Impact of Replacing Smear Microscopy with Xpert MTB/RIF for Diagnosing Tuberculosis in Brazil: A Stepped-Wedge Cluster-Randomized Trial

Betina Durovni1,2, Valeria Saraceni1,3, Susan van den Hof4,5, Anete Trajman2,6, Marcelo Cordeiro-Santos3,7, Solange Cavalcante1,8, Alexandre Menezes9, Frank Cobelens4,5

1 Rio de Janeiro Municipal Health Secretariat, Rio de Janeiro, Brazil, 2 Programa de Pós-graduação em Clínica Médica, Rio de Janeiro Federal University, Rio de Janeiro, Brazil, 3 Programa de Pós-graduação em Doenças Infecciosas, Tropical Medicine Foundation Dr. Heitor Vieira Dourado, Manaus, Brazil, 4 KNCV Tuberculosis Foundation, The Hague, The Netherlands, 5 Department of Global Health, Academic Medical Center and Amsterdam Institute for Global Health and Development, Amsterdam, The Netherlands, 6 Montreal Chest Institute, McGill University, Montreal, Canada, 7 Amazonas State University, Manaus, Brazil, 8 Oswaldo Cruz Foundation, Instituto de Pesquisa Evandro Chagas, Rio de Janeiro, Brazil, 9 Global Health Strategies, Rio de Janeiro, Brazil

Abstract

Background: Abundant evidence on Xpert MTB/RIF accuracy for diagnosing tuberculosis (TB) and rifampicin resistance has been produced, yet there are few data on the population benefit of its programmatic use. We assessed whether the implementation of Xpert MTB/RIF in routine conditions would (1) increase the notification rate of laboratory-confirmed pulmonary TB to the national notification system and (2) reduce the time to TB treatment initiation (primary endpoints).

Methods and Findings: We conducted a stepped-wedge cluster-randomized trial from 4 February to 4 October 2012 in 14 primary care laboratories in two Brazilian cities. Diagnostic specimens were included for 11,705 baseline (smear microscopy) and 12,522 intervention (Xpert MTB/RIF) patients presumed to have TB. Single-sputum-sample Xpert MTB/RIF replaced two-sputum-sample smear microscopy for routine diagnosis of pulmonary TB. In total, 1,137 (9.7%) tests in the baseline arm and 1,777 (14.2%) in the intervention arm were positive (p < 0.001), resulting in an increased bacteriologically confirmed notification rate of 59% (95% CI = 31%, 88%). However, the overall notification rate did not increase (15% vs. 31% CI = −6%, 37%), and we observed no change in the notification rate for those without a test result (−3%, 95% CI = −37%, 30%). Median time to treatment decreased from 11.4 d (interquartile range [IQR] = 8.5–14.5) to 8.1 d (IQR = 3.4–9.0) (p = 0.04), although not among confirmed cases (median 7.5 [IQR = 4.9–10.0] versus 7.3 [IQR = 3.4–9.0], p = 0.51). Prevalence of rifampicin resistance detected by Xpert was 3.3% (95% CI = 2.4%, 4.3%) among new patients and 7.4% (95% CI = 4.3%, 11.7%) among retreatment patients, with a 98% (95% CI = 87%, 99%) positive predictive value compared to phenotypic drug susceptibility testing. Missing data in the information systems may have biased our primary endpoints. However, sensitivity analyses assessing the effects of missing data did not affect our results.

Conclusions: Replacing smear microscopy with Xpert MTB/RIF in Brazil increased confirmation of pulmonary TB. An additional benefit was the accurate detection of rifampicin resistance. However, no increase on overall notification rates was observed, possibly because of high rates of empirical TB treatment.

Trial registration: ClinicalTrials.gov NCT01363765

Please see later in the article for the Editors’ Summary.
Introduction

The battle against tuberculosis (TB), a leading cause of death worldwide [1], has been hampered by a lack of accurate and rapid diagnostic tests, including those for drug resistance. The automated real-time PCR-based Xpert MTB/RIF assay (Xpert; Cepheid, Sunnyvale, California, US) can detect in 2 h the presence of a Mycobacterium tuberculosis–specific sequence of the rpoB gene as well as mutations in this gene responsible for most cases of phenotypic rifampicin resistance [2]. Xpert has proved to be feasible [3,4], accurate [5], and cost-effective [6–8] under field conditions in different settings, including at point of care in peripheral clinics. Since the World Health Organization (WHO) endorsed the use of Xpert in populations with high rates of drug-resistant TB and HIV co-infection [9] in 2010, more than 85 peer-reviewed papers have reported the assay’s accuracy for different specimens and populations [10].

However, the true clinical and public health performance of diagnostic tests is influenced by the treatment decisions made based on test results, and on delays in processing samples and reporting results [11]. For scaling up new diagnostics, decision-makers need pragmatic randomized controlled trials with patient-relevant endpoints, such as time to treatment initiation and treatment outcomes [12,13]. A recent randomized controlled trial in sub-Saharan Africa [4] was the first to demonstrate that despite a higher proportion of TB confirmation for Xpert than for smear microscopy (83% versus 50%), overall TB detection did not increase, because of the high rates of empirical treatment. In addition, Xpert diagnosis did not result in decreased morbidity at 2 and 6 mo of treatment. Studies conducted in different populations (populations with high HIV co-infection rates [14] and hospitalized patients [15]) also failed to show improvement in clinical outcomes, despite the reduced time to TB diagnosis with Xpert [14,15]. In both cases, rates of empirical treatment were also very high.

Moreover, substantial controversies remain about where Xpert capability should be located (peripheral clinics versus centralized laboratories), Xpert’s role in increasing detection of drug-susceptible as well as multidrug-resistant (MDR) TB, and the optimal management of Xpert rifampicin-resistant cases before confirmatory phenotypic drug susceptibility testing (DST) results are available [10]. Also, the benefits of implementation of Xpert in routine medical care remain to be established. Despite the limited available evidence of the programmatic benefits of the adoption of the assay, by September 2013, 95 out of the 145 countries eligible for concessional prices had procured cartridges for the public sector [16].

In the context of a pilot rollout project in Brazil, we conducted a pragmatic trial to evaluate the effect of replacing two-sample smear examinations by one-sample Xpert on pulmonary TB notification to the national notification system and time to treatment initiation in routine public health practice. The trial’s design, analysis, and reporting adhered to the principles of the CONSORT statement for pragmatic trials [17].

Methods

Ethics

The study was approved by the Brazil National Ethics Commission (Conselho Nacional de Ética em Pesquisa – CONEP #494/2011), the Rio de Janeiro Municipal Health Department Review Board (CETEMPS #236/11), and the Tropical Medicine Foundation of Amazonas Review Board (CEP FMT/HVD, 24 November 2011). The need for informed consent was waived by the ethical boards because this was a pilot implementation of a diagnostic test in routine practice, and only routine reporting data were used for the analysis.

Study Setting and Participants

With 82,775 TB patients notified to Brazil’s national notification system (Sistema de Informação de Agravos de Notificação [SINAN]) in 2012, Brazil is one of the 22 high-TB-burden countries [18]. Sputum smear examination (stained for acid-fast bacilli) is the mainstay of pulmonary TB diagnosis, with mycobacterial culture and DST recommended for specific subpopulations, in particular for previously treated patients [19]. An estimated 26% of new patients start treatment on clinical/radiological grounds, without bacteriological confirmation, and in over 70% of retreatment patients no culture or DST is performed [20]. The Brazilian National TB Program recommends empirical treatment while awaiting culture results if, despite a course of broad antibiotics, symptoms persist and there is a high clinical suspicion despite negative smear results (Figure S1) [19]. Rates of co-infection with HIV (9.7%) and of rifampicin resistance (<2%) in 2010; Drauilio Barreira, Director of the Brazilian National TB Program, personal communication) are relatively low.

The study was conducted in the cities of Manaus and Rio de Janeiro, which notified 1,315 and 4,959 new pulmonary TB cases [21], respectively, in 2011. In Rio de Janeiro (2010 population: 6,320,446) [22], Xpert was introduced in all 11 public primary care laboratories. In Manaus (2010 population: 1,802,014) [22], Xpert was introduced in three public laboratories, including an HIV referral hospital and a TB referral center. These laboratories cover 70% of TB diagnoses in both cities.

Patients whose sputum samples were sent to the study laboratories for diagnosis of pulmonary TB between 4 February and 4 October 2012 were eligible. There were no exclusion criteria, but in the Xpert arm, samples considered insufficient or inadequate for Xpert processing according to the manufacturer’s guidance [23] were tested only by smear examination. A sputum sample was considered insufficient for Xpert testing if its volume was less than 1 ml, and was considered inadequate for Xpert testing if on macroscopic examination it did not contain sputum or was blood-stained (as this may inhibit the PCR reaction) [24].

Study Design

This trial was a group-based comparison with phased introduction of Xpert to replace sputum smears as the initial diagnostic test for new pulmonary TB (Figure 1) [25]. A stepped-wedge design was chosen as it allowed a randomized comparison within a pilot project before national rollout. The units of comparison were TB laboratories and the clinics that use their services. The 14 trial laboratories were randomly assigned to the order in which they entered the intervention. To prevent imbalanced randomization with respect to important confounding variables as a result of the relatively small number of units, we applied restrained randomization based on the size of the monthly case load (low [n = 2], intermediate [n = 10], and high [n = 2]) of the laboratories, and on the estimated HIV prevalence (low [n = 12] and high [n = 2]) among the patients [26]. Allocation was not concealed, but laboratory staff and physicians were blinded to the order of entry into the intervention until Xpert was introduced.

