contamination of cultures (i.e., only one of four cultures had positive results after >28 days to growth in MGIT or <20 colonies in Löwenstein–Jensen medium) or if the patient died or was lost to follow-up.

MTB/RIF TEST

The MTB/RIF test was performed as described previously[19,20] (Fig. 2). Two laboratory technicians were trained as operators and passed proficiency testing after four runs per person. Sample reagent was added in a 2:1 ratio to untreated sputum and in a 3:1 ratio to decontaminated sputum pellets. The additional sample reagent in pellets was necessary to meet the volume requirements for the assay sample. The closed sputum container was manually agitated twice during a 15-minute period at room temperature before 2 ml of the inactivated material was transferred to the test cartridge (equivalent to 0.7 ml of untreated sputum or 0.5 ml of decontaminated pellet). Cartridges were inserted into the test platform, which was located in the microscopy room or another general-purpose laboratory space. The electronic results were sent directly from the MTB/RIF test system to the central database.

Figure 1 (facing page). Enrollment and Outcomes.
Patients were enrolled at centers that have diverse populations with a high prevalence of tuberculosis. In Lima, Peru, patients with suspected tuberculosis were enrolled at 30 primary care clinics with a high rate of tuberculosis case notification, a rate of coinfection with the human immunodeficiency virus (HIV) of less than 3%, and a low rate of multidrug resistance. In Cape Town and Durban, South Africa, patients were enrolled at primary care tuberculosis clinics located within informal settlements with a high incidence of tuberculosis and an estimated rate of HIV coinfection of 70% and a rate of multidrug resistance of 4%. In Mumbai, India, patients with complicated tuberculosis and a rate of multidrug resistance as high as 50% were enrolled at a tertiary care center. In Baku, Azerbaijan, prisoners were enrolled on arrival at a tuberculosis screening and treatment facility, which reports a high rate of multidrug resistance (25%) among patients with tuberculosis and a rate of HIV coinfection of approximately 6%. LJ denotes Löwenstein–Jensen, MGIT mycobacteria growth indicator tube, and NALC–NaOH *N*-acetyl-L-cysteine and sodium hydroxide.

SEQUENCING

Bidirectional sequencing was performed on the 81-bp *rpoB* core region of culture isolates in all rifampin-resistant and discordant strains with forward (CGTGGAGGCGATCACACCGCAGAC) and reverse (AGCTCCAGCCCGGCACGCTCACGT) primers with the use of the BigDye Terminator Cycle Sequencing kit, according to the manufacturer's recommendations, in a 3130xl Genetic Analyzer (Applied Biosystems). Traces were analyzed with ABI sequence-analysis software, version 5.2.0.

STATISTICAL ANALYSIS

Sensitivity and specificity for the MTB/RIF test were estimated for a single direct test, a single test on a pelleted sample, the combination of two tests (one direct and one pelleted), and the combination of three tests (one direct and two pelleted). Combinations were classified as positive if at least one of the component test results was positive. The indeterminate rate was the number of tests classified as "invalid," "error," or "no result" divided by the total number performed. When results were indeterminate and sufficient sample remained, the assay was repeated once, and the second result was used for analysis. For analyzing the single direct test and the combination of three tests, Wilson's binomial method was used to calculate 95% confidence intervals.[21] For all other intrapatient MTB/RIF results, and for comparisons across subgroups and testing methods, generalized estimating equations were used for calculating confidence intervals to account for within-patient clustering.[22]

RESULTS

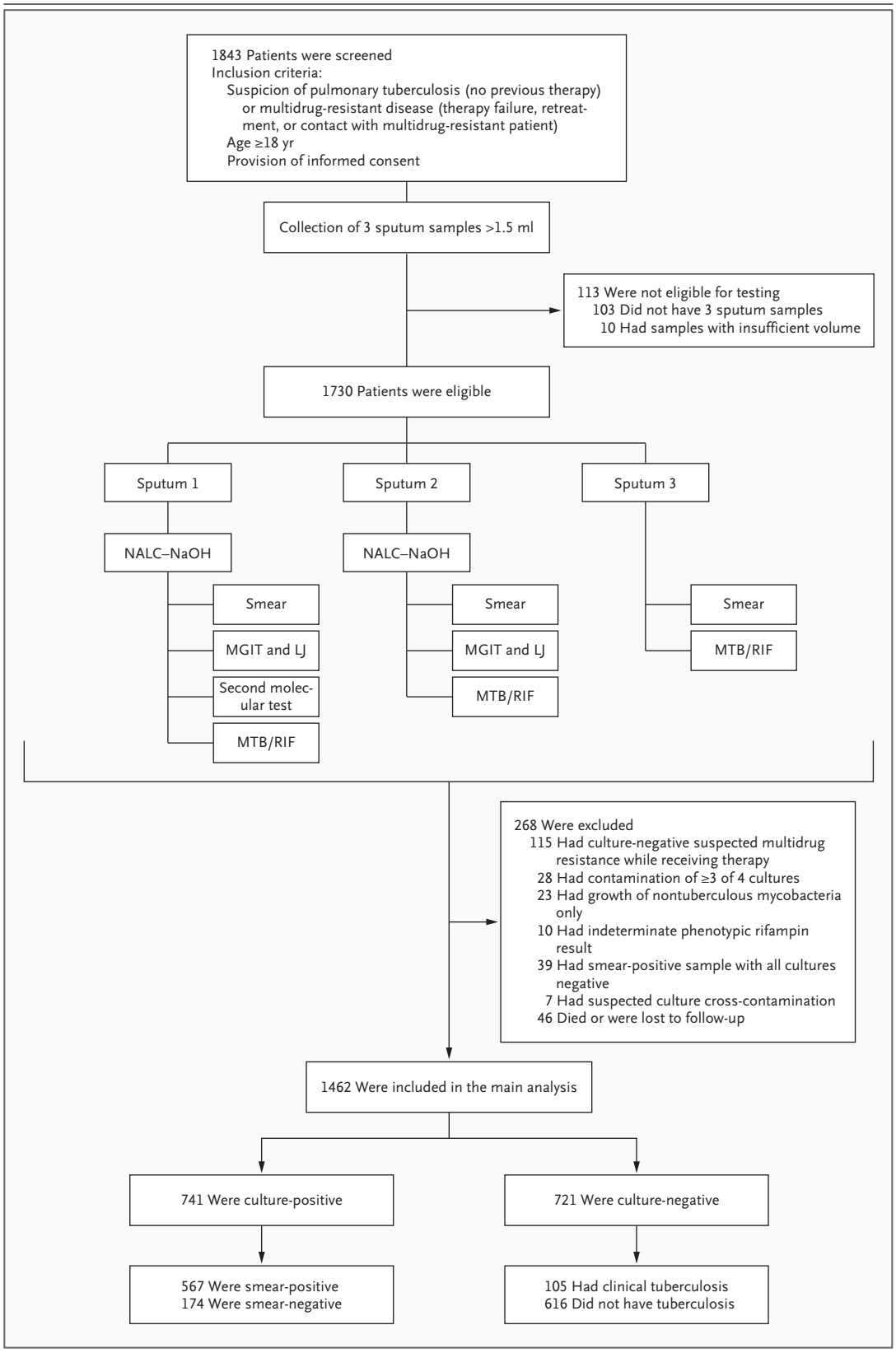
PATIENTS

Of the 1462 patients (4386 samples) included in the analysis, 567 (38.8%) had smear- and culture-positive tuberculosis; 174 (11.9%) had smear-negative, culture-positive tuberculosis; 105 (7.2%) had clinically defined tuberculosis; and 616 (42.1%) had no clinical evidence of tuberculosis (Table 1). Of patients with culture-positive samples, 207 of 741 (27.9%) were found to have multidrug resistance on conventional drug-susceptibility testing. A total of 113 patients were not eligible for testing because of an inadequate number of sputum samples (in 103 patients) or an inadequate volume of sputum samples (in 10). A total of 268 patients were excluded from the analysis for a variety of reasons, including 115 who had culture-negative samples but were receiving tuberculosis treatment at enrollment because of suspected multidrug resistance (Fig. 1).

