Editorial

The role of line probe assays in the Xpert MTB/RIF Ultra era

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Comment on: Nathavitharana RR, Cudahy PG, Schumacher SG, *et al.* Accuracy of line probe assays for the diagnosis of pulmonary and multidrug-resistant tuberculosis: a systematic review and meta-analysis. Eur Respir J 2017;49(1). pii: 1601075.

Received: 15 May 2017; Accepted: 30 May 2017; Published: 22 June 2017.

doi: 10.21037/jlpm.2017.06.01

View this article at: http://dx.doi.org/10.21037/jlpm.2017.06.01

Tuberculosis (TB) remains an important public health problem with an estimated 10.4 million new cases diagnosed in 2015 (1). Of these, approximately 4.3 million cases went undiagnosed or unreported, posing a major hurdle to the eradication of TB (1). The control of TB is further threatened by the HIV epidemic and the emergence and spread of multidrug-resistant (MDR) TB, defined as resistance to rifampicin and isoniazid. In 2015, there were an estimated 480,000 new cases of MDR-TB and an additional 100,000 people were diagnosed with rifampicin-resistant TB. These cases result in continued transmission in communities and health care settings due to the detection and treatment gap, with only 1 million cases (30% of the 3.4 million bacteriologically confirmed cases or 10% of all cases globally) having had a drug susceptibility test for rifampicin in 2015. The WHO has therefore included early diagnosis of TB and universal drug susceptibility testing as one of its core priorities for global TB control (2).

In the past decade, the world has experienced exciting developments in the field of TB diagnostics, resulting in the first major breakthrough in TB diagnostics in the past 100 years. This in turn has resulted in an increase in industry interest, with more than 50 diagnostic companies and assay developers currently engaged in TB technologies (3). In 2008, the WHO endorsed the use of molecular line probe assays for the detection of resistance to rifampicin and isoniazid in patients with smear positive or culture positive TB (4). In 2011, the WHO recommended replacement of smear microscopy by the Xpert® MTB/RIF (Xpert) assay (Cepheid, Sunnyvale, USA), a molecular test that allows rapid diagnosis of *Mycobacterium tuberculosis* complex and simultaneous detection of resistance to rifampicin (5). As of 31 December 2016, over 23 million Xpert cartridges have been procured in the public sector of 130 countries eligible for concessional pricing (6). Since March 2017, the next-generation Xpert® Ultra assay was introduced to overcome the imperfect sensitivity in smear-negative, pediatric and HIV-associated TB of the first generation Xpert assay, and to correct some of its limitations in the identification of rifampicin resistance (7). Overall, sensitivity of the Xpert Ultra assay was 5% (95% CI: +2.7, +7.8) higher than that of the first generation Xpert. Sensitivity increases were the highest among smear-negative culture-positive patients (+17%, 95% CI: 10, 25).

Currently, the WHO recommends that all individuals presenting with symptoms or signs of TB should be screened with Xpert (Ultra) and that all individuals diagnosed with rifampicin resistant TB initiate an empiric MDR-TB treatment regimen (8). Treatment should subsequently be optimized following confirmatory testing for rifampicin resistance and drug susceptibility testing for isoniazid and second-line anti-TB drugs (8). Given the rapid changes in the TB diagnostic arena, it is unclear what the role of the different TB diagnostics is, especially with regards to the line probe assays and culture-based drug susceptibility assays.

In 2017, Nathavitharana *et al.* published a systematic review and meta-analysis of the performance of the line probe assays Genotype MTBDR*plus*V1, Genotype

MTBDR*plus*V2 and Nipro NTM+MDRTB (9). They found an overall good performance for the detection of resistance to rifampicin, with a pooled sensitivity of 96.7% and a pooled specificity of 98.8%. The performance for detection of isoniazid resistance was also good, with high pooled specificity (99.2%) but somewhat lower pooled specificity (90.2%). This was likely due to mutations outside of the probe hotspots or other mechanisms of isoniazid resistance. The pooled sensitivity for detection of *M. tuberculosis* was high (94%) when assays were done on smear positive specimens, but disappointingly low (44%) when the assays were performed on smear negative specimens. The number of studies that included smear negative specimens was however limited and there was substantial heterogeneity between studies. Overall, the authors concluded that line probe assays could play "an adjunctive role for the appropriate early management of MDR-TB"; however, what this role entails was not made explicit. The question where line probe assays fit into the current TB diagnostic algorithm therefore remains unclear.