In the smear microscopy arm, up to two sputum smears per patient were examined by conventional light microscopy based on direct Ziehl-Neelsen staining, as per routine. In the Xpert arm, usually only one of the submitted samples was examined. The second sample was processed for Xpert testing only if the first one
was inadequate or insufficient, or if an error in processing occurred. If the first sample was processed successfully, the second sample was discarded. Results were reported back to the requesting clinic. Patients with an Xpert rifampicin-resistant result were referred to a referral center, and provisionally started on first-line treatment while awaiting confirmation by phenotypic DST, in line with existing Brazilian National TB Program guidelines (Lowenstein-Jensen medium or Mycobacteria Growth Indicator Tube [BD Microbiology Systems, Cockeysville, Maryland, US], according to the referral laboratory routine). This policy at the time of the study was based on the low drug resistance prevalence in Brazil and the expected low positive predictive value (PPV) of an Xpert rifampicin resistance result. Sample size was calculated based on a laboratory-confirmed TB notification rate of 50/100,000/year, an average cluster population of 500,000, a coefficient of variation of 0.25, and an additional design effect due to the cluster design of 1.5 [26,27]. The study was powered to be able to detect a 60% increase in laboratory-confirmed pulmonary TB with a 5% type I error and an 80% type II error in the 8-mo study period.

All laboratories started off providing samples in the smear microscopy arm. Two laboratories then switched overnight to the Xpert arm every month, so that in the eighth (final) month of the trial, all units were in the Xpert arm (Figure 1). Fourth generation Xpert cartridges (G4) were used.

Primary endpoints were (1) the notification rate of laboratory-confirmed pulmonary TB to SINAN by any of the clinics relying on study laboratories’ services, measured by the difference and the ratio of rates in the intervention versus the baseline period, and (2) time to treatment initiation, estimated by the notification date minus the laboratory result date. In Brazil, notification of TB to SINAN is mandatory, and is done at the time of treatment initiation, such that notification can be considered to indicate that, and when, a patient started treatment.

Secondary endpoints were the following notification rates: for pulmonary TB despite a negative test result, for pulmonary TB without any laboratory result reported, and for overall pulmonary TB irrespective of laboratory test result. Additional endpoints were the rate of Xpert tests positive for rifampicin resistance and the proportion of patients with a rifampicin-resistant Xpert result confirmed by conventional DST (PPV).

Data Collection, Management, and Analysis

Data were collected from the routine laboratory reporting system (Gerenciamento de Ambiente Laboratorial [GAL]) and SINAN.

GAL contains details and results of all diagnostic tests ordered in the public laboratory system, entered by the laboratories. SINAN contains demographic and clinical data on all patients starting TB treatment, entered by the treating physicians or nurses. Entries in GAL were checked periodically for discrepancies against the regular TB laboratory logbooks and Xpert machines’ logs; errors were corrected directly in GAL. When the sample collection period was completed, GAL records related to diagnostic testing were extracted and allocated to the smear microscopy or Xpert arm according to sample processing dates. For the smear microscopy arm, any [first or second] positive test was considered a positive result. Pulmonary TB notifications for the study period were extracted from SINAN and checked manually for inconsistencies.

For Rio de Janeiro, pulmonary TB cases notified by clinics outside the municipal primary care network were excluded. For Manaus, all notified pulmonary TB cases were included. Pulmonary TB cases in Manaus that were notified in SINAN...
but not identified in GAL were assigned to one of the three participating laboratories by adding to each laboratory a number of notified cases proportional to the number notified with laboratory confirmation, stratified by month, sex, and age group.

The databases were linked using RECLINK [28] by name, date of birth, and sex; additional manual linkage was performed using the following algorithm in Stata version 12 (Stata Corp, College Station, Texas, US); patients were considered identical if (1) sex, clinic, and date of birth were the same, and name was similar except for missing given names, abbreviations, or different spelling, or (2) sex and clinic were the same; name was similar except for missing given names, abbreviations, or different spelling; there was a 0- to 14-d difference in result report date in GAL and start of treatment according to SINAN; and date of birth differed for day only, month only, or year only, or the date and month were swapped (e.g., 11 April and 4 November).

Culture and DST results for patients with Xpert rifampicin-resistant samples were obtained from the Brazilian MDR TB reporting system by manual linkage [29].

Analyses were performed in Stata version 12. Numbers of laboratory diagnoses of TB and TB notifications were calculated for the smear microscopy and Xpert arms, stratified by municipality, age group, sex, HIV co-infection, and study month. Since the trial did not follow cohorts of patients, the units of analysis were not individual patients but populations with their number of notified cases. We therefore constructed an aggregated database of the number of TB notifications and population denominators for each of the 896 strata combining laboratory (n = 14), study month (n = 8), sex (n = 2), and age group (n = 4).

For calculation of diagnostic and notification rates, population denominators took into account projected growth during the study period based on age- and sex-specific projected growth rates (separately for Rio de Janeiro and Manaus) [30] and were adjusted for variations in monthly number of days clinics were open by weighting the number of person-months for the proportion of patients with suspected TB with samples examined each month out of the total number examined by the laboratory during the whole study period, stratified by sex and age group.

The primary analyses were cluster-averaged, i.e., they compared the means of the cluster-specific notification rates between the 14 Xpert and the 14 smear microscopy cluster periods by their ratios and differences. Since the cluster-averaged method does not allow likelihood-based approaches for multivariable analysis, we adjusted the resulting rate ratio for potential confounding variables using a population-averaged quasi-likelihood method [26]. This method consisted of fitting a multivariable Poisson regression model that included all covariates except the notification rate (i.e., the endpoint of interest), and then comparing the model residuals for both trial arms because often two samples per patient were examined. In follow-up and duplicate samples (Figure 2). The number of duplicate samples excluded was larger in the smear microscopy arm because often two samples per patient were examined. In total, 11,705 specimens in the smear microscopy arm and 12,522 in the Xpert arm were included in the primary analysis.

We excluded any laboratory diagnosis made in the Xpert arm by smear examination, for two reasons. First, this approach would best reflect the situation of only Xpert being available, and therefore would best quantify the impact on TB notification of replacing smear examination with Xpert testing. Second, this approach would err on the conservative side with regard to the magnitude of increase in notification of laboratory-confirmed TB due to the use of Xpert. Unlike trials in which the endpoint is derived by dividing the number of diagnosed patients (numerator) by the number of tested patients (denominator), the endpoint in the present trial had as the denominator the population served by the study laboratories. Therefore, excluding patients diagnosed by smear microscopy in the intervention arm from the numerator did not affect the denominator, such that after excluding the smear-diagnosed patients, the notification rate for the intervention arm would by definition be lower than that without this exclusion, bringing the notification rate ratio for the intervention compared to the baseline arm closer to one. For the primary endpoints, we also show the intention to treat (ITT) analysis, including laboratory diagnoses made through smear examination in the Xpert arm.

Results

During the study period, the 14 laboratories examined 34,758 sputum specimens. Excluded were 4,731 (28.8%) specimens examined in the smear microscopy arm, and 5,800 (31.7%) examined in the Xpert arm, mostly those obtained for treatment follow-up and duplicate samples (Figure 2). The number of duplicate samples excluded was larger in the smear microscopy arm because often two samples per patient were examined. In total, 11,705 specimens in the smear microscopy arm and 12,522 in the Xpert arm were included in the primary analysis.

There were 1,137 (9.7%) positive smear examinations and 1,777 (14.2%) positive Xpert examinations (p < 0.001; Table 1). The proportion of positive examinations varied among the laboratories from 3.4% to 15.4% for smears, and from 7.4% to 21.9% for Xpert, with a median increase of 60.5% (range 1.4% to 181.6%) in positivity when using Xpert.

Primary Endpoints

Over the study period, 4,960 patients were notified with pulmonary TB to SINAN by clinics served by the study laboratories. Of these, 2,216 patients (47.6%) could be linked to positive test results (76.0% of all 2,914 positive test results) and 529 (11.4%) to negative test results (25.5% of 2,134 negative results). The remaining 1,915 (41.1%) notified patients could not be linked to any study period test result (Figure 2). Conversely, 695 positive tests could not be linked to cases in the notification system, 303 (26.6% [95% CI = 24.0%, 29.2%]) in the baseline and 392 (22.1%...
(95% CI = 20.2%, 24.0%) in the intervention arm (p = 0.003). We do not know whether these patients were treated without notification.

There was no difference in sex, age, or TB treatment history among these three groups (positive, negative, or no test results) or between the smear microscopy and Xpert arms. In both arms, HIV-infected patients were notified with laboratory-negative pulmonary TB more often than patients with negative or unknown HIV status were (Table 1).

Table 2 shows the unadjusted results for the notification rate of laboratory-confirmed TB (primary endpoint). The cluster-averaged laboratory-confirmed TB notification rate was 30.5/100,000/year in the smear microscopy arm versus 48.7/100,000/year in the Xpert arm, for a notification rate ratio of 1.59 (95% CI = 1.31, 1.88) and a notification rate difference of 18.2/100,000/year (95% CI = 9.4, 26.8) favoring the Xpert arm. Notification rates for laboratory-confirmed TB were higher for men than for women, and highest in the age group 15–59 y; these patterns were consistent across both arms. In the ITT analysis (Tables S1 and S2), which includes laboratory diagnosis by smear microscopy in the Xpert arm, the TB notification rate ratio was slightly higher: 1.67 (95% CI = 1.39, 1.96). The reasons for examining these 2,170 Xpert-arm specimens by smear microscopy were as follows: insufficient volume (1,151; 53.0%), inadequate

---

**Figure 2. Flowchart showing study inclusion in baseline (smear examination) and intervention (Xpert MTB/RIF) arms.** Bold arrows indicate cross-linkage between databases. doi:10.1371/journal.pmed.1001766.g002
### Table 1. Numbers and characteristics of laboratory-reported and notified TB cases, by intervention arm.