SENSITIVITY AND SPECIFICITY

Case Detection

Among patients with culture-positive tuberculosis, the overall sensitivity of the MTB/RIF test was



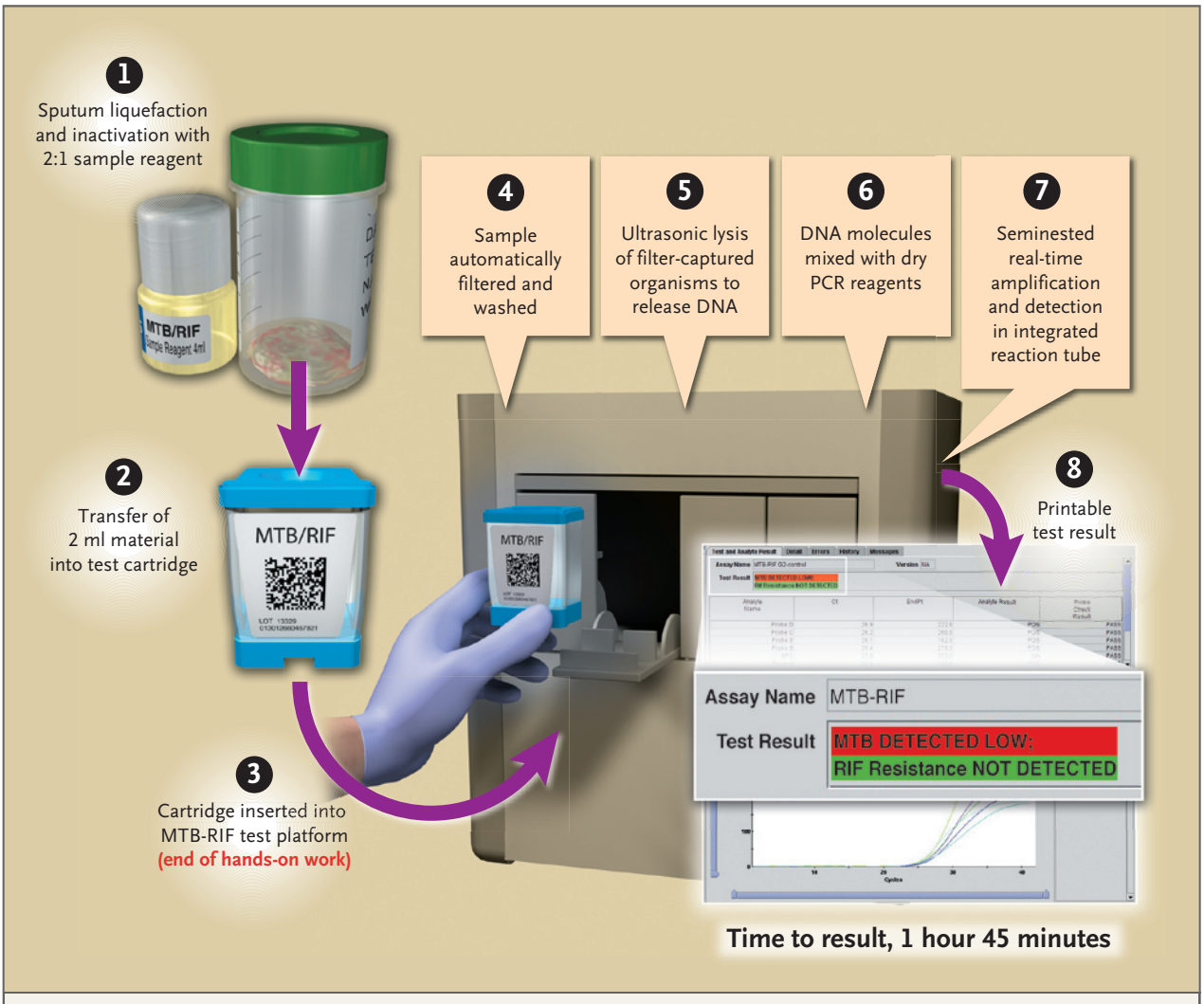


Figure 2. Assay Procedure for the MTB/RIF Test.

Two volumes of sample treatment reagent are added to each volume of sputum. The mixture is shaken, incubated at room temperature for 15 minutes, and shaken again. Next, a sample of 2 to 3 ml is transferred to the test cartridge, which is then loaded into the instrument. All subsequent steps occur automatically. The user is provided with a printable test result, such as "MTB detected; RIF resistance not detected." PCR denotes polymerase chain reaction.

97.6%. The sensitivity was 99.8% for smear- and culture-positive cases and 90.2% for smear-negative, culture-positive cases, with no significant variation in overall sensitivity across sites ($P=0.24$ by chi-square test) (Table 2). Testing of multiple specimens per patient had a modest effect over the yield of a single assay performed directly on sputum. The sensitivity of a single direct MTB/RIF test for culture-confirmed tuberculosis was 92.2% and rose to 96.0% with the additional testing of a pelleted sample. For the detection of smear-negative, culture-positive tuberculosis, the sensitivity of the assay was 72.5% for one test, 85.1% for two tests, and 90.2% for three tests. A single, direct MTB/RIF test identified a greater proportion of culture-positive patients than did a single Löwenstein–Jensen culture (Table 1 in the Supplementary Appendix). Among HIV-positive patients with pulmonary tuberculosis, the sensitivity of the MTB/RIF test was 93.9%, as compared with 98.4% in HIV-negative patients ($P=0.02$). There was no significant difference in sensitivity between tests on untreated sputum and those on decontaminated pellet ($P=0.16$).

