Reply to “Molecular Diagnosis of Rifampin-Monoresistant Tuberculosis in Indian Patients: Problems with a Discordance Analysis”

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We read with interest the remarks made by Dr. Alland and colleagues (1), in response to our recent observations published in the *Journal of Clinical Microbiology* (2). We would like to offer responses to their comments below. Dr. Alland and colleagues consider that our findings favored the line probe assay (LPA) over the Xpert MTB/RIF assay because according to them (i) our findings are different from what they expect and (ii) we did not include all 405 samples in drug susceptibility testing, which should have been tested first by the gold standard MGIT960 liquid culture and drug susceptibility test (DST) and then the Xpert MTB/RIF test. We would like to put on record that in this study our primary aim was not to compare Hain’s LPA with Xpert MTB/RIF on sputum samples. Indeed, the study was a part of the Programmatic Management of Drug-Resistant Tuberculosis (PMDT), which is a national tuberculosis (TB) control program (3). According to the PMDT guidelines, all sputum samples from patients not responding to antitubercular treatment and suspected to have developed drug-resistant tuberculosis are first tested by smear examination, and smear-positive samples are subjected to Hain’s LPA. The smear-negative samples are cultured, and if culture becomes positive, these cultures are subjected to LPA. In fact, when we got very high (22.2%) rates of positivity of rifampin (RIF) monoresistance in these samples, we started suspecting the LPA results. Our obvious concern was whether this high RIF monoresistance had something to do with the geographical area or was due to the special category of patients whom we were treating, but the most important question before us was the quality of results that we were returning. Therefore, first we concentrated our efforts on validating the LPA results, especially the RIF monoresistance. Since these samples were RIF monoresistant, we decided that Xpert MTB/RIF could give us the answer fastest as it detects only rifampin resistance. We tested all rifampin-monoresistant samples and statistically calculated the number of RIF-sensitive samples as a control for testing by Xpert MTB/RIF. To our surprise, there was a gross discrepancy between the two test methods. Therefore, we had to go to the gold standard (MGIT960 culture DST system) to resolve the discrepant results. We were not satisfied with that only, so we further subjected these isolates to sequencing of the 81-bp region of the rpoB gene. The study design is given in the flow chart of the original publication (2). Hence, it is absolutely incorrect to assume that our results were biased toward one test system.

We might agree with the concern raised by Alland et al. to some extent that this study would not have detected isolates that were RIF resistant if tested first by Xpert MTB/RIF but RIF sensitive by LPA. However, Alland et al. have ignored the data presented in the paper which show that 5.1% of samples reported as RIF sensitive by LPA were found resistant by Xpert MTB/RIF. These data can be extrapolated to answer their concern, if these discrepant results are considered truly resistant (taking Xpert MTB/RIF as the standard) and falsely sensitive by LPA, irrespective of which test was done first. We would argue that this rate (5.1%) of disagreement in misidentifying the RIF resistance by LPA was indeed exactly the same (6%; 95% confidence interval [CI], 3% to 13%) as that summarized in the recently published Cochrane review (4). With the clarification offered above, it is clear that our aim was to see what percentage of RIF-monoresistant results given by LPA had concordance with Xpert MTB/RIF results. This algorithm was followed as a real-life situation. As of now, LPA is the primary test in all TB laboratories under the national tuberculosis control programs of most countries to detect drug resistance under PMDT services (2, 3, 5), while Xpert MTB/RIF is yet to be rolled out in the national TB control programs. Alland et al. cannot deny that if Xpert MTB/RIF is rolled out in all primary care TB laboratories, we might be missing a large number of RIF monoresistance cases, if the resistance results are not verified by other methods. We feel that our conclusions would not have differed even if this study was done simultaneously on Xpert MTB/RIF and LPA after culture DST, as suggested by Alland et al. Moreover, to do that, thousands of samples would have been required to find these many monoresistant and heteroresistant *M. tuberculosis* isolates. However, as suggested by Alland et al., another study may be performed on a large number of samples, under the TB control program, targeting all the suggested criteria.

High drug resistance has been reported in Beijing strains compared to Central Asian (CAS) strains of *M. tuberculosis* (6); therefore, the 22 isolates (1 became contaminated) for which sequences are published in our paper were characterized by spoligotyping and mycobacterial interspersed repetitive-unit–variable-number tandem-repeat (MIRU-VNTR) analysis. However, we did not find any Beijing strains in these samples. Of the 22 isolates, 16 were CAS (14 of CAS-1_Delhi [SIT 26] and 2 of SIT 846), 5 were Orphan (SIT 27), and 1 belonged to the MANU2 (SIT 1976) genotype.

The sputum samples included in this study were received from eight districts of Punjab, which is a northwestern state of India. All were relapse cases on category II treatment or on category I treatment for more than 2 months. Details of these patients and plau-
sible reasons for the high rates of RIF mono- and heteroresistance have already been reported by us (3). It seems that Alland et al. have not read that publication. This paper clearly showed several differences in Delhi and Punjab strains, including significantly high RIF mono- and heteroresistance in Punjab samples compared to Delhi samples. We also reported that the mutation rate at codons 530 to 533 in RIF-monoresistant strains from Delhi was very low in comparison to the very high mutation rate (43.2%) at these codons in samples from the Punjab region. If this has some relation to the low RIF resistance detection by Xpert MTB/RIF, we need to study that. We suspect that the high resistance rate in Punjab samples, besides being due to samples coming from patients already on category II treatment and from treatment failure cases, could be related to the high incidence of RIF-heteroresistant \textit{M. tuberculosis} populations, which might reflect ongoing transmission of these strains at the community level (7).

Regarding the reason for excluding 72 multidrug-resistant TB (MDR-TB) samples, it was simply because Xpert MTB/RIF does not detect isoniazid (INH) resistance. Hence, we did not want to complicate the results and delay the study. We also think that it would have not served our purpose. Otherwise, it has been made amply clear in several studies that Xpert MTB/RIF has lower sensitivity (80 to 90%) than does phenotypic DST (8) and the performance goes down further if the sample has mixed (sensitive and resistant) populations of \textit{M. tuberculosis} (9). These authors report that MGIT liquid culture DST is highly sensitive in that it is able to detect the presence of even a 1% RIF-heteroresistant population in a mixed population in a sample, and LPA can detect mutations in \textit{rpoB} in as low as a 5% heteroresistant population while sequencing detects resistance only if 50% of the population is resistant. In the case of Xpert MTB/RIF, it is previously documented that for the 531 TTG mutation and the 533 CCG mutation, the assay can detect the presence of resistance with 95% certainty only when the mixture has 65.6% 531 TTG DNA and 100% 533 CCG mutated DNA. These findings support our findings (10). Other workers have also reported that Xpert MTB/RIF has wide variability (60 to 100%) in detecting RIF resistance (cited by 7 articles). The company (11) or its representatives (12) have been raising concerns about recent research publications that do not favor Xpert MTB/RIF, but these concerns are being answered well by unbiased research such as that recently published by us as well as by others (13).

REFERENCES

11. Mudur G. 2014. Manufacturer stands by Xpert tuberculosis test after India study questions its reliability. BMJ 348:g3338. http://dx.doi.org/10.1136/bmj.g3338.