| Category       | Baseline Arm (Smear Examination) | Intervention Arm (Xpert MTB/RIF) |
|----------------|----------------------------------|----------------------------------|
|                | Number of Tests Performed | Positive Test Result | Notified and Started on Treatment | Number of Tests Performed | Positive Test Result | Notified and Started on Treatment |
|                |                          | Positive Test Result | Negative Test Result | No Test Result | All | Positive Test Result | Negative Test Result | No Test Result | All |
| Total          | 11,705                  | 1,137 (9.7%)         | 831 (40.5%)          | 313 (15.3%) | 906 (44.2%) | 2,050 (100%) | 12,522                  | 1,777 (14.2%)         | 1,385 (53.1%) | 216 (8.3%) | 1,009 (38.7%) | 2,610 (100%) |
| Sex            | Male                  | 6,487                | 749 (65.9%)         | 555 (66.8) | 202 (64.5%) | 1,314 (64.1%) | 6,679                  | 1,181 (66.5%)         | 910 (65.7%) | 135 (62.5%) | 637 (63.1%) | 1,682 (64.4%) |
|                | Female                | 5,218                | 388 (34.1%)         | 276 (33.2) | 111 (35.5%) | 5,843 (33.9%) | 475 (34.3%)          | 81 (37.5%)           | 372 (36.9%) | 928 (35.6%) |
| Age group      |< 15 y                | 400                  | 34 (3.0%)           | 25 (3.0%)  | 3 (1.0%)    | 28 (3.1%) | 449                  | 24 (1.4%)            | 14 (1.0%) | 4 (1.9%) | 38 (3.8%) | 56 (2.1%) |
|                | 15–39 y               | 4,786                | 603 (53.0%)         | 453 (44.9%) | 165 (52.7%) | 450 (49.7%) | 1,068                  | 521 (50.1%)          | 768 (55.5%) | 113 (52.3%) | 514 (50.9%) | 1,395 (53.4%) |
|                | 40–59 y               | 4,228                | 371 (32.7%)         | 257 (30.9%) | 108 (35.4%) | 317 (35.0%) | 682 (33.3%)          | 4,414              | 596 (33.5%) | 455 (29.9%) | 70 (32.4%) | 310 (30.7%) | 835 (32.0%) |
|                | ≥ 60 y                | 2,291                | 129 (11.3%)         | 96 (11.6%)  | 37 (11.8%)  | 111 (12.2%) | 244 (11.9%)          | 2,602              | 190 (10.7%) | 148 (10.7%) | 29 (13.4%) | 147 (14.6%) | 324 (12.4%) |
| City           | Rio de Janeiro        | 9,747                | 1,037 (91.2%)       | 756 (91.0%) | 228 (72.8%) | 755 (83.3%) | 1,739                  | 6,989              | 1,171 (65.9%) | 865 (82.5%) | 81 (75.7%) | 720 (71.8%) | 1,666 (63.8%) |
|                | Manaus                | 1,958                | 100 (8.8%)          | 75 (9.0%)  | 85 (27.2%)  | 151 (16.7%) | 311 (15.2%)          | 5,533              | 606 (34.1%) | 369 (20.6%) | 51 (23.6%) | 206 (20.4%) | 626 (24.0%) |
| HIV status*    | HIV positive          | N/A                  | N/A                  | 54 (65.6%) | 58 (18.9%)  | 73 (9.9%) | 187 (98.9%)          | N/A | N/A | 87 (63.3%) | 50 (23.1%) | 55 (5.5%) | 192 (74.7%) |
|                | HIV negative          | N/A                  | N/A                  | 284 (34.2%) | 97 (31.0%)  | 276 (36.5%) | 657 (34.6%)          | N/A | N/A | 369 (26.6%) | 51 (23.6%) | 206 (20.4%) | 626 (24.0%) |
| HIV status*    | HIV unknown           | N/A                  | N/A                  | 493 (59.3%) | 158 (50.5%) | 405 (53.6%) | 1,056                  | N/A | N/A | 929 (67.1%) | 115 (53.2%) | 748 (74.1%) | 1,790 (68.6%) |
| TB treatment history | New TB               | N/A                  | N/A                  | 688 (82.8%) | 266 (85.0%) | 0          | 954 (46.5%)          | N/A | N/A | 1,833 (85.4%) | 194 (89.8%) | 0 | 1,377 (52.8%) |
|                | Retreatment           | N/A                  | N/A                  | 142 (17.1%) | 47 (15.0%)  | 0          | 189 (92.9%)          | N/A | N/A | 202 (14.6%) | 15 (6.9%) | 0 | 217 (8.3%) |
| Unknown        | N/A                  | 1 (0.1%)             | 0                    | 906 | 907 (44.2%) | N/A | N/A                  | 0 | 7 (3.2%) | 1,009 | 1,016 (38.9%) |

Percentages in parentheses are column percentages, except for total cases, for which the percentages are row percentages.

*Excluding 452 notified TB cases not linked to a specified study arm.

N/A, not available.

doi:10.1371/journal.pmed.1001766.t001
### Table 2. Notifications of laboratory-confirmed pulmonary TB by arm (baseline and intervention), by sex, age, municipality, and baseline smear-positive rate.

| Category                  | Baseline Arm (Smear Examination) | Intervention Arm (Xpert MTB/RIF) | Notification Rate Ratio | Notification Rate Difference |
|---------------------------|----------------------------------|----------------------------------|-------------------------|------------------------------|
|                           | Population (Person-Years)        | Population (Person-Years)        | Overall*                | Cluster-Averagedb (95% CI)   | Overall*                | Cluster-Averagedb (95% CI)   | Overall*                | Cluster-Averagedb (95% CI)   |
|                           | Notification Rates (per 100,000 Population per Year) | Notification Rates (per 100,000 Population per Year) |                           |                               |                           |                               |                           |                               |
| Total                     | 2,799,071                        | 2,647,008                        | 1.76                    | 1.59 (1.31, 1.88)            | 22.6                     | 18.1 (9.4, 26.8)             |
| Sex                       |                                  |                                  |                         |                              |                           |                               |                           |                               |
| Male                      | 1,465,981                        | 1,404,926                        | 1.71                    | 1.60 (1.31, 1.90)            | 26.9                     | 22.6 (11.6, 33.7)            |
| Female                    | 1,333,090                        | 1,242,082                        | 1.85                    | 1.54 (1.16, 1.92)            | 17.5                     | 12.4 (3.6, 21.2)             |
| Age group                 |                                  |                                  |                         |                              |                           |                               |                           |                               |
| <15 y                     | 594,291                          | 571,447                          | 0.58                    | 0.43 (0.00, 1.11)            | −1.8                     | −2.1 (−4.6, 0.4)             |
| 15–39 y                   | 1,178,082                        | 1,079,807                        | 1.85                    | 1.53 (1.19, 1.87)            | 32.7                     | 22.5 (8.0, 36.9)             |
| 40–59 y                   | 680,888                          | 640,892                          | 1.88                    | 1.85 (1.45, 2.26)            | 33.3                     | 31.1 (16.3, 46.0)            |
| ≥60 y                     | 345,811                          | 354,862                          | 1.50                    | 1.40 (0.66, 2.13)            | 14.0                     | 12.4 (−10.5, 35.3)           |
| City                      |                                  |                                  |                         |                              |                           |                               |                           |                               |
| Rio de Janeiro            | 2,497,035                        | 1,725,565                        | 1.65                    | 1.51 (1.19, 1.83)            | 19.8                     | 16.4 (6.1, 16.3)             |
| Manaus                    | 302,036                          | 921,443                          | 2.27                    | 1.99 (1.01, 2.98)            | 31.6                     | 24.3 (0.3, 48.5)             |
| Baseline smear-positive ratec |                                  |                                  |                         |                              |                           |                               |                           |                               |
| <27.5                     | 1,292,644                        | 1,241,463                        | 2.15                    | 1.85 (1.22, 2.48)            | 26.9                     | 18.9 (4.9, 33.0)             |
| 27.5–36.4                 | 659,537                          | 471,307                          | 1.77                    | 1.85 (1.44, 2.27)            | 22.5                     | 25.1 (13.0, 37.2)            |
| ≥36.5                     | 846,890                          | 934,238                          | 1.39                    | 1.29 (0.89, 1.69)            | 15.6                     | 11.7 (−4.4, 27.6)            |

*Overall notification rates: number of notified cases divided by population size, multiplied by 100,000.

bCluster-averaged rates: mean of cluster-specific notification rates.
cLab-specific rate of positive smear examinations in the first study month, per 100,000 population per year.

doi:10.1371/journal.pmed.1001766.t002
material (e.g., saliva) (200; 9.2%), and logistical obstacles (819; 37.7%). The last referred to a single laboratory where the problems were solved after the first month of implementation.

Thirteen of the 14 laboratories showed an increase in laboratory-confirmed TB notification rate with the switch to Xpert (Table 3), although the difference was not significant for five of these laboratories. The laboratory-specific notification rate ratios ranged significantly from 0.95 (95% CI = 0.65, 1.37) to 2.95 (95% CI = 1.48, 5.36), with a median of 1.53. Possible changes over time in the effectiveness of the intervention were examined by plotting the notification rate ratio of laboratory-confirmed TB against the number of months since the switch from smear examination to Xpert (Figure S2) and by plotting the difference of the notification rate ratio in the intervention and baseline arms (Figure S3). During any of the study months, the laboratory-confirmed TB notification rate based on Xpert exceeded that based on smear examination (Figure S3), and the notification rate for laboratory-confirmed TB based on Xpert remained stable over time (Figure S2). The notification rate for overall pulmonary TB increased for 11 laboratories and decreased for three, with the laboratory-specific notification rate ratio ranging from 0.76 to 1.75 (median 1.16; Figure S4).

We performed sensitivity analyses in which we assumed that an incrementing proportion of the laboratory-positive patients for whom no notification record was found was identified as “not notified” because of failed linkage between the GAL and SINAN databases. Of these analyses, the one assuming 100% failed database linkage equals the analysis comparing between the intervention and baseline arms the rates of positive laboratory tests irrespective of notification. There was no significant change in rate ratio (Figure 3). This means that the missing notifications of patients with positive laboratory results, whether because of failed database linkage or because of failed notification, occurred at random and did not bias our primary endpoints.