The estimated specificity was 99.2% for a single direct MTB/RIF test, 98.6% for two MTB/RIF tests,

Table 1. Demographic and Clinical Characteristics of the Patients, Diagnosis Group at Enrollment, and Distribution in Final Diagnostic Category.*

Variable	Lima, Peru (N=341)	Baku, Azerbaijan (N=353)	Cape Town, South Africa (N=380)	Durban, South Africa (N=346)	Mumbai, India (N=310)	All Patients (N=1730)
Demographic or clinical characteristic						
Median age (range) — yr	31 (18–79)	37 (20–69)	36 (18–80)	32 (18–68)	30 (17–88)	34 (17–88)
Female sex — no./total no. (%)	138/319 (43.3)	0/251	119/349 (34.1)	186/313 (59.4)	90/230 (39.1)	533/1462 (36.5)
HIV infection — no./total no. (%)	3/179 (1.7)	9/193 (4.7)	159/209 (76.1)	217/304 (71.4)	4/91 (4.4)	392/976 (40.2)
History of tuberculosis — no./total no. (%)	75/316 (23.7)	137/251 (54.6)	148/344 (43.0)	138/306 (45.1)	173/230 (75.2)	671/1447 (46.4)
Diagnosis group at enrollment						
Suspicion of pulmonary tuberculosis — no./total no. (%)	293/341 (85.9)	131/353 (37.1)	223/380 (58.7)	272/346 (78.6)	91/310 (29.4)	1010/1730 (58.4)
Suspicion of multidrug-resistant tuberculosis not receiving therapy — no./total no. (%)	41/341 (12.0)	149/353 (42.2)	136/380 (35.8)	59/346 (17.1)	60/310 (19.4)	445/1730 (25.7)
Suspicion of multidrug-resistant tuberculosis receiving therapy — no./total no. (%)	7/341 (2.1)	73/353 (20.7)	21/380 (5.5)	15/346 (4.3)	159/310 (51.3)	275/1730 (15.9)
Distribution in final diagnostic category						
Smear- and culture-positive tuberculosis — no./total no. (%)	199/341 (58.4)	80/353 (22.7)	96/380 (25.3)	30/346 (8.7)	162/310 (52.3)	567/1730 (32.8)
Smear-negative and culture-positive tuberculosis — no./total no. (%)	12/341 (3.5)	69/353 (19.5)	52/380 (13.7)	15/346 (4.3)	26/310 (8.4)	174/1730 (10.1)
Clinical tuberculosis — no./total no. (%)	6/341 (1.8)	32/353 (9.1)	12/380 (3.2)	49/346 (14.2)	6/310 (1.9)	105/1730 (6.1)
No tuberculosis — no./total no. (%)	102/341 (29.9)	70/353 (19.8)	189/380 (49.7)	219/346 (63.3)	36/310 (11.6)	616/1730 (35.6)
Indeterminate — no./total no. (%)	22/341 (6.5)	102/353 (28.9)	31/380 (8.2)	33/346 (9.5)	80/310 (25.8)	268/1730 (15.5)
Culture-negative disease with suspected multidrug resistance receiving therapy	2/22 (9.1)	51/102 (50.0)	3/31 (9.7)	10/33 (30.3)	49/80 (61.3)	115/268 (42.9)
Contamination of ≥3 of 4 cultures	0/22	28/102 (27.5)†	0/31	0/33	0/80	28/268 (10.4)
Single culture positive with >28 days (MGIT) or <20 colonies (LJ)	0/22	0/102	3/31 (9.7)	2/33 (6.1)	2/80 (2.5)	7/268 (2.6)
Smear-positive, culture-negative tuberculosis	14/22 (63.6)	5/102 (4.9)	4/31 (12.9)	12/33 (36.4)	4/80 (5.0)	39/268 (14.6)
Nontuberculous mycobacteria only	2/22 (9.1)	4/102 (3.9)	1/31 (3.2)	2/33 (6.1)	14/80 (17.5)	23/268 (8.6)
Discrepant phenotypic drug-susceptibility testing	2/22 (9.1)	5/102 (4.9)	2/31 (6.5)	0/33	1/80 (1.3)	10/268 (3.7)
Death or loss to follow-up	2/22 (9.1)	9/102 (8.8)	18/31 (58.1)	7/33 (21.2)	10/80 (12.5)	46/268 (17.2)

* HIV denotes human immunodeficiency virus, LJ Löwenstein–Jensen, and MGIT mycobacteria growth indicator tube.

† In Azerbaijan, there was a delay of up to 8 days between sputum collection and processing because of air transport to the laboratory in Germany, which resulted in an overall culture contamination rate of 13.9%, as compared with less than 2% for other sites.

Table 2. Overall Sensitivity and Specificity of the MTB/RIF Test, According to the Number of Tests per Patient, as Compared with Three Smears and Four Cultures.*

Site and No. of Tests	Sensitivity			Specificity
	All Culture-Positive	Smear-Positive and Culture-Positive	Smear-Negative and Culture-Positive	No Tuberculosis
Site				
Lima, Peru				
Correct — no./total no. (%)	209/211 (99.1)	199/199 (100)	10/12 (83.3)	102/102 (100)
95% CI	96.6–99.7	98.1–100.0	55.2–95.3	96.4–100.0
Baku, Azerbaijan				
Correct — no./total no. (%)	144/149 (96.6)	80/80 (100.0)	64/69 (92.8)	68/70 (97.1)
95% CI	92.4–98.6	95.4–100.0	84.1–96.9	90.2–99.2
Cape Town, South Africa				
Correct — no./total no. (%)	142/148 (95.9)	95/96 (99.0)	47/52 (90.4)	186/189 (98.4)
95% CI	91.4–98.1	94.3–99.8	79.4–95.8	95.4–99.5
Durban, South Africa				
Correct — no./total no. (%)	43/45 (95.6)	30/30 (100.0)	13/15 (86.7)	213/219 (97.3)
95% CI	85.2–98.8	88.6–100.0	62.1–96.3	94.2–98.7
Mumbai, India				
Correct — no./total no. (%)	185/188 (98.4)	162/162 (100.0)	23/26 (88.5)	35/36 (97.2)
95% CI	95.4–99.5	99.7–100.0	71.0–96.0	85.8–99.5
No. of MTB/RIF tests				
3 Samples (2 pellet and 1 direct)				
Correct — no./total no. (%)	723/741 (97.6)	566/567 (99.8)	157/174 (90.2)	604/616 (98.1)
95% CI	96.2–98.5	99.0–100.0	84.9–93.8	96.6–98.9
2 Samples (1 pellet and 1 direct)				
Correct — no./total no. (%)†	1423/1482 (96.0)	1127/1134 (99.4)	296/348 (85.1)	1215/1232 (98.6)
95% CI	94.6–97.1	98.6–99.7	79.7–89.2	97.5–99.2
1 Sample (direct)				
Correct — no./total no. (%)	675/732 (92.2)	551/561 (98.2)	124/171 (72.5)	604/609 (99.2)
95% CI	90.0–93.9	96.8–99.0	65.4–78.7	98.1–99.6

* Site-specific performance is shown for three MTB/RIF test results per patient (two pellet samples plus one direct sample). The sensitivity of the test did not differ significantly between patients who were suspected of having pulmonary tuberculosis and those suspected of having multidrug-resistant tuberculosis (P=0.96). (For details, see Table 3 in the Supplementary Appendix.) Of 105 patients with culture-negative samples who were treated for tuberculosis on the basis of clinical symptoms, 29.3% had positive results on the MTB/RIF test (data not shown), but no further analysis was done during this study.