Xpert Ultra is currently the most sensitive, rapid and simple tool for diagnosis of rifampicin resistant TB and is therefore recommended by WHO as the initial test for assessment of TB in all individuals with presumptive TB. Line probe assays take longer to perform and, due to their technical complexity, can only be executed at reference or regional laboratories. Line probe assays can thus only play an adjunctive role in the current TB diagnostic landscape. One potential adjunctive role is confirmation of rifampicin resistance detected by Xpert (Ultra). Confirmation of rifampicin resistance is important to avoid unnecessary treatment with a longer and more toxic regimen in cases of administrative error or in the presence of a "silent" mutation in the *rpoB* region. The advantage of a repeat Xpert test is the simplicity and speed of the assay. The use of a line probe assay for confirmation of rifampicin resistance would have a much longer turn-around time as it requires transportation to a centralized laboratory. Repeating a test using the same assay addresses administrative errors, but does not address other causes of false positive results. Whether using a line probe assay can overcome this problem is unclear as both tests (Xpert and line probe assays) are molecular assays based on the detection of mutations in the 81 bp rifampicin resistance determining region of the *rpoB* gene. Studies have reported opposing results between line probe and Xpert with regards to the identification of rifampicin resistance when either culture-based phenotypic drug susceptibility tests or sequencing was used as a reference standard (10). While this may point to erroneous calling of drug resistance by one of the two tests, it can result in confusion in clinical practice as neither test is considered a gold standard. The advantage of the use of a line probe assay could lie in the ability to simultaneously detect resistance to isoniazid. Knowledge on the presence of *inhA* promoter or *katG* mutations could help guide treatment, albeit only partially as further tests would be needed to determine the optimally effective regimen for each individual patient. Fortunately, the same crude DNA extract could be used for the detection of second-line resistance using the Genotype MTBDR*sl* line probe assay. While awaiting these results, the presence of an *inhA* promoter mutation would suggest the usefulness of the inclusion of high-dose isoniazid whereas the efficacy of high-dose isoniazid in patients with *katG* mutant strains is uncertain. Furthermore, strains with *inhA* promoter mutations are typically resistant to ethionamide (and prothionamide) (11), one of the seven drugs (kanamycin, high-dose moxifloxacin, prothionamide (or ethionamide), clofazimine, high-dose INH, pyrazinamide and ethambutol) included in the initial phase of the shorter MDR-TB regimen recommended by WHO in May 2016 (12). Another alternative for confirmation of rifampicin resistance detected by an initial Xpert (Ultra) assay is culture-based phenotypic drug susceptibility. The advantage of a phenotypic culture-based would lie in its ability to detect resistance independent of the underlying resistance mechanism and the potential to use the same culture to subsequently test for other first and second line drugs. Clear disadvantages remain, including slower turn-around time, the technical infrastructure needs of a centralized laboratory, and frequent contamination of liquid cultures. To date, no study has compared the clinical usefulness of a repeat Xpert (Ultra) assay versus a line probe assay or culture-based drug susceptibility assay for confirmation of rifampicin resistance detected by Xpert (Ultra).

Another potential role of line probe assays is the detection of isoniazid resistance in patients diagnosed with rifampicin sensitive TB who respond poorly to standard first-line treatment. Multiple studies have shown poor treatment outcomes for isoniazid mono-resistant cases when treated with the standardised TB treatment regimen (13-15). Furthermore, individuals with isoniazid mono-resistant TB treated with a standard first line treatment regimen are at increased risk to progress to MDR-TB. It has been

suggested that unrecognized isoniazid resistance might contribute to the emergence of MDR-TB in settings with high prevalence of isoniazid resistance (15). To date, no studies have assessed the value of performing a line probe assay in individuals who respond poorly while receiving treatment for rifampicin sensitive TB diagnosed by Xpert (Ultra).

In conclusion, while line probe assays have good performance for detection of rifampicin and isoniazid resistance in smear positive sputum samples and culture isolates, more research is needed to determine their role in TB diagnostics algorithms in the Xpert Ultra era.

Acknowledgments

Funding: M De Vos and A Van Rie are supported by funding from the Fonds voor Wetenschappelijk Onderzoek-Research Foundation Flanders (G0F8316N). M De Vos is supported by the United States National Institute of Health (R01AI099532); A Van Rie by the United States National Institute of Health (R01 AI099026).

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by Section Editor Maoshui Wang (Department of Lab Medicine, Shandong Provincial Chest Hospital, Jinan, China).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2017.06.01). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/jlpm.2017.06.01
Cite this article as: Van Rie A, De Vos M. The role of line probe assays in the Xpert MTB/RIF Ultra era. J Lab Precis Med 2017;2:32.