Treatment was initiated before sputum sample processing in 417 (36.5%) and 585 (36.5%) of those patients notified with a bacteriological test result in the smear microscopy and Xpert arms, respectively. Overall, the cluster-averaged time interval between sputum processing (generally the same day as sputum collection) and start of treatment decreased from a median of 11.4 d (interquartile range [IQR] = 8.5–14.5) in the smear microscopy arm to 8.1 d (IQR = 5.4–9.3) in the Xpert arm (p = 0.04; Figure 4, left) for the per-protocol analysis, and to 8.6 d (IQR 5.4–9.7, p = 0.04; Figure 4, right) for the ITT analysis. Stratification of time intervals by bacteriological confirmation status showed no decrease for laboratory-confirmed TB notifications (median 7.5 [IQR = 4.9–10.0] and 7.3 [IQR = 3.4–9.0], p = 0.51) or for laboratory-negative TB notifications (median 21.5 [IQR = 13.5–25.6] and 14.0 [IQR 0.8–21.7], p = 0.07).

Secondary Endpoints

In contrast to the increase in the cluster-averaged laboratory-confirmed notification rate with the switch to Xpert, there were no significant changes in the cluster-averaged notification rate of laboratory-negative pulmonary TB (notification rate ratio 0.61, 95% CI = 0.31, 1.25), non-tested pulmonary TB (notification rate ratio 0.97, 95% CI = 0.63, 1.30), or overall pulmonary TB (notification rate ratio 1.15, 95% CI = 0.94, 1.37; Table 4). With multivariable adjustment, only the laboratory-negative TB notification rate ratio decreased significantly (0.52, 95% CI = 0.21, 0.84, p = 0.004; Table 4). Results for secondary endpoints stratified by patients’ and laboratories’ characteristics are presented in Tables 5–8. Across the laboratories, the population-based rates of positive test results regardless of notification (Figure S5) showed a distribution similar to that of the laboratory-confirmed TB notifications (Figure S4).

**Discussion**

This pragmatic trial showed that in a setting where laboratory diagnosis for pulmonary TB is largely restricted to sputum smear examination, implementing Xpert on a single sputum specimen increased laboratory-confirmed TB rates by 50% (31%–88%) and reduced time to treatment initiation from 11 to 8 d. The increase in TB confirmation was robust to potential confounding as well as to potential selection bias due to non-linkage of laboratory and notification databases, and was sustained over the study period. However, the overall notification rate of pulmonary TB—regardless of test results—did not increase significantly. In addition, because of the characteristics of the test, Xpert could accurately and promptly detect rifampicin resistance, which necessitates second-line drug treatment. Even in this setting with low drug resistance prevalence, the PPV for rifampicin resistance was high, although more than one-third of patients had no confirmatory DST result. Since culture with phenotypic DST is not routinely done in the country, these resistant cases would probably only be detected after treatment failure.

Our findings suggest that in this primary care setting, Xpert placed in laboratories is more useful for confirming pulmonary TB than for increasing TB detection. TB confirmation is relevant because it can potentially prevent many patients with respiratory symptoms who do not have TB from receiving unnecessary treatment and from having their true diagnosis delayed, although this remains to be demonstrated. The absence of a significant effect on detection of overall TB, a finding also recently reported from sub-Saharan Africa [1,32], is probably largely explained by the large proportion of patients who were started on empirical treatment [33]. Empirical treatment for TB is a medical decision...
| Laboratory | Baseline Arm (Smear Examination) | Intervention Arm (Xpert MTB/RIF) | Notification Rate Ratio (95% CI) | p-Value |
|------------|---------------------------------|---------------------------------|---------------------------------|---------|
|            | Population Notifications        | Notification Rate per 100,000 per Year (95% CI) | Population Notifications | Notification Rate per 100,000 per Year (95% CI) |         |
| 1          | 71,136                          | 27                               | 38.0 (25.1, 55.2)              | 373,387 | 219                               | 58.6 (51.1, 66.9) | 1.55 (1.03, 2.40) | 0.026  |
| 2          | 19,631                          | 6                                | 30.5 (11.2, 66.6)              | 168,531 | 78                                | 46.2 (36.6, 57.8) | 1.51 (0.67, 4.25) | 0.331  |
| 3          | 128,993                         | 28                               | 21.7 (14.5, 31.4)              | 289,819 | 121                               | 41.7 (34.7, 49.9) | 1.92 (1.27, 3.01) | 0.001  |
| 4          | 196,975                         | 57                               | 29.0 (21.9, 37.5)              | 646,545 | 401                               | 62.1 (56.1, 68.4) | 2.14 (1.62, 2.88) | 0.000  |
| 5          | 172,172                         | 92                               | 53.4 (43.1, 65.6)              | 248,345 | 171                               | 68.9 (58.9, 79.9) | 1.29 (0.99, 1.68) | 0.048  |
| 6          | 85,430                          | 12                               | 14.0 (7.3, 24.6)               | 106,367 | 41                                | 38.6 (27.6, 52.3) | 2.74 (1.41, 5.74) | 0.001  |
| 7          | 162,646                         | 55                               | 33.8 (25.4, 44.0)              | 133,615 | 65                                | 48.7 (37.6, 62.1) | 1.44 (0.99, 2.10) | 0.047  |
| 8          | 166,765                         | 62                               | 37.2 (28.5, 47.7)              | 123,472 | 59                                | 47.8 (36.4, 61.7) | 1.29 (0.88, 1.87) | 0.169  |
| 9          | 212,759                         | 73                               | 34.3 (26.9, 43.2)              | 162,631 | 53                                | 32.6 (24.5, 42.6) | 0.95 (0.65, 1.37) | 0.779  |
| 10         | 296,101                         | 93                               | 31.4 (25.3, 38.4)              | 147,148 | 87                                | 59.1 (47.3, 72.9) | 1.88 (1.39, 2.55) | 0.000  |
| 11         | 514,874                         | 124                              | 24.1 (20.1, 28.7)              | 116,858 | 37                                | 31.6 (22.3, 43.7) | 1.31 (0.89, 1.91) | 0.151  |
| 12         | 366,373                         | 82                               | 22.4 (17.8, 27.7)              | 81,875  | 26                                | 31.8 (20.7, 46.5) | 1.42 (0.88, 2.23) | 0.129  |
| 13         | 181,159                         | 39                               | 21.5 (15.3, 29.4)              | 22,012  | 14                                | 63.6 (34.8, 106.7)| 2.95 (1.48, 5.56) | 0.002  |
| 14         | 224,056                         | 81                               | 36.1 (28.7, 44.9)              | 26,402  | 13                                | 49.3 (26.2, 84.2) | 1.36 (0.70, 2.46) | 0.305  |
| Total/average | 2,799071                        | 831                              | 30.5 (24.9, 36.1)              | 2,647,008 | 1,385                          | 48.7 (41.5, 55.8) | 1.59 (1.31, 1.88) | <0.001|

The numbers of the laboratories reflect the sequence of introduction of the Xpert intervention (two labs at a time).

doi:10.1371/journal.pmed.1001766.t003

Table 3. Notifications of pulmonary tuberculosis by laboratory according to arm (baseline versus intervention).
that depends on pretest probability, the patient’s clinical condition, and test availability. The sensitivity and specificity of empirical treatment based on the WHO algorithm [34] vary substantially [35]. The pooled specificity for this algorithm in smear-negative patients was 69% in a meta-analysis [35], suggesting that a great number of patients falsely diagnosed with TB on clinical and radiological grounds could benefit from better diagnosis. In our study, 44.2% (95% CI = 43.9%, 47.9%) of patients in the smear microscopy arm and 38.7% (95% CI = 37.2%, 47.8%) in the Xpert arm were notified to SINAN as TB cases without a laboratory test, and an additional 15.3% (95% CI = 14.7%, 15.9%) and 8.3% (95% CI = 7.8%, 8.8%), respectively, with a negative test result. These groups of patients contain an unknown proportion of individuals who do not have TB, and this proportion ideally could be reduced by using Xpert. Due to Xpert’s higher sensitivity, health-care workers can more confidently withhold TB treatment when the test result is negative, in particular for patients with no HIV infection, requesting the patient to come back for further TB diagnostics if the symptoms remain. We have no data to show how much extra delay to treatment initiation this would entail. The study period may have been too short to expect such a change in health-care workers’ behavior, thus limiting our ability to show this possible benefit of Xpert.

There are alternative explanations for at least some of the non-laboratory-confirmed notifications. A positive test result may have been issued outside the study laboratories, such as at hospitals or small primary care laboratories, or database linkage may have failed despite the various algorithms used to address the lack of unique patient identifiers. Indeed, incomplete linkage has been described in previous studies in Brazil [36,37]. Finally, dropout between diagnosis and treatment may be underreported [38]. Incomplete linkage and notification are also likely causes of the high proportion of positive laboratory results for which no disease notification could be found [36,37]. We have no details about the patients who had a positive test but could not be retrieved in the notification database. The crude proportion of these missing patients decreased from the baseline to the intervention period. The rate ratio for laboratory-positive notifications (1.59, 95% CI = 1.31, 1.88; Figure 4) was equal to the rate ratio for positive laboratory results (1.60, 95% CI = 1.33, 1.86; Figure S5), and we observed similarity in age and sex distribution between patients with and without laboratory results, both suggesting that missing data happened at random. Together these data suggest that dropout between diagnosis and treatment was lower in the intervention than in the baseline arm, although the true magnitude of the difference cannot be established. Although initial loss to follow-up declined, it still was substantial, and likely added to transmission in the community. However, Xpert implementation has the potential to diminish transmission by reducing time to treatment initiation and initial loss to follow-up.
Despite its limitations, reliance on routine reporting data allowed a highly pragmatic trial design, closely resembling routine clinical practice. In particular, it obviated the need for individual informed consent, which would have made a trial of this size unfeasible and would have carried a risk of non-participation. In addition, the stepped-wedge trial design had the advantage of allowing an assessment of the effectiveness of this diagnostic intervention during its implementation with a limited number of laboratories, while a parallel cluster-randomized design would have required a larger number of randomization units [26]. The design has, however, potential for bias, in particular when assignment of the outcome to a study arm is not straightforward, such as with delayed treatment effects, or when conditions that affect the outcome change over time [39]. We believe that neither possibility for bias applies to our study. The primary endpoint of disease notification occurred within weeks after the diagnostic test, so that mis-assignment is unlikely.

