High positive predictive value of Xpert in a low rifampicin resistance prevalence setting

To the Editor:

Although drug susceptible tuberculosis (TB) is a curable disease, spread of rifampicin-resistant TB, including multidrug-resistant (MDR)- and extensively drug-resistant (XDR)-TB, is a threat to disease control worldwide [1], in particular in Eastern Europe [2]. XpertMTB/RIF (Xpert; Cepheid Inc., Sunnyvale, CA, USA) is an accurate [3] and affordable [4] automated PCR-based assay that detects DNA of *Mycobacterium tuberculosis* and resistance to rifampicin in <2 h [5].

While the World Health Organization (WHO) has endorsed the use of Xpert for TB diagnosis as a replacement for sputum smear examination [6], concerns have been raised about the low positive predictive value (PPV) of rifampicin-resistant Xpert results in patients who have never previously been treated for TB and in countries with a low prevalence of rifampicin-resistance and MDR-TB [5]. WHO guidelines currently call for initiation of MDR-TB treatment with second-line drugs when resistance is repeatedly detected by Xpert, while confirmatory testing is performed [6].

Xpert detects mutations in the *M. tuberculosis rpoB* gene, which is responsible for >95% of rifampicin resistance, with a well-established 98% specificity [3, 7]. Compared with the earlier version [3, 7], the latest generation (G4) assay has a reduced risk of false positive results for rifampicin resistance [8]. The probe B sequence was modified so that the probe/wild-type target hybrid remains stable at elevated annealing temperatures, further improving specificity to 99.8% under research conditions [8].

However, the PPV of Xpert for rifampicin and multidrug resistance during programmatic use in low MDR-TB prevalence countries has not been widely explored; estimates are based on prevalence and specificity [5]. A recent report on the use of G4 Xpert assays under programmatic conditions in South Africa showed an overall 99.5% PPV for rifampicin resistance compared with resistance mutations detected by line-probe assay (MTBDRplus; Hain Lifescience, Nehren, Germany) in smear-positive specimens, and compared with phenotypic drug susceptibility testing (DST) in smear-negative specimens [9]. However, drug resistance levels and PPV were not established separately for new and retreatment patients.

We aimed to evaluate the PPV of Xpert under programmatic conditions for new and retreatment TB patients in Brazil, a country with a low prevalence of rifampicin resistance [10]. As part of a pilot implementation study in 14 primary care laboratories in Rio de Janeiro and Manaus, 30 000 G4 Xpert tests were performed under routine conditions on sputum specimens from patients for whom diagnostic sputum examination was requested. The first 15 000 tests were used as part of a laboratory-randomised trial of TB case detection [11]. The national TB programme recommendations during the study were to refer patients with a positive Xpert rifampicin resistance signal to one of three designated MDR-TB centres for culture and phenotypic DST and start them on standard first-line drugs (FLD) treatment while awaiting the results. The MDR-TB centres used solid (Löwenstein–Jensen or Ogawa) or liquid (Mycobacteria Growth Indicator Tube (MGIT)) media for culture, and proportional or automated methods for phenotypic DST, according to their routine practice.

Information on cases of rifampicin resistance identified in the Xpert systems records were gathered from the MDR-TB centres, the national TB-reporting database (SINAN) and the national drug-resistant TB information system (SITETB). The PPV of Xpert for rifampicin resistance and for MDR-TB was calculated using phenotypic DST as the reference standard.

The study was approved by the National Ethics Board (CONEP, approval number 494/2011), the Rio de Janeiro Municipal Health Department Review Board (CEP SMS, approval number 236/11) and the Tropical Medicine Foundation of Manaus Review Board (CEP FMT/HVD, November 24, 2011).

During the study, 150 patients were identified with a positive rifampicin resistance Xpert signal, of whom 55 (37%) had been previously treated, 94 (63%) were new cases and one had no information regarding treatment history. Cultures were obtained for 139 patients (93%), of which 19 remained negative (11 (12%) among new cases and eight (15%) among retreatment cases) and three were contaminated. In nine out of

the 19 patients the negative results were from cultures requested more than 7 days after treatment initiation. For the 117 positive cultures 102 phenotypic DST results were obtained: six isolates had insufficient material and nine results were missing.

Overall, 92 out of 102 had rifampicin resistance confirmed by phenotypic DST, giving a PPV of 90.2% (95% CI 82.7–95.2%). Among new cases, the PPV for rifampicin resistance was 90.6% (95% CI 80.7–96.5%) and among retreatment cases, 89.5% (95% CI 75.2–97.1%) (table 1). For multidrug resistance, the PPVs were 82.8% (95% CI 71.3–91.1%) and 81.6% (95% CI 65.7–92.3%), respectively. The PPV tended to be higher with MGIT (46 out of 53 or 94.7%; 95% CI 73.9–99.9%) and Ogawa (18 out of 19 or 93.3%; 95% CI 77.9–98.2%) when compared to Löwenstein–Jensen media (28 out of 30 or 86.6%; 95% CI 74.7–94.5%), but numbers are too small to enable a rigorous comparison.