† The denominator for patients with two tests includes two observations per patient. The first observation is a combination of the first sputum sample (pellet) and third sputum sample (direct). The second observation is a combination of the second sputum sample (pellet) and the third sputum sample (direct). The calculation of the confidence interval (CI) accounts for within-patient correlation and the use of the third sputum sample two times.

and 98.1% for three MTB/RIF tests. At sites performing alternative nucleic acid–amplification testing, the sensitivity of the MTB/RIF test performed directly on sputum was higher than that of Amplicor (94.6% vs. 86.8%, P<0.01) and similar to that of ProbeTec (83.7% vs. 83.9%, P=0.96) performed on extracted DNA from sputum pellets. The specificity of the MTB/RIF test did not differ significantly from that of Amplicor or Probetec (Table 2 in the Supplementary Appendix).

Detection of Multidrug Resistance

Table 3 shows the sensitivity and specificity of the MTB/RIF test for the detection of rifampin and

Table 3. Sensitivity and Specificity of the MTB/RIF Test for the Detection of Rifampin and Multidrug Resistance, as Compared with Phenotypic Drug-Susceptibility Testing Alone and in Combination with Sequencing of Discrepant Cases, According to Site.*

Site and Total	Phenotypic Drug-Susceptibility Testing†		Phenotypic Drug-Susceptibility Testing and Discrepant Resolution by Sequencing†	
	Sensitivity for Rifampin Resistance	Specificity for Rifampin Resistance	Sensitivity for Rifampin Resistance	Specificity for Rifampin Resistance
Lima, Peru — no./total no. (%)	16/16 (100.0)	190/193 (98.4)	19/19 (100.0)	190/190 (100.0)
Baku, Azerbaijan — no./total no. (%)	47/49 (95.9)	90/94 (95.7)	51/52 (98.1)	90/90 (100.0)
Cape Town, South Africa — no./total no. (%)	15/16 (93.8)	126/126 (100.0)	15/15 (100.0)	126/126 (100.0)
Durban, South Africa — no./total no. (%)	3/3 (100.0)	38/38 (100.0)	3/3 (100.0)	38/38 (100.0)
Mumbai, India — no./total no. (%)	119/121 (98.3)	61/64 (95.3)	121/122 (99.2)	62/62 (100.0)
Total for rifampin resistance				
Correct — no./total no. (%)	200/205 (97.6)	505/515 (98.1)	209/211 (99.1)	506/506 (100.0)
95% CI — %	94.4–99.0	96.5–98.9	96.6–99.7	99.2–100.0
Total for multidrug resistance				
Correct — no. /total no. (%)	195/200 (97.5)		197/199 (99.0)	
95% CI — %	94.3–98.9		96.4–99.7	

* Multidrug resistance is defined as resistance to both rifampin and isoniazid. Of 723 culture-positive samples, 720 were analyzed for rifampin resistance because results on the MTB/RIF test were indeterminate in 3 cases. During blinded sequencing of 15 discrepant samples, *rpoB* mutations were identified in 9 samples that were rifampin-sensitive on phenotypic drug-susceptibility testing. A wild-type allele was identified in 1 sample, which had been reported as resistant on phenotypic drug-susceptibility testing. Mixed infections were identified in 3 samples and were excluded from the analysis after discrepant resolution. In 2 samples, sequencing confirmed the phenotypic result: *rpoB* mutation 516 GTC was detected in 1, and 531 TTG in the other.

† This is the reference standard for the comparison with the MTB/RIF test.

multidrug resistance (resistance to both rifampin and isoniazid). For 15 of 718 patients for whom results on the MTB/RIF test were discrepant on phenotypic testing, sequencing confirmed resistance-associated *rpoB* mutations in nine strains that were identified as rifampin-sensitive on drug-susceptibility testing, determined the presence of a wild-type allele in one strain deemed rifampin-resistant on drug-susceptibility testing, and identified 3 patients with mixed infection containing wild-type and mutant strains in the same culture. Taking sequencing results into account, the MTB/RIF test correctly detected rifampin resistance in 209 of 211 patients (99.1% sensitivity) and in all 506 patients with rifampin susceptibility (100% specificity).

The *rpoB* mutations found in this study were representative of the global situation: 16 different mutations were identified, but a limited number, notably in codons 516, 526 and 531, accounted for almost all resistant strains.

Using the South African samples, we compared the performance of the direct Genotype MTBDRplus assay with that of the MTB/RIF test. In smear-positive sputum samples, the MTBDRplus assay showed a sensitivity equivalent to that of the MTB/RIF test. However, in samples from smear-negative, culture-positive patients, for which the MTBDRplus assay is not indicated, the MTBDRplus assay provided a false negative result in 37 of 67 samples (55.2%).

In a subgroup of 115 patients with culture-negative tuberculosis who had suspected multidrug resistance and were receiving tuberculosis treatment (and who were excluded from the main analysis), 51 had positive results on the MTB/RIF test, and rifampin resistance was detected in 8. We observed that all 8 patients were later started on second-line therapy for treatment failure by physicians who were unaware of the results on MTB/RIF testing. In comparison, none of 8 randomly selected patients from the same cohort with positive results on MTB/RIF testing that did not detect rifampin resistance were given second-line tuberculosis treatment. Although the manufacturer currently recommends that the MTB/RIF test be used for patients with suspected tuberculosis who have not received treatment, our data provide a first

indication that the test also detects multidrug-resistant tuberculosis in patients who are receiving therapy, even after culture conversion.

INDETERMINATE RATE

The MTB/RIF test was indeterminate in 192 of 5190 tests performed (3.7%), a rate that was lower than the overall culture-contamination rate (5.5%) in 381 of 6920 MGIT and Löwenstein–Jensen cultures ($P<0.001$). Allowing for one repeat test, the indeterminate rate dropped to 1.2% (63 of 5190 tests). Valid results were obtained in 129 of 139 repeat tests (92.8%). No patient had indeterminate results on all samples tested. A total of 20 of 2072 samples (1.0%) with positive results had an indeterminate result for rifampin resistance. These indeterminate rifampin results all occurred in smear-negative, culture-positive sputum samples with a very late cycle threshold (35 to 37 cycles) in the MTB/RIF test. A software change allowing the assay to analyze results for up to 40 cycles would have eliminated 19 of the 20 indeterminate results without affecting the specificity of the assay.

DISCUSSION

In our study, an assay that was designed for point-of-treatment use in low-income countries accurately detected pulmonary tuberculosis and screened for rifampin resistance. This assay identified more than 97% of all patients with culture-confirmed tuberculosis who met the inclusion criteria, including more than 90% of patients with smear-negative disease. Performance both for case detection and discrimination of rifampin resistance was similar across diverse sites, suggesting that the findings are likely to be widely applicable. In view of the low sensitivity of smear microscopy for the diagnosis of tuberculosis in patients with HIV infection, the increased sensitivity of the MTB/RIF test — notably, among patients with smear-negative tuberculosis — at the two South African sites with 60 to 80% prevalence of HIV infection is encouraging.

There are several reasons why the findings of this study might not translate widely into improved care for patients with tuberculosis. First, only reference facilities were used in the study, and it is not certain that our findings would be replicated in microscopy centers, health posts, and other point-of-treatment settings where temperature and electricity supply will be more variable and training issues will be more relevant. However, qualitative questionnaires that were completed during the study suggested that users considered 2 to 3 days a sufficient duration of training for technicians without previous molecular experience (as compared with 2 weeks for Ziehl–Neelsen microscopy). The relative simplicity of the MTB/RIF test, plus its hands-on time of under 15 minutes and its unambiguous readout, is advantageous, whereas the need for annual calibration was identified as a challenge for implementation at peripheral laboratories, especially in rural areas. Large-scale projects to show the feasibility and effect of MTB/RIF testing at such sites are under way.