Because of the small number of clusters in our study, we opted for the most robust and conservative statistical approach based on cluster-averaged rather than overall rates [26]. This primary analysis did not allow adjustment for time effects. An increase in effectiveness over time after the switch to Xpert could indicate a learning curve effect, while a decrease over time would suggest that the excess cases notified in the Xpert arm compared to the smear microscopy arm reflect detection of a temporary “backlog” of prevalent TB cases not identified by smear examination, rather than recent incident cases. However, Figures S1 and S2 show no consistent pattern of change with time since the start of using Xpert, making it unlikely that these effects occurred. Furthermore, the secondary analysis based on overall rates supported the primary analysis results, and adjustment for time effects only increased the effect of Xpert on notification rates.

Another possible source of bias was the unexpected high proportion of insufficient-volume samples in the Xpert arm, which had to be examined microscopically. However, the ITT analysis in which such smear examinations, when positive, were included only increased the effect of Xpert, and to limited extent. Indeed, recent data suggest that Xpert sensitivity is unaffected by sputum volume [40].

There was substantial variation in notification rate ratios for laboratory-confirmed TB across the 14 study laboratories. The largest relative and absolute increases were observed for the laboratories with relatively low notification rates in the smear microscopy arm. Since the sensitivity of Xpert is less operator-dependent than that of smear examination [3], especially for specimens with low bacterial load, these differences between the laboratories probably reflect differences in the operator-dependent sensitivity of smear examination. This difference would imply that Xpert improves TB case detection most where smear laboratory performance is suboptimal, e.g., because of high workload or inexperienced technicians, even though this may not translate into improvement of case finding for reasons such as empirical treatment and dropout between diagnosis and treatment.

Figure 4. Box-and-whisker plot of cluster-averaged time interval between processing of sputum and start of first-line drug treatment in the baseline (smear examination) and intervention (Xpert MTB/RIF) arms. Delays are shown for three groups: (1) all TB patients notified for whom a sputum test was performed, (2) TB patients notified with bacteriological confirmation, and (3) TB patients notified without bacteriological confirmation. Left: per-protocol analysis; right: ITT analysis.

doi:10.1371/journal.pmed.1001766.g004

Xpert Implementation in Brazil

PLOS Medicine | www.plosmedicine.org

December 2014 | Volume 11 | Issue 12 | e1001766
Cluster-averaged notification rates, rate differences, and rate ratios for laboratory-confirmed TB, TB with negative test result, TB with no testing, and overall pulmonary TB.

### Table 4: Cluster-averaged notification rates, rate differences, and rate ratios for laboratory-confirmed TB, TB with negative test result, and TB with no testing.

| Category | Baseline (Smear Microscopy) | Intervention (Xpert) | Unadjusted Rate Ratio (95% CI) | Adjusted* Rate Ratio (95% CI) |
|----------|-----------------------------|----------------------|---------------------------|-------------------------------|
|          | Notification Rate (95% CI)  |                      |                           |                               |
|          |                              |                      |                           |                               |
| Laboratory-confirmed, notifications | 30.5 (26.3, 34.8)       | 48.7 (41.5, 55.8)    | 1.59 (1.31, 1.88)         |                               |
|          |                              |                      |                           |                               |
| Laboratory-confirmed, negative test result | 36.9 (31.7, 42.9)       | 51.3 (46.2, 56.6)    | 1.38 (1.17, 1.59)         |                               |
|          |                              |                      |                           |                               |
| Laboratory-confirmed, no test | 121 (61, 180)           | 151 (104, 210)       | 1.25 (1.05, 1.48)         |                               |
|          |                              |                      |                           |                               |
| Laboratory-confirmed, non-laboratory result | 35.8 (31.4, 40.5)       | 40.3 (34.6, 45.9)    | 1.14 (1.00, 1.28)         |                               |
|          |                              |                      |                           |                               |
| Laboratory-confirmed, positive smear | 208 (182, 233)          | 260 (228, 292)       | 1.26 (1.12, 1.41)         |                               |
|          |                              |                      |                           |                               |
| Laboratory-confirmed, negative smear | 167 (137, 197)          | 201 (166, 233)       | 1.28 (1.09, 1.52)         |                               |
|          |                              |                      |                           |                               |
| Overall, notifications | 79.6 (65.7, 93.5)        | 91.7 (80.2, 103.2)   | 1.15 (1.00, 1.32)         |                               |
|          |                              |                      |                           |                               |
| Positive smear examinations | 41.5 (34.1, 49.8)       | 51.3 (46.2, 56.6)    | 1.25 (1.05, 1.48)         |                               |
|          |                              |                      |                           |                               |
| Positive smear non-laboratory examinations | 26 (13.4, 39.6)         | 30.7 (23.1, 39.1)    | 1.20 (1.02, 1.41)         |                               |

* Notification rate ratio is for intervention (Xpert) compared to baseline (smear examination) arm.

**Note:** All rates are adjusted for sex, age, municipality, and baseline smear-positive rate, quasi-likelihood population-averaged method.

In conclusion, this programmatic study showed the effectiveness of replacing smear microscopy with Xpert for TB case confirmation and reduction of time to treatment initiation at the population level. These results support the Brazilian Ministry of Health’s decision to adopt Xpert as a replacement for smear microscopy in 92 municipalities that cover more than 55% of new TB cases. However, important challenges remain in order to take full advantage of the potential of this technology in pragmatic conditions, such as reducing more dramatically treatment initiation delays and avoiding unnecessary empirical treatment.
Table 5. Notifications of pulmonary tuberculosis despite negative laboratory test.

| Category | Baseline Period (Smear Examination) | Intervention Period (Xpert MTB/RIF) | Notification Rate Ratio |
|----------|-------------------------------------|------------------------------------|-------------------------|
|          | Population (Person-Years) | Notification Rate (per 100,000 Population per Year) | Cluster-Averaged (95% CI) | Overall (95% CI) |
| Total    | 2,799,071 11.2 | 12.1 (8.1, 18.0) 2,647,008 8.2 | 0.73 0.61 (0.01, 1.23) |
| Sex      | Male | 1,465,981 13.7 | 15.1 (7.2, 22.9) 1,420,626 9.6 | 4.9 (1.6, 8.1) 0.70 0.64 (0.01, 1.13) |
|          | Female | 1,333,090 8.3 | 8.8 (3.4, 13.9) 1,247,382 6.5 | 4.7 (1.6, 8.1) 0.78 0.55 (0.01, 1.20) |
| Age group | <15 y | 594,291 0.5 | 0.5 (0.0, 1.1) 571,447 0.7 | 0.0 (0.0, 0.6) 1.39 0.47 (0.01, 1.79) |
|          | 15–39 y | 1,178,082 14.0 | 15.8 (7.5, 24.2) 1,079,807 10.5 | 0.75 0.58 (0.01, 1.26) |
|          | 40–59 y | 680,888 15.8 | 16.7 (6.5, 26.9) 640,892 10.9 | 0.92 (0.34, 1.40) 0.69 0.68 (0.01, 1.14) |
|          | 60 y | 345,881 10.7 | 12.3 (3.2, 23.3) 354,882 8.2 | 0.76 0.76 (0.01, 1.42) |
| City     | Rio de Janeiro | 2,497,035 9.1 | 9.3 (0.2, 10.3) 1,725,565 4.7 | 4.1 (2.4, 5.9) 0.51 0.45 (0.01, 1.24) |
|          | Manaus | 302,036 21.1 | 22.5 (0.0, 76.5) 927,445 14.6 | 191 (0.0, 57.9) 0.52 0.35 (0.01, 0.74) |
|          | Baseline smear-positive rate | 1.29 (0.0, 3.7) 1.29 (0.0, 3.7) | 1.29 (0.0, 3.7) 1.29 (0.0, 3.7) |
|          | 27–36.4 | 659,537 8.3 | 6.5 (0.0, 13.5) 471,307 7.0 | 4.7 (1.7, 7.8) 0.84 0.83 (0.01, 1.97) |
|          | 36.5 | 846,890 10.4 | 7.1 (0.0, 13.5) 934,238 4.7 | 4.7 (1.7, 7.8) 0.84 0.83 (0.01, 1.97) |

Overall notification rates: number of notified cases divided by population size, multiplied by 100,000. Cluster-averaged rates: mean of cluster-specific notification rates.

aLaboratory-specific rate of positive smear examinations in the first study month, per 100,000 population per year.
Table 6. Notifications of pulmonary tuberculosis without recorded laboratory test.