Among the 10 rifampicin-susceptible cases, other drug-resistance was present in six cases, including one case of resistance to pyrazinamide and another to isoniazid, ethambutol and streptomycin. Four isolates were fully susceptible to all FLD (table 1).

Because of the current national recommendations to await phenotypic culture results, the median (interquartile range) delay from Xpert result to initiation of second-line drug treatment was 143 (89–206) days: 133 (87–188) days among new cases and 151 (83–221) days for retreatment cases.

Our findings suggest that the excellent specificity of the Xpert G4 assay previously suggested in the research context [8] is retained in routine practice. Assuming 95% sensitivity and 3–4% prevalence of rifampicin resistance, the PPV for 99.8% specificity would be 94–95%. Both we and others [9, 12] have found a PPV of around 90% in real-world studies in settings with such prevalence. However, the true PPV of Xpert may be even higher: the finding of resistance profiles that are rare in rifampicin-susceptible strains (pyrazinamide monoresistance or combined resistance to three FLD) in our study suggests that phenotypic DST may have shown false-negative rifampicin results in these cases and thereby underestimated the PPV of Xpert. Indeed, false-negative results for rifampicin resistance in phenotypic DST have been reported using Löwenstein–Jensen medium [13]. Our study was not powered to show differences in PPV by culture media. Regardless of the technique employed, genotypic rifampicin resistance not detected phenotypically may have clinical and epidemiological relevance [14].

Our results have limitations. First, culture and phenotypic DST could not be performed for all Xpert rifampicin-resistant samples. This points out the fragility of the programmatic laboratory system, discussed elsewhere [11]. The missing DST data likely occurred at random, possibly with the exception of the negative cultures for patients under treatment, which may point to susceptibility to rifampicin and/or isoniazid. Under the extreme assumption that all 19 patients with negative cultures carried rifampicin-susceptible bacilli, the PPV would reduce to 76.0% (92 out of 121). In addition, no claims can be made with regard to the specificity and negative predictive value of Xpert for rifampicin resistance because only samples with a positive Xpert result were cultured.

The main strength of this study is its highly pragmatic design, thus reflecting findings from true programmatic conditions. In addition, we could link results to the history of treatment using the information system. In the South African study [9], the PPV could not be stratified by TB treatment history as treatment history was not retrieved for all Xpert-confirmed TB patients. Our findings suggest that, even

TABLE 1 DST results (positive predictive value for resistance) according to previous tuberculosis treatment history among 102 patients with a positive Xpert MTB/RIF resistance signal and available DST results

	New cases	Retreatment	Overall
Subjects n	64	38	102
Rifampicin resistance confirmed	58 (90.6)	34 (89.5)	92 (90.2)
Rifampicin + isoniazid (MDR)[#]	53 (82.8)	31 (81.6)	84 (82.4)
Rifampicin + other (not isoniazid)	1 (1.6)	1 (2.6)	2 (1.9)
Rifampicin only	4 (6.3)	2 (5.3)	6 (5.9)
Rifampicin-susceptible	6 (9.4)	4 (10.5)	10 (9.8)
Other (not rifampicin) drugs	3 (4.7)	3 (7.9)	6 (5.9)
Fully susceptible	3 (4.7)	1 (2.6)	4 (3.9)

Data are presented as n (%), unless otherwise stated. DST: drug susceptibility test; MDR: multidrug resistant. [#]: with or without resistance to additional first-line drugs.

among new cases, Xpert has a very high PPV for rifampicin resistance. This has relevant programmatic implications for policy recommendations in Brazil and other countries with low TB resistance rates. Our findings suggest that in case of a G4 Xpert (the only commercially available version since 2011) rifampicin resistance signal, even among previously untreated TB patients, second-line drug treatment could be started immediately and, where necessary, the regimen adapted after DST results are available. Optimisation of accuracy for rifampicin resistance detection and assays for rapid detection of resistance to other drugs are needed, however, if appropriate treatment regimens are to be reliably based on molecular-based tests. Rapid detection and treatment of MDR- and XDR-TB are essential steps to achieve the Millennium Development Goals by 2050, which include TB elimination [2, 15].

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High predictive value of Xpert for rifampicin resistance even in new TB cases from a low MDR-TB prevalence setting http://ow.ly/A1mUa

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