Second, to achieve great simplicity of use, the MTB/RIF test uses sophisticated technology, which is costly to manufacture. Although FIND has negotiated concessionary pricing for public-sector programs in low-income countries and is working to further lower the costs of testing, the costs of instruments and tests will still be considerably higher than those for microscopy, which is all that is currently available in peripheral health care settings in many countries. However, MTB/RIF testing could be less costly than implementation of culture and drug-susceptibility testing.

Globally, ineffective tuberculosis detection and the rise of multidrug resistance and extensively drug-resistant tuberculosis have led to calls for dramatic expansion of culture capability and drug-susceptibility testing in countries in which the disease is endemic.[23] Unfortunately, the infrastructure and trained personnel required for such testing are not available except in a limited number of reference centers, and results of testing are often not available for at least 4 months, which dramatically reduces its clinical utility.[24,25] The complexity of standard nucleic acid–amplification tests prevents the expansion of this method. The MTB/RIF test automates DNA extraction, amplification, and detection inside a test cartridge that is never reopened, with little chance of amplicon contamination. Specimen processing is simplified to a single nonprecise step that both liquefies and inactivates sputum, which results in a reduction in viable tubercle bacilli of 6 to 8 logs and eliminates the necessity for a biosafety cabinet. Data from a recent study confirm that the MTB/RIF assay generates no infectious aerosols.[26] These features of simplicity and safety of use could allow for cost-effective and highly sensitive detection of tuberculosis and drug resistance outside reference centers,

which would increase access to testing and decrease delays in diagnosis, without the need to build large numbers of laboratories equipped for advanced biosafety.

Supported by FIND (for clinical evaluation and assay development) and grants from the National Institutes of Health (AI52523), Cepheid, and the Bill and Melinda Gates Foundation (for assay development).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the clinical and laboratory teams at the partner sites for their efforts in the implementation, conduct, and timely completion of the study; Justine de Grandpré and Dr. Ranald Sutherland for their continuous technical support and project management during assay development; and FIND logistics officer Nora Champouillon and data manager Pierrick Gonnet for facilitating the project.

REFERENCES

1. Global tuberculosis control — epidemiology, strategy, financing: WHO report. Geneva: World Health Organization, 2009. (WHO/HTM/TB/2009.411.)
2. Perkins MD, Cunningham J. Facing the crisis: improving the diagnosis of tuberculosis in the HIV era. J Infect Dis 2007; 196:Suppl 1:S15-S27.
3. Getahun H, Harrington M, O'Brien R, Nunn P. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. Lancet 2007;369:2042-9.
4. Havlir DV, Getahun H, Sanne I, Nunn P. Opportunities and challenges for HIV care in overlapping HIV and TB epidemics. JAMA 2008;300:423-30.
5. Uys PW, Warren RM, van Helden PD. A threshold value for the time delay to TB diagnosis. PLoS One 2007;2(1):e757.
6. Farmer P, Bayona J, Becerra M, et al. The dilemma of MDR-TB in the global era. Int J Tuberc Lung Dis 1998;2:869-76.
7. Van Rie A, Enarson D. XDR tuberculosis: an indicator of public-health negligence. Lancet 2006;368:1554-6.
8. Palomino JC. Molecular detection, identification and drug resistance detection in Mycobacterium tuberculosis. FEMS Immunol Med Microbiol 2009;56: 103-11.
9. Urdea M, Penny LA, Olmsted SS, et al. Requirements for high impact diagnostics in the developing world. Nature 2006; 444:Suppl 1:73-9.
10. El-Hajj HH, Marras SA, Tyagi S, Kramer FR, Alland D. Detection of rifampin resistance in Mycobacterium tuberculosis in a single tube with molecular beacons. J Clin Microbiol 2001;39:4131-7.
11. Piatek AS, Tyagi S, Pol AC, et al. Molecular beacon sequence analysis for detecting drug resistance in Mycobacterium tuberculosis. Nat Biotechnol 1998;16:359-63.
12. Raja S, Ching J, Xi L, et al. Technology for automated, rapid, and quantitative PCR or reverse transcription-PCR clinical testing. Clin Chem 2005;51:882-90.
13. Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative: Standards for Reporting of Diagnostic Accuracy. Clin Chem 2003; 49:1-6.
14. Kent PT, Kubica GP, eds. Public health mycobacteriology: a guide for the Level III laboratory. Atlanta: Centers for Disease Control, 1985.
15. Laboratory services in tuberculosis control. Geneva: World Health Organization, 1998. (WHO/TB/98.258.)
16. Cohn ML, Waggoner RF, McClatchy JK. The 7H11 medium for the cultivation of mycobacteria. Am Rev Respir Dis 1968;98:295-6.
17. Hillemann D, Rüsch-Gerdes S, Richter E. Application of the Capilia TB assay for culture confirmation of Mycobacterium tuberculosis complex isolates. Int J Tuberc Lung Dis 2005;9:1409-11.
18. Siddiqi SH, Ruesch-Gerdes S. MGIT procedure manual for BACTEC MGIT 960 TB System. Franklin Lakes, NJ: Beckton, Dickinson, July 2006.
19. Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 2010;48:229-37.
20. Blakemore R, Story E, Helb D, et al. Evaluation of the analytical performance of the Xpert MTB/RIF Assay. J Clin Microbiol 2010;48:2495-501.
21. Agresti A, Coull B. Approximate is better than "exact" for interval estimation of binomial proportions. Am Stat 1998; 52:119-26.
22. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 1986;42:121-30.
23. Stop TB Partnership: the global plan to stop TB 2006–2015. Geneva: World Health Organization, 2006.
24. Yagui M, Perales MT, Asencios L, et al. Timely diagnosis of MDR-TB under program conditions: is rapid drug susceptibility testing sufficient? Int J Tuberc Lung Dis 2006;10:838-43.
25. Urbanczik R, Rieder HL. Scaling up tuberculosis culture services: a precautionary note. Int J Tuberc Lung Dis 2009; 13:799-800.
26. Banada PP, Sivasubramani SK, Blakemore R, et al. Containment of bioaerosol infection risk by the Xpert MTB/RIF assay and its applicability to point-of-care settings. J Clin Microbiol 2010 August 18 (Epub ahead of print).