| Category                  | Baseline Period (Smear Examination) | Intervention Period (Xpert MTB/RIF) | Notification Rate Ratio |
|---------------------------|------------------------------------|------------------------------------|-------------------------|
|                           | Population (Person-Years) | Overall | Cluster-Averaged (95% CI) | Population (Person-Years) | Overall | Cluster-Averaged (95% CI) | Overall | Cluster-Averaged (95% CI) |
|                           | Notification Rate (per 100,000 Population per Year) | | | Notification Rate (per 100,000 Population per Year) | | | |
| Total                     | 2,799,071 | 32.4 | 36.9 (26.8, 47.1) | 2,647,008 | 38.1 | 35.8 (27.6, 43.9) | 1.18 | 0.61 (<0.01, 1.23) |
| Sex                       |          |      |                    | | | | |
| Male                      | 1,465,981 | 38.1 | 45.5 (32.2, 58.8) | 1,404,926 | 45.3 | 43.8 (34.7, 52.8) | 1.19 | 0.64 (<0.01, 1.33) |
| Female                    | 1,333,090 | 26.2 | 28.2 (19.0, 37.4) | 1,242,082 | 30.0 | 26.8 (18.9, 34.7) | 1.14 | 0.55 (<0.01, 1.20) |
| Age group                 |          |      |                    | | | | |
| <15 y                     | 594,291  | 4.7  | 4.8 (1.1, 8.5)     | 571,447 | 6.6  | 5.8 (2.4, 9.2)     | 1.41 | 0.47 (<0.01, 1.75) |
| 15–39 y                   | 1,178,082 | 38.3 | 46.2 (29.9, 62.5) | 1,079,807 | 47.7 | 46.6 (36.9, 56.3) | 1.25 | 0.58 (<0.01, 1.26) |
| 40–59 y                   | 680,888  | 46.6 | 51.5 (34.7, 68.3) | 640,892 | 48.4 | 43.8 (33.2, 54.4) | 1.04 | 0.68 (<0.01, 1.42) |
| ≥60 y                     | 345,811  | 32.1 | 35.5 (18.4, 52.7) | 354,862 | 41.4 | 39.9 (23.6, 56.2) | 1.29 | 0.76 (<0.01, 1.69) |
| City                      |          |      |                    | | | | |
| Rio de Janeiro            | 2,497,035 | 30.2 | 34.3 (21.7, 46.8) | 1,725,565 | 41.7 | 37.6 (27.3, 47.9) | 1.38 | 0.45 (0.24, 0.66) |
| Manaus                    | 302,036  | 50.0 | 46.7 (29.2, 64.2) | 921,443 | 31.4 | 29.2 (19.1, 39.2) | 0.63 | 0.85 (<0.01, 2.74) |
| Baseline smear-positive rate* | |      |                    | | | | |
| <27.5                     | 1,292,644 | 27.3 | 34.4 (14.7, 54.1) | 1,241,463 | 34.7 | 33.6 (26.4, 40.9) | 1.27 | 0.66 (<0.01, 1.82) |
| 27.5–36.4                 | 659,537  | 35.3 | 38.7 (40.7, 73.2) | 471,307 | 43.3 | 37.2 (0.0, 80.1) | 1.23 | 0.83 (<0.01, 1.97) |
| ≥36.5                     | 846,890  | 38.0 | 38.0 (13.7, 62.2) | 934,238 | 39.9 | 36.7 (27.3, 46.1) | 1.05 | 0.39 (0.10, 0.69) |

Overall notification rates: number of notified cases divided by population size, multiplied by 100,000. Cluster-averaged rates: mean of cluster-specific notification rates.

*Laboratory-specific rate of positive smear examinations in the first study month, per 100,000 population per year.

doi:10.1371/journal.pmed.1001766.t006
Table 7. Notifications of overall pulmonary tuberculosis, regardless of test result.

| Category          | Baseline Period (Smear Examination) | Intervention Period (Xpert MTB/RIF) | Notification Rate Ratio | Notification Rate Difference |
|-------------------|-------------------------------------|-------------------------------------|-------------------------|-----------------------------|
|                   | Population (Person-Years)           | Population (Person-Years)           | Overall                 | Overall                     |
|                   | Overall Cluster-Averaged            | Overall Cluster-Averaged            | (95% CI)                | (95% CI)                    |
| Total             | 2,799,071                           | 2,647,008                           | 1.35                    | 25.3                        |
|                   | 73.2                                | 98.6                                | 1.15 (0.94, 1.37)       | 12.2 (−50, 29.3)            |
| Sex               |                                     |                                     |                         |                             |
| Male              | 1,465,981                           | 1,404,926                           | 1.34                    | 30.1                        |
|                   | 89.7                                | 119.7                               | 1.16 (0.94, 1.38)       | 15.6 (−60, 37.0)            |
| Female            | 1,333,090                           | 1,242,082                           | 1.35                    | 19.5                        |
|                   | 55.2                                | 74.7                                | 1.12 (0.85, 1.38)       | 7.1 (−8.8, 22.9)            |
| Age group         |                                     |                                     |                         |                             |
| <15 y             | 594,291                             | 571,447                             | 1.04                    | 0.4                         |
|                   | 9.4                                 | 9.8                                 | 0.84 (0.18, 1.51)       | −1.4 (−7.4, 4.6)            |
| 15–39 y           | 1,178,082                           | 1,079,807                           | 1.43                    | 38.6                        |
|                   | 90.6                                | 129.2                               | 1.15 (0.91, 1.40)       | 16.2 (−9.4, 41.5)           |
| 40–59 y           | 680,888                             | 640,892                             | 1.30                    | 30.2                        |
|                   | 100.1                               | 130.3                               | 1.17 (0.86, 1.49)       | 18.1 (−14.6, 51.0)          |
| ≥60 y             | 345,811                             | 354,862                             | 1.29                    | 20.7                        |
|                   | 70.6                                | 91.3                                | 1.18 (0.60, 1.75)       | 13.9 (−31.6, 59.3)          |
| City              |                                     |                                     |                         |                             |
| Rio de Janeiro    | 2,497,035                           | 1,725,565                           | 1.39                    | 27.0                        |
|                   | 69.6                                | 96.6                                | 1.19 (0.92, 1.46)       | 14.6 (−57, 34.8)            |
| Manaus            | 302,036                             | 921,443                             | 1.00                    | −0.5                        |
|                   | 102.9                               | 102.4                               | 1.04 (0.58, 1.49)       | 3.4 (−39.1, 45.9)           |
| Baseline smear-   |                                     |                                     |                         |                             |
| positive rate a   |                                     |                                     |                         |                             |
| <27.5             | 1,292,644                           | 1,241,463                           | 1.51                    | 32.5                        |
|                   | 63.9                                | 96.4                                | 1.16 (0.68, 1.63)       | 11.7 (−240, 47.5)           |
| 27.5–36.4         | 659,537                             | 471,307                             | 1.40                    | 29.2                        |
|                   | 72.9                                | 102.1                               | 1.30 (0.71, 1.90)       | 22.6 (−21.4, 66.7)          |
| ≥36.5             | 846,890                             | 934,238                             | 1.14                    | 11.9                        |
|                   | 88.0                                | 99.9                                | 1.05 (0.71, 1.38)       | 4.3 (−25.2, 33.6)           |

Overall notification rates: number of notified cases divided by population size, multiplied by 100,000. Cluster-averaged rates: mean of cluster-specific notification rates.

*Laboratory-specific rate of positive smear examinations in the first study month, per 100,000 population per year.
### Table 8. Positive laboratory test result for tuberculosis, irrespective of notification.

| Category | Baseline Period (Smear Examination) | Intervention Period (Xpert MTB/RIF) | Notification Rate Ratio | Notification Rate Difference |
|----------|-------------------------------------|-------------------------------------|-------------------------|-----------------------------|
|          | Population (Person-Years) | Notification Rate (per 100,000 Population per Year) | Overall | Cluster-Averaged (95% CI) | Population (Person-Years) | Notification Rate (per 100,000 Population per Year) | Overall | Cluster-Averaged (95% CI) |
|          | Overall | Cluster-Averaged (95% CI) | Overall | Cluster-Averaged (95% CI) | Overall | Cluster-Averaged (95% CI) | Overall | Cluster-Averaged (95% CI) |
| Total    | 2,799,071 | 44.3 | 46.0 (38.6, 54.8) | 2,647,008 | 66.2 | 66.2 (56.5, 74.8) | 1.49 | 1.41 (1.16, 1.66) | 21.9 | 19.1 (7.4, 30.7) |
| Sex      | Male    | 1,465,981 | 55.8 | 57.3 (47.7, 66.9) | 1,404,926 | 83.5 | 82.0 (70.1, 94.0) | 1.50 | 1.43 (1.18, 1.69) | 27.7 | 24.7 (10.2, 39.4) |
|          | Female  | 1,333,090 | 31.8 | 35.2 (28.2, 42.1) | 1,242,082 | 46.7 | 46.8 (37.8, 55.7) | 1.47 | 1.33 (0.63, 1.64) | 14.9 | 11.7 (0.9, 224) |
| Age group| <15 y   | 594,291 | 4.4 | 3.5 (1.2, 5.8) | 571,447 | 6.3 | 5.9 (2.5, 9.3) | 1.44 | 1.69 (0.57, 2.81) | 1.9 | 2.4 (−1.5, 6.3) |
|          | 15–39 y | 1,178,082 | 55.8 | 60.6 (48.2, 73.0) | 1,079,807 | 87.9 | 87.2 (73.4, 101.0) | 1.57 | 1.44 (1.15, 1.73) | 32.0 | 26.6 (9.0, 44.3) |
|          | 40–59 y | 680,888 | 63.7 | 65.5 (52.1, 79.0) | 640,892 | 92.4 | 92.2 (78.8, 105.6) | 1.45 | 1.41 (1.13, 1.68) | 28.6 | 26.8 (8.7, 44.8) |
|          | ≥60 y   | 345,811 | 35.9 | 40.9 (27.5, 54.4) | 354,862 | 50.4 | 52.6 (30.9, 74.3) | 1.41 | 1.29 (0.69, 1.88) | 14.6 | 11.7 (−12.7, 36.0) |
| City     | Rio de Janeiro | 2,497,035 | 45.1 | 48.8 (39.5, 58.0) | 1,725,565 | 67.7 | 68.3 (27.3, 79.1) | 1.50 | 1.40 (1.12, 1.67) | 22.5 | 19.5 (6.1, 32.9) |
|          | Manaus  | 302,036 | 37.1 | 38.7 (3.9, 73.5) | 921,443 | 63.5 | 56.1 (19.1, 88.8) | 1.71 | 1.45 (0.65, 2.25) | 26.5 | 17.4 (−13.4, 48.3) |
| Baseline smear-positive rate * | <27.5  | 1,292,644 | 35.0 | 33.5 (25.2, 41.6) | 1,241,463 | 58.9 | 51.2 (38.4, 63.9) | 1.68 | 1.53 (1.16, 1.91) | 23.9 | 17.8 (5.2, 30.4) |
|          | 27.5–36.4 | 659,537 | 54.5 | 55.6 (46.4, 64.7) | 471,307 | 69.2 | 67.8 (51.8, 84.0) | 1.27 | 1.22 (0.94, 1.50) | 14.6 | 12.2 (−3.3, 27.6) |
|          | ≥36.5   | 846,890 | 50.8 | 51.8 (26.3, 77.4) | 934,238 | 80.4 | 81.0 (68.4, 93.3) | 1.58 | 1.56 (1.14, 1.98) | 29.6 | 29.2 (7.4, 51.0) |

Overall notification rates: number of notified cases divided by population size, multiplied by 100,000. Cluster-averaged rates: mean of cluster-specific notification rates.