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

Catharina C Boehme, Mark P Nicol, Pamela Nabeta, Joy S Michael, Eduardo Gotuzzo, Rasim Tahirli, Ma Tarcela Gler, Robert Blakemore, William Worodria, Christen Gray, Laurence Huang, Tatiana Caceres, Rafail Mehdiyev, Lawrence Raymond, Andrew Whitelaw, Kalaiselvan Sagadevan, Heather Alexander, Heidi Albert, Frank Cobelens, Helen Cox, David Alland, Mark D Perkins

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.**

Funding **Foundation for Innovative New Diagnostics, Bill & Melinda Gates Foundation, European and Developing Countries Clinical Trials Partnership (TA2007.40200.009), Wellcome Trust (085251/B/08/Z), and UK Department for International Development.**

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

Lancet 2011; 377: 1495–1505

Published Online April 19, 2011 DOI:10.1016/S0140-6736(11)60438-8

See Comment page 1467

Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland (C C Boehme MD, P Nabeta MD, C Gray MPH, H Alexander PhD, H Albert PhD, M D Perkins MD); **National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa** (M P Nicol PhD, A Whitelaw MD); **Division of Medical Microbiology and Institute for Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa** (Prof M P Nicol, A Whitelaw); **Christian Medical College, Vellore, India** (Prof J S Michael MD, Kalaiselvan S MSc); **Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru** (E Gotuzzo MD, T Caceres BSc); **Special Treatment Institution, Baku, Azerbaijan** (R Tahirli MD); **Tropical Disease Foundation, Manila, Philippines** (M T Gler MD); **New Jersey Medical School and Division of Infectious Diseases, University of Medicine and Dentistry of New Jersey, Newark, NJ, USA** (R Blakemore BSc, Prof D Alland MD); **Department of Medicine, Faculty of Medicine, Makerere University, Kampala, Uganda** (W Worodria MMed); **Uganda Ministry of Health, Kampala, Uganda** (W Worodria); **Division of Pulmonary and Critical Care Medicine, and HIV/AIDS Division, San Francisco General Hospital, University of California, San Francisco, CA, USA** (Prof L Huang MD);

Azerbaijan Ministry of Justice, Baku, Azerbaijan (R Mehdiyev MD); **Lung Center of the Philippines, Manila, Philippines** (L Raymond MD); **Centers for Disease Control and Prevention, Division of Tuberculosis Elimination, Atlanta, GA, USA** (H Alexander); **Foundation for Innovative New Diagnostics (FIND), Kampala, Uganda** (H Albert); **Department of Global Health and Amsterdam Institute of Global Health and Development, Academic Medical Center, Amsterdam, Netherlands** (F Cobelens PhD); **and Médecins Sans Frontières, Cape Town, South Africa** (H Cox PhD)

Correspondence to:
Dr Catharina Cora Boehme, Foundation for Innovative New Diagnostics (FIND), Avenue de Budé 16, 1202 Geneva, Switzerland
catharina.boehme@finddiagnostics.org

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 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**

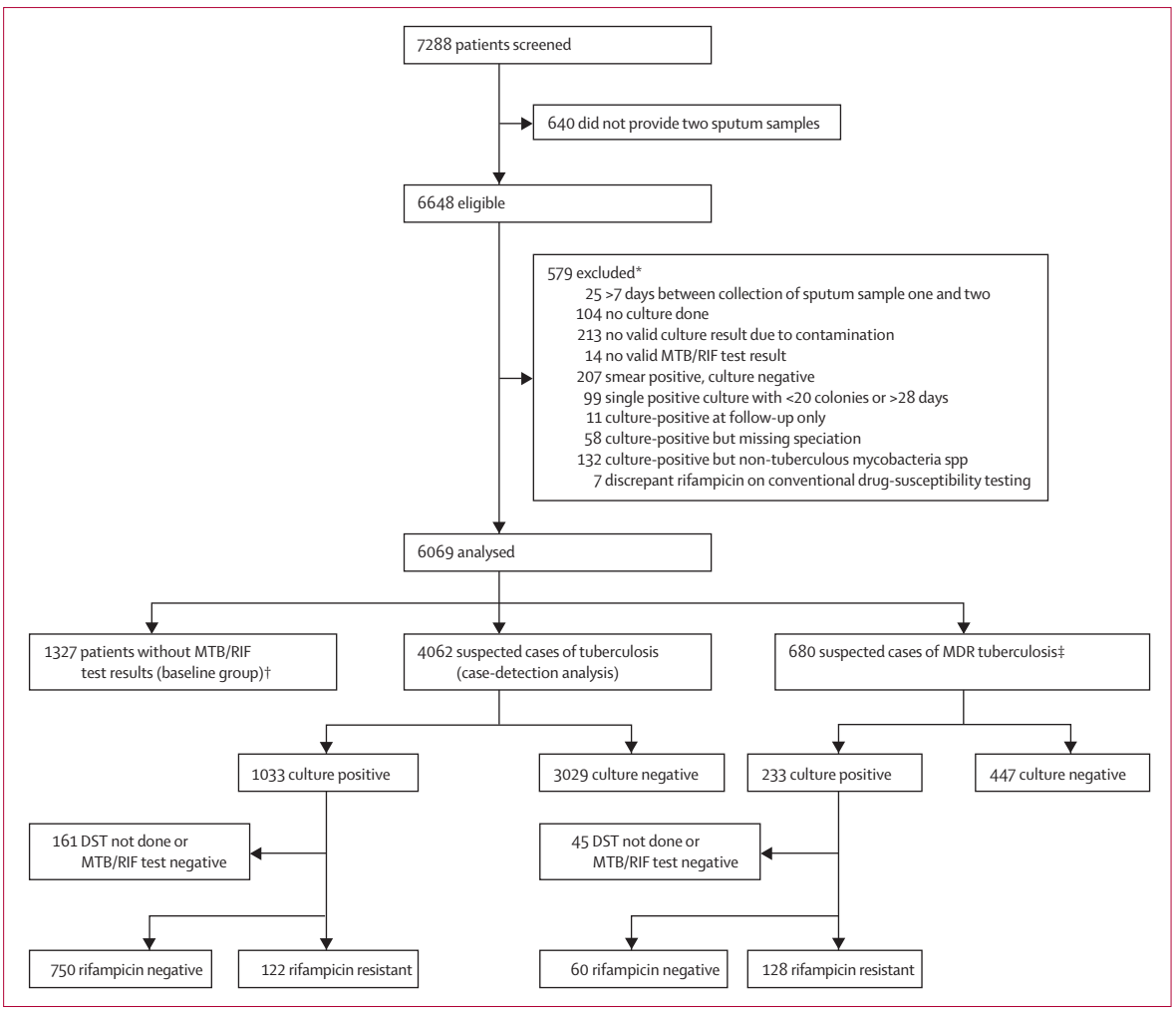


Figure 1: **Study profile**
MTB=*Mycobacterium tuberculosis*. RIF=rifampicin. MDR=multidrug resistant. DST=drug-susceptibility testing. *Some patients met several exclusion criteria and are listed more than once. †In South Africa only. ‡680 suspected cases of MDR tuberculosis were not included in the 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 $p<0{\cdot}05$ 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**