*Laboratory-specific rate of positive smear examinations in the first study month, per 100,000 population per year.

doi:10.1371/journal.pmed.1001766.t008
Supporting Information

Figure S1 Algorithms for TB investigation and treatment. (A) Study algorithm for sputum sample processing in the intervention arm. (B) National algorithm for Xpert-based pulmonary TB investigation in Brazil. (C) National algorithm for smear-based pulmonary TB investigation in Brazil. (TIF)

Figure S2 Notification rates of laboratory-confirmed TB for the intervention arm, by month since start of using Xpert. Dots denote notification rates based on Xpert. Solid line: linear trend for notification rates (decline 0.74/100,000/year for each month; correlation coefficient 0.262, \( p = 0.95 \)). Vertical bars: 95% confidence intervals for the notification rates. (TIF)

Figure S3 Difference between intervention (Xpert) and baseline (smear examination) arm in cluster-averaged notification rates of laboratory-confirmed TB, by study month. Point estimates represent cluster-averaged notification rate differences between intervention and baseline arms. Values greater than zero denote higher notification rates for intervention than for baseline. Vertical bars: 95% confidence intervals for the cluster-averaged notification rate differences. Horizontal bar: notification rate difference for entire study period (18.1/100,000/year). Month 1 and month 8 had baseline-only and intervention-only observations, respectively. (TIF)

Figure S4 Notification rates for baseline (smear examination) and intervention (Xpert) arms, by study laboratory. Cluster-specific notification rates (i.e., of all clinics that use the services of a particular study laboratory) of overall TB irrespective of laboratory confirmation. Laboratory number corresponds to the sequence of transition from baseline (smear examination) to intervention (Xpert) arm. Laboratories 2, 4, and 6 were situated in Manaus, all others in Rio de Janeiro. (TIF)

Figure S5 Positivity rate per laboratory, irrespective of notification. Rate per 100,000 population per year for positive laboratory diagnoses, irrespective of notification, per laboratory. Laboratory number corresponds to the sequence of transition from baseline (smear examination) to intervention (Xpert) arm. Laboratories 2, 4, and 6 were situated in Manaus, all others in Rio de Janeiro. (TIF)

Table S1 Numbers and characteristics of laboratory-reported and notified TB cases, by intervention arm, including 54 smear results in the intervention arm (ITT analysis). (DOCX)

Table S2 Notifications of laboratory-confirmed pulmonary TB by arm (baseline and intervention), by sex, age, municipality, and baseline smear-positive rate, including 54 smear results in the intervention arm (ITT analysis). (DOCX)

Table S3 Secondary analysis: unadjusted and multivariably adjusted notification rate ratios for laboratory-confirmed TB, TB with negative test result, TB with no testing, and overall pulmonary TB, using a mixed multilevel model. (DOCX)

Table S4 Notification rate ratios of laboratory-confirmed TB adjusted for calendar time, comparing the Xpert to the smear microscopy arm, stratified by baseline smear-positive rate (time-adjusted mixed multilevel model). The time-adjusted mixed multilevel model for laboratory-confirmed notifications showed significant interactions between intervention status and municipality, and between intervention status and baseline smear positivity rate. The interaction with municipality no longer contributed significantly to the model likelihood when the interaction with baseline rate was included in the model, whereas the interaction with baseline rate continued to contribute significantly (\( p < 0.001 \)) even when the interaction with municipality was included. Hence, we concluded that the underlying interaction was between intervention status and baseline smear positivity rate. This table shows that the notification rate ratios for laboratory-confirmed TB decreased from 1.97 in the lowest baseline category to 1.28 in the highest baseline category. (DOCX)

Table S5 Cluster-averaged analysis excluding month 1 and month 8. Excluding the data for months 1 and 8, which related to baseline-only and intervention-only observations, respectively, did not affect the cluster-averaged notification rate ratio for laboratory-confirmed TB (1.60, 95% CI 1.25, 1.96, \( p < 0.01 \)), although the notification rate ratio adjusted by quasi-likelihood population-averaged analysis was lower (1.48, 95% CI 1.17, 1.79, \( p < 0.01 \)). These exclusions slightly increased the unadjusted and adjusted cluster-averaged notification rate ratios for overall TB. (DOCX)

Text S1 Trial protocol. (DOC)

Text S2 CONSORT checklist. (DOC)

Author Contributions

Conceived and designed the experiments: BD VS SvDH AT SC MCS AM FC. Performed the experiments: BD VS SvDH AT SC MCS AM FC. Analyzed the data: VS SvDH AT FC. Contributed reagents/materials/analysis tools: VS SvDH FC. Wrote the first draft of the manuscript: BD VS SvDH AT SC MCS AM FC. The paper: VS SvDH AT SC MCS AM FC. Wrote the paper: BD VS SvDH AT SC MCS AM FC. ICMJE criteria for authorship read and met: BD VS SvDH AT SC MCS AM FC. Agree with manuscript results and conclusions: BD VS SvDH AT SC MCS AM FC. Data manager: VS.

References

1. Raviglione M, Murali B, Floyd K, Lonnroth K, Getahun H, et al. (2012) Scaling up interventions to achieve global tuberculosis control: progress and new developments. Lancet 379: 1902–1913. doi:10.1016/S0140-6736(12)60127-2

2. Boehme CC, Nabeta P, Hilleman D, Nicol MP, Shenai S, et al. (2010) Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 363: 1005–1015. doi:10.1056/NEJMoa0907847

3. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, et al. (2011) Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 377: 1495–1505. doi:10.1016/S0140-6736(11)60438-8