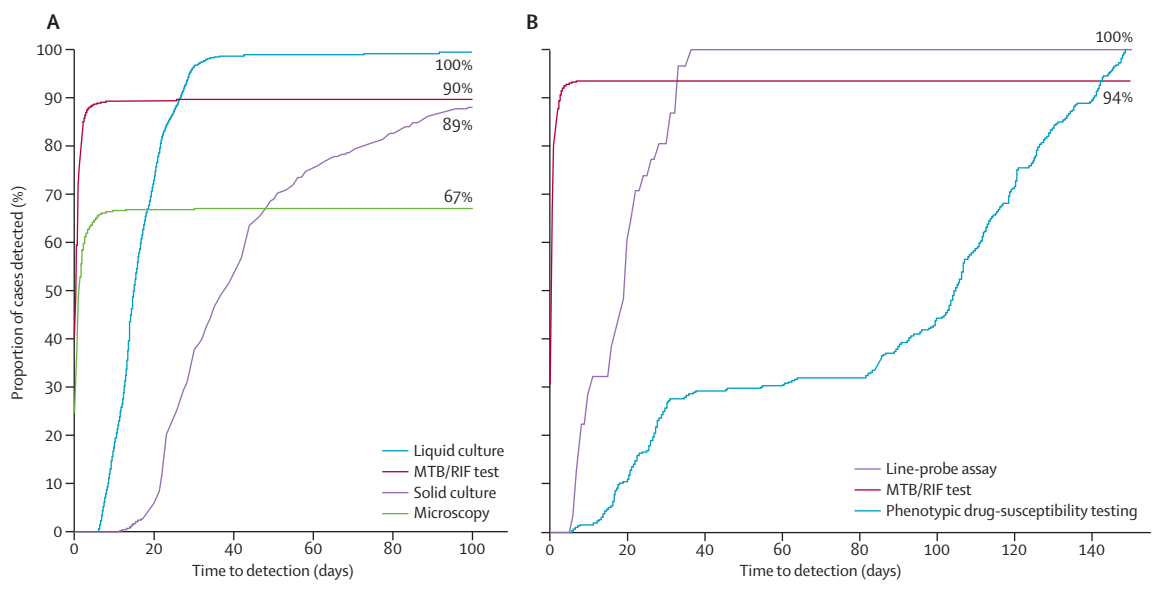


Figure 2: **Proportion of tuberculosis cases detected by each method in culture-positive patients**
Percentages are the maximum proportion of cases detected by every method. (A) Tuberculosis case detection. (B) Detection of rifampicin resistance. Time to detection was defined as time between date of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

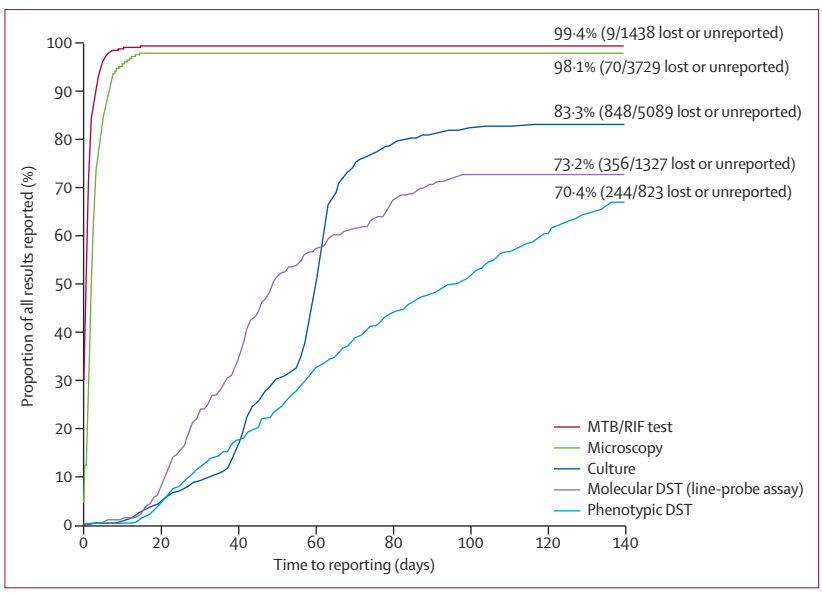


Figure 3: **Proportion of results reported to the clinics for each method from date of first sputum sample**
Percentages are the maximum proportion of results received by the clinic within 30 days of recorded date of smear microscopy, MTB/RIF test, or culture, or within 150 days of sputum collection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

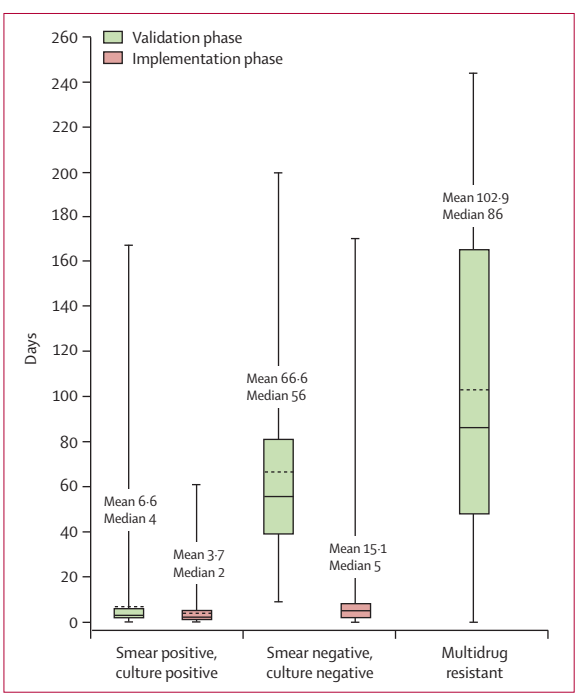


Figure 4: **Time to treatment during validation phase (treatment based on conventional methods only) and implementation phase (treatment based on MTB/RIF test and conventional methods) for patients with smear-positive, culture-positive tuberculosis, smear-negative, culture-positive tuberculosis, or multidrug-resistant 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

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.

Acknowledgments

We thank the study teams for their efforts to complete data collection in time for the WHO expert review meeting, the National tuberculosis Control Programmes and Laboratory Services and Médecins Sans Frontieres for their support in the planning and conduct of the study, and Sean O'Brien from Duke University (Durham NC, USA) for his support during data analysis.

References

1 Bruchfeld J, Aderaye G, Palme IB, Bjorvatn B, Kallenius G, Lindquist L. Sputum concentration improves diagnosis of tuberculosis in a setting with a high prevalence of HIV. *Trans R Soc Trop Med Hyg* 2000; **94:** 677–80.

2 Perkins MD, Cunningham J. Facing the crisis: improving the diagnosis of tuberculosis in the HIV era. *J Infect Dis* 2007; **196:** S15–27.

3 Steingart KR, Ramsay A, Pai M. Optimizing sputum smear microscopy for the diagnosis of pulmonary tuberculosis. *Expert Rev Anti Infect Ther* 2007; **5:** 327–31.

4 World Health Organization. Global tuberculosis control, 2010. http://whqlibdoc.who.int/publications/2010/9789241564069_eng.pdf (accessed March 18, 2011).

5 Behr MA, Warren SA, Salamon H, et al. Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. *Lancet* 1999; **353:** 444–49.

6 Havlir DV, Barnes PF. Tuberculosis in patients with human immunodeficiency virus infection. *N Engl J Med* 1999; **340:** 367–73.

7 Millen SJ, Uys PW, Hargrove J, van Helden PD, Williams BG. The effect of diagnostic delays on the drop-out rate and the total delay to diagnosis of tuberculosis. *PLoS One* 2008; **3:** e1933.

8 Garin B, Glaziou P, Kassa-Kelembho E, Yassibanda S, Mbelesso P, Morvan J. High mortality rates among patients with tuberculosis in Bangui, Central African Republic. *Lancet* 1997; **350:** 1298.

9 Harries AD, Nyangulu DS, Kang'ombe C, et al. Treatment outcome of an unselected cohort of tuberculosis patients in relation to human immunodeficiency virus serostatus in Zomba Hospital, Malawi. *Trans R Soc Trop Med Hyg* 1998; **92:** 343–47.

10 Mukadi YD, Maher D, Harries A. Tuberculosis case fatality rates in high HIV prevalence populations in sub-Saharan Africa. *AIDS* 2001; **15:** 143–52.

11 Pronyk PM, Kahn K, Hargreaves JR, et al. Undiagnosed pulmonary tuberculosis deaths in rural South Africa. *Int J Tuberc Lung Dis* 2004; **8:** 796–99.

12 Lanjewar DN, Duggal R. Pulmonary pathology in patients with AIDS: an autopsy study from Mumbai. *HIV Med* 2001; **2:** 266–71.

13 World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. http://whqlibdoc.who.int/publications/2010/9789241599191_eng.pdf (accessed Feb 15, 2011).

14 Farmer P, Bayona J, Becerra M, et al. The dilemma of MDR-TB in the global era. *Int J Tuberc Lung Dis* 1998; **2:** 869–76.

15 Fischl MA, Daikos GL, Uttamchandani RB, et al. Clinical presentation and outcome of patients with HIV infection and tuberculosis caused by multiple-drug-resistant bacilli. *Ann Intern Med* 1992; **117:** 184–90.

16 Van Rie A, Enarson D. XDR tuberculosis: an indicator of public-health negligence. *Lancet* 2006; **368:** 1554–56.

17 Banada PP, Sivasubramani SK, Blakemore R, et al. Containment of bioaerosol infection risk by the Xpert MTB/RIF assay and its applicability to point-of-care settings. *J Clin Microbiol* 2010; **48:** 3551–57.

18 Helb D, Jones M, Story E, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol* 2010; **48:** 229–37.

19 Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med* 2010; **363:** 1005–15.

20 World Health Organization. Services in tuberculosis control part III: Culture. http://whqlibdoc.who.int/hq/1998/WHO_TB_98.258_(part3).pdf (accessed April 6, 2011).

21 Hillemann D, Rüsch-Gerdes S, Richter E. Application of the Capilia TB assay for culture confirmation of *Mycobacterium tuberculosis* complex isolates. *Int J Tuberc Lung Dis* 2005; **9:** 1409–11.

22 World Health Organization. Tuberculosis country profiles. http://www.who.int/tb/country/data/profiles/en/index.html (accessed March 21, 2011).

23 Médecins Sans Frontières, Western Cape Department of Health, City of Cape Town Department of Health, City of Cape Town Department of Health and University of Cape Town, Centre for Infectious Disease Epidemiology and Research. Providing HIV/TB care at the primary health care level. Khayelitsha annual activity report 2008–2009. Sea Point, South Africa: Médecins Sans Frontières, 2010.

24 English R, Information Management Office. Boland/Overberg region annual health status report 2007/2008. Worcester, UK: Boland/Overberg regional office, 2008.

25 Central TB Division, Directorate General of Health Services Ministry of Health and Family Welfare. Revised national tuberculosis control programme, performance report, India, 2010. http://www.tbcindia.org/pdfs/Perf%203Q%202010.pdf (accessed March 21, 2011).

26 Tropical Disease Foundation, Department of Health Republic of the Philippines. 2007 Nationwide Tuberculosis Prevalence Survey.

27 Asencios L, Quispe N, Mendoza-Ticona A, et al. Vigilancia nacional de la resistencia a medicamentos antituberculosos, Peru 2005–2006. *Rev Peru Med Exp Salud Publica* 2009; 26: 278–87.

28 Wright A, Zignol M, Van Deun A, et al. Epidemiology of antituberculosis drug resistance 2002–07: an updated analysis of the global project on anti-tuberculosis drug resistance surveillance. *Lancet* 2009; **373:** 1861–73.

29 Médecins Sans Frontières. Scaling up diagnosis and treatment of drug-resistant tuberculosis in Khayelitsha, South Africa. An integrated, community-based approach. Cape Town, South Africa, Médecins Sans Frontières, 2011.

30 Lukoye D, Cobelens FGJ, Ezati N, et al. Rates of anti-tuberculosis drug resistance in Kampala-Uganda are low and not associated with HIV infection. *PLoS One* 2011; **6:** e16130.

31 Philippine Nationwide Tuberculosis Drug Resistance Survey Team. Nationwide drug resistance survey of tuberculosis in the Philippines. *Int J Tuberc Lung Dis* 2009; **13:** 500–07.

32 Mendoza-Ticona A, Iglesias-Quilca D. Tuberculosis en pacientes con VIH/SIDA. *Acta Med Per* 2008; **25:** 247–54.

33 Stop TB Department World Health Organization European Region. Accelerating the implementation of collaborative TB/HIV activities in the WHO European Region; Vienna, Austria; July 16–17, 2010.

34 Marlowe EM, Novak Weekley SM, Cumpio J, et al. Evaluation of the cepheid Xpert MTB/RIF assay for the direct detection of *Mycobacterium tuberculosis* complex from respiratory specimens. *J Clin Microbiol* 2011; published online Feb 2. DOI:10.1128/JCM.02214-10.

35 Armand S, Vanhuls P, Delcroix G, Courcol R, Lemaître N. Comparison of the Xpert MTB/RIF test with an IS6110-TagMan real-time PCR assay for direct detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens. *J Clin Microbiol* 2011; published online March 16. DOI:10.1128/JCM.02157-10.

36 Moure R, Muñoz L, Torres M, Santin M, Martin R, Alcaide F. Rapid detection of *Mycobacterium tuberculosis* complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. *J Clin Microbiol* 2011; **49:** 1137–39.

37 Long Q, Li Y, Wang Y, et al. Barriers to accessing TB diagnosis for rural-to-urban migrants with chronic cough in Chongqing, China: a mixed methods study. *BMC Health Serv Res* 2008; **8:** 202.

38 Khan MS, Khan S, Godfrey-Faussett P. Default during TB diagnosis: quantifying the problem. *Trop Med Int Health* 2009; **14:** 1437–41.

39 Rao NA, Anwer T, Saleem M. Magnitude of initial default in pulmonary tuberculosis. *J Pak Med Assoc* 2009; **59:** 223–25.

40 Botha E, Den Boon S, Verver S, et al. Initial default from tuberculosis treatment: how often does it happen and what are the reasons? *Int J Tuberc Lung Dis* 2008; **12:** 820–23.

41 Squire SB, Belaye AK, Kashoti A, et al. 'Lost' smear-positive pulmonary tuberculosis cases: where are they and why did we lose them? *Int J Tuberc Lung Dis* 2005; **9:** 25–31.

42 Stop TB Department WHO. Roadmap for rolling out Xpert MTB/RIF for rapid diagnosis of TB and MDR-TB. http://www.who.int/tb/laboratory/roadmap_xpert_mtb-rif.pdf (accessed Feb 15, 2011).