4. Theron G, Zijenah L, Chanda D, Clowes P, Rachow A, et al. (2014) Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for...
tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. Lancet 3: 423–443. doi:10.1016/S0140-6736(13)62073-5
5. Steingart KR, Schiller I, Homem DJ, Pai M, Boehme CC, et al. (2014) Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev 1: CD009593. doi:10.1002/14651858.CD009593.pub3
6. Choi HW, Miele K, Dowdy D, Shah M (2013) Cost-effectiveness of Xpert® MTB/RIF for diagnosing pulmonary tuberculosis in the United States. J Int Tuberc Lung Dis 17: 1320–1325. doi:10.5588/jitld.13.0095
7. Menzies NA, Cohen T, Lin H-H, Murray M, Salomon JA (2012) Population health impact and cost-effectiveness of tuberculosis diagnosis with Xpert MTB/RIF: a dynamic simulation and economic evaluation. PLoS Med 9: e1001347. doi:10.1371/journal.pmed.1001347
8. Vassal A, van Kampen S, Sohn H, Michael JS, John KR, et al. (2011) Rapid diagnosis of tuberculosis with the Xpert MTB/RIF assay in high burden countries: a cost-effectiveness analysis. PLoS Med 8: e1001120. doi:10.1371/journal.pmed.1001120
9. World Health Organization (2012) Tuberculosis diagnostics Xpert MTB/RIF test. WHO endorsement and recommendations. Available: http://who.int/tb/featxers/factsheet_xpert_may2011 update.pdf. Accessed 9 November 2013.
10. World Health Organization (2011) Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Available: http://www.stop-tuberculosis.org/wp-content/uploads/documents/WHO%20Policy%20Statement%20on%20Xpert%20MTB-RIF%202013%20for%20publication.pdf. Accessed 13 November 2014.
11. Small PM, Pai M (2010) Tuberculosis diagnosis—time for a game change. N Engl J Med 363: 1070–1071.
12. Schunemann HJ, Oxman AD, Brozek J, Glasziou P, Jaeschke R, et al. (2008) Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. BMJ 336: 1106–1110. doi:10.1136/bmj.39500.677199.AE
13. Cochedis F, van den Ho S, Pai M, Squire SB, Ramsay A, et al. (2012) Which new diagnostics for tuberculosis, and when? J Infect Dis 205 Suppl (2): S191–S196. doi:10.1093/infdis/jis189.
14. Hanrahan GF, Scifab K, Derry CB, Dansey H, Clouse K, et al. (2013) Time to treatment and patient outcomes among TB suspects screened by a single point-of-care Xpert MTB/RIF at a primary care clinic in Johannesburg, South Africa. PLoS ONE 8: e65421. doi:10.1371/journal.pone.0065421
15. Yoon C, Cantamachii A, Davis JL, Wordersi W, den Boon S, et al. (2012) Impact of Xpert MTB/RIF testing on tuberculosis management and outcomes in hospitalized patients in Uganda. PLoS ONE 7: e68599. doi:10.1371/journal.pone.0068599
16. World Health Organization (2014) TB diagnostics and laboratory strengthening: WHO monitoring of Xpert MTB/RIF roll-out. Available: http://www.who.int/tb/laboratory/mtbrirollout/en/. Accessed 31 October 2014.
17. Zwerenstien M, Terweeck S, Gagnier JJ, Altman DG, Tunis S, et al. (2008) Improving the reporting of pragmatic trials: an extension of the CONSORT statement. BMJ 337: a2390.
18. Vassall A, van Kampen S, Sohn H, Michael JS, John KR, et al. (2011) Rapid tuberculosis mortality surveillance to identify programme errors and improve tuberculosis reporting. Int J Tuberc Lung Dis 15: 929–932.
19. Oliveira LM, Paiheiror RS (2011) Obstos e interações por tuberculose não notificados no Município do Rio de Janeiro. Rev Saude Publica 45: 31–39.
20. Hayes RJ, Moulton LH (2009) Cluster randomized trials. Boca Raton (Florida): Chapman & Hall/CRC.
21. Moulton LH, Gohb J, Durovni B, Cavalcante SC, Pacheco AG, et al. (2007) Statistical design of THRs: a phased implementation clinic-randomized study of a tuberculosis preventive therapy intervention. Clin Trials 4: 190–199. doi:10.1177/1740745070769097
22. Camargo KR Jr, Cooli CM (2000) [Reclf: an application for database linkage implementing the probabilistic record linkage method] Cad Saude Publica 16: 439–447.
23. Sistema de Informação de Tratamentos Especiais de Tuberculose (2014) SITETB. Available: http://www.sitetb.org. Accessed 13 November 2014.
24. Brazilian Ministry of Health (2013) Programa Nacional de Controle da Tuberculose (2013) Programa Nacional de Controle da Tuberculose. Available: http://www.stoptb.org/download.pdf?ua=1. Accessed 13 November 2014.
25. World Health Organization (2007) Improving the diagnosis and treatment of smear-negative pulmonary and extra-pulmonary tuberculosis among adults and adolescents: recommendations for HIV-prevalent and resource-constrained settings. Available: http://www.who.int/hiv/pub/thb/pulmonary/en/. Accessed 5 March 2014.
26. Wahuismi S, Bwanga F, De Costa A, Hale M, Joloba M, et al. (2013) Meta-analysis to compare the accuracy of GeneXpert, MODS and the WHO 2007 algorithm for diagnosis of smear-negative pulmonary tuberculosis. BMC Infect Dis 13: 507.
27. Selig L, Guedes R, Kritski A, Spector N, Lapa E Silva JR, et al. (2009) Uses of tuberculosis mortality surveillance to identify programme errors and improve tuberculosis reporting. Int J Tuberc Lung Dis 13: 507–532.
28. Oliveira LM, Filheiror RS (2011) Obstos e internações por tuberculose não notificados no Município do Rio de Janeiro. Rev Saude Publica 45: 31–39.
29. Harrles AD, Rusan IE, Chuang GY, Hinderaker E, Enarsson DA (2009) Registering initial defaulters and reporting on their treatment outcomes. Int J Tuberc Lung Dis 13: 801–803.
30. Rhode DA, Murray DM, Andridge RR, Pennell ML, Hade EM (2011) Studies with staggered starts: multiple baseline designs and group-randomized trials. Am J Public Health 101: 2161–2169. doi:10.2105/ajph.2011.300264
31. Theron G, Peter J, vans Zyll-Rui, Mishra H, Streicher E, et al. (2011) Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. Am J Respir Crit Care Med 184: 13–140. doi:10.1164/rccm.201101-0036OC.
32. Foundation for Innovative New Diagnostics (2011) Performance of Xpert MTB/RIF version G4 assay. Geneva: Foundation for Innovative New Diagnostics. Available: http://www.stop-tuberculosis.org/wp-content/assets/documents/map/findxpert-cartridge.pdf. Accessed 29 January 2014.
33. Osman M, Simpson JA, Caldwell J, Bosman M, Nicol MP (2014) GeneXpert MTB/RIF version G4 for identification of rifampin-resistant tuberculosis in a programmatic setting. J Clin Microbiol 52: 635–637. doi:10.1128/JCM.02517-13.
34. Van Deun A, Aun KM, Bola V, Lebeke R, Hossain MA, et al. (2013) Rilampid drug resistance tests for tuberculosis: challenging the gold standard. J Clin Microbiol 51: 2633–2640. doi:10.1128/JCM.00533-13.
35. Ruluf SB, Kumar P, Singh A, Pragati S, Baisoni V, et al. (2014) Comparison of Xpert MTB/RIF with line probe assay for detection of rifampin-resistant Mycobacterium tuberculosis. J Clin Microbiol 52: 1846–1852. doi:10.1128/JCM.03065-13.
36. Khaw N, Choi SM, Lee J, Park KS, Lee C-H, et al. (2013) Diagnostic accuracy and turnaround time of the Xpert MTB/RIF assay in routine clinical practice. PLoS ONE 8: e77456. doi:10.1371/journal.pone.0077456.
37. Durovni B, Sazareu V, Cordeiro-Santos M, Cavalcante SC, Soares E, et al. (2014) Operational lessons drawn from pilot implementation of Xpert MTB/Rif in Brazil. Bull World Health Organ 92: 613–617.
Editors’ Summary

Background. Tuberculosis—a contagious bacterial disease that usually infects the lungs—is a global public health problem. Each year, about 8.6 million people develop active tuberculosis and at least 1.3 million people die from the disease, mainly in resource-limited countries. Mycobacterium tuberculosis, the bacterium that causes tuberculosis, is spread in airborne droplets when people with active disease cough or sneeze. The characteristic symptoms of tuberculosis include cough, weight loss, and night sweats. Diagnostic tests for tuberculosis include sputum smear microscopy (microscopic analysis of mucus coughed up from the lungs), the growth (culture) of M. tuberculosis from sputum samples, and molecular tests (for example, the Xpert MTB/RIF test) that rapidly and accurately detect M. tuberculosis in sputum and determine its antibiotic resistance. Tuberculosis can be cured by taking several antibiotics daily for at least six months, although the emergence of multidrug-resistant tuberculosis is making the disease increasingly hard to treat.

Why Was This Study Done? Quick, accurate diagnosis of active tuberculosis is essential to reduce the global tuberculosis burden, but in most high-burden settings diagnosis relies on sputum smear analysis, which fails to identify many infected people. Mycobacterial culture correctly identifies more infected people but is slow, costly, and rarely available in resource-limited settings. In late 2010, therefore, the World Health Organization recommended the routine use of the Xpert MTB/RIF assay (Xpert) for tuberculosis diagnosis, and several resource-limited countries are currently scaling up the use of Xpert in their national tuberculosis control programs. However, although Xpert works well in ideal conditions, little is known about its performance in routine (real-life) settings. In this pragmatic stepped-wedge cluster-randomized trial, the researchers assess the impact of replacing smear microscopy with Xpert for the diagnosis of tuberculosis in Brazil, an upper-middle-income country with a high tuberculosis burden. A pragmatic trial asks whether an intervention works under real-life conditions; a stepped-wedge cluster-randomized trial sequentially and randomly rolls out an intervention to groups (clusters) of people.

What Did the Researchers Do and Find? The researchers randomly assigned 14 tuberculosis diagnosis laboratories in two cities to switch at different times from smear microscopy to Xpert for tuberculosis diagnosis. Specifically, at the start of the eight-month trial, all the laboratories used smear microscopy for tuberculosis diagnosis. At the end of each month, two laboratories switched to using Xpert, so that in the final month of the trial, all the laboratories were using Xpert. During the trial, 11,705 samples from patients with symptoms consistent with tuberculosis were examined using smear microscopy (baseline arm), and 12,522 samples were examined using Xpert (intervention arm). The researchers obtained the results of these tests from a database of all the diagnostic tests ordered in the Brazilian public laboratory system, and they obtained data on tuberculosis notifications during the trial period from the national notification system.

In total, 9.7% and 14.2% of the tests in the baseline and intervention arm, respectively, were positive, and the laboratory-confirmed tuberculosis notification rate was 1.59 times higher in the Xpert arm than in the smear microscopy arm. However, the overall notification rate (which included people who began treatment on the basis of symptoms alone) did not increase during the trial. The time to treatment (the time between the laboratory test date and the notification date, when treatment usually starts in Brazil) was about 11 days and eight days in the smear microscopy and Xpert arms, respectively.

What Do These Findings Mean? The findings indicate that, in a setting where laboratory diagnosis for tuberculosis was largely restricted to sputum smear examination, the implementation of Xpert increased the rates of laboratory-confirmed pulmonary (lung) tuberculosis notifications and reduced the time to treatment initiation, two endpoints of public health relevance. However, implementation of Xpert did not increase the overall notification rate of pulmonary tuberculosis (probably because of the high rate of empiric tuberculosis treatment in Brazil), although it did facilitate accurate and rapid detection of rifampicin resistance. The accuracy of these findings may be limited by certain aspects of the trial design, and further studies are needed to evaluate the possible effects of Xpert beyond diagnosis and the time to treatment initiation. Nevertheless, these findings suggest that replacing smear microscopy with Xpert has the potential to increase the confirmation (but not detection) of pulmonary tuberculosis and to reduce the time to treatment initiation at the population level in Brazil and other resource-limited countries.

Additional Information. Please access these websites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.1001766.

- The World Health Organization (WHO) provides information (in several languages) on tuberculosis, on tuberculosis diagnostics, and on the rollout of Xpert; further information about WHO’s endorsement of Xpert is included in a Strategic and Technical Advisory Group for Tuberculosis report; the “Global Tuberculosis Report 2013” provides information about tuberculosis around the world, including Brazil
- The Stop TB Partnership is working towards tuberculosis elimination and provides patient stories about tuberculosis (in English and Spanish); the Tuberculosis Vaccine Initiative (a not-for-profit organization) also provides personal stories about tuberculosis
- The US Centers for Disease Control and Prevention provides information about tuberculosis and its diagnosis (in English and Spanish)
- The US National Institute of Allergy and Infectious Diseases also has detailed information on all aspects of tuberculosis
- More information about this trial is available