New Xpert MTB/XDR: added value and future in the field

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Xpert MTB/XDR is a new rapid molecular test for drug resistant tuberculosis [1].


Introduction
The recent launch of the new Xpert MTB/XDR assay (Cepheid Inc., Sunnyvale, CA, USA) by the Foundation for Innovative New Diagnostics [1] was widely welcomed by the tuberculosis (TB) community, given the critical need for new and better diagnostics to guide the treatment of drug resistant tuberculosis (DR-TB). Xpert MTB/XDR detects resistance to isoniazid (target genes: inhA promoter, katG, fabG1, oxyR-aphC intergenic region), ethionamide (inhA promoter), fluoroquinolones (gyrA and gyrB), and second-line injectables (rrs and eis promoter) and is positioned as an add-on or "reflex test" in patients with rifampicin resistance detected by Xpert MTB/RIF or Ultra [2, 3].

The spread of DR-TB strains threatens recent gains in global TB control [4], with evidence that the majority of patients with rifampicin resistant (RR-TB) or multi-drug resistant (MDR-TB) TB acquire their infection through person-to-person transmission. Inadequate diagnostic and treatment options have hampered an effective global response. The use of Xpert MTB/RIF as a rapid and sensitive frontline TB detection test has been shown to improve patient outcomes and is cost effective [5], but data for RR/MDR-TB are lacking; partly hampered by the poor treatment options available in the past. We provide a brief overview of the perceived benefits, limitations and remaining challenges with the new test (table 1).

Benefits/added value
Existing Xpert MTB/RIF and Ultra assays only detect rifampicin resistance, which prevents RR/MDR-TB differentiation and provides no further treatment guidance. Expanded drug susceptibility testing (DST) is required to guide optimised RR/MDR-TB treatment, which is essential to improve patient outcome, reduce the duration of infectiousness and limit the risk of drug resistance amplification. Drug resistance amplification is a particular concern with the promotion of new bedaquiline containing all-oral short-course regimens [6]. Culture and phenotypic DST is the current gold standard, but is rarely available in TB endemic settings. In the absence of phenotypic DST, molecular techniques such as line probe assays (e.g. MTBDRsl) are recommended. However, these assays have poor sensitivity in sputum smear negative patients and require specialised infrastructure that is not available outside well-equipped central laboratories [7].
A key benefit of the Xpert MTB/XDR assay is that it requires the same sample processing steps as Xpert MTB/RIF and Ultra, which have demonstrated robustness in field conditions. The familiarity, portability and user-friendly format of the Xpert MTB/XDR assay makes it attractive for decentralisation. It also has minimal biosafety requirements, offers rapid turn-around times and can be operated by health personnel with limited training. Results are available in less than 90 min, compared to several weeks with traditional culture-based DST or several days with line probe assays performed at a central laboratory level.

Rapid fluoroquinolone resistance determination is critical, given its pivotal role in RR/MDR-TB treatment and importance in protecting companion second-line drugs like bedaquiline [8, 9]. The 2020 World Health Organization (WHO) recommendations for the treatment of RR/MDR-TB recognise the key contribution of later generation fluoroquinolones in shorter duration all-oral regimens [6]. In initial evaluations, Xpert MTB/ XDR was able to identify fluoroquinolone resistance with 91.4% sensitivity and 98.5% specificity compared to phenotypic DST [3]. This is close to WHO targets for diagnostic sensitivity (>95%) and specificity (>98%) of rapid DST [10]. However, the assays’ diminished ability to detect mutations (S91P/A90V, D94A) causing low level fluoroquinolone resistance, especially in hetero-resistant strain populations [3], is a concern and highlights the need for further careful monitoring of discrepant geno/phenotypic DST results in different settings.

**Limitations/challenges**

Apart from cartridge costs, the new assay requires GeneXpert instrument modules with 10 colour multiplex technology, hence the current Xpert instruments will require major refitting and recalibration. This presents a significant challenge, particularly to low- and middle-income countries that may need to shoulder additional shipping and servicing expenses. Although the assay is attractive for decentralisation,
its optimal placement in different settings needs to be guided by local diagnostic algorithms, as well as local feasibility and cost-effectiveness. Poor infrastructure, cost and logistical constraints will limit point-of-care placement in most high burden countries [5]. Another imperative to consider is the ability to provide adequate RR/MDR-TB treatment in all settings with diagnostic access [11]. In recent years, MDR-TB programme up-scaling and increased access to high quality second-line TB drugs through the Global Drug Facility successfully narrowed the RR/MDR-TB diagnosis–treatment gap resulting from Xpert MTB/RIF roll-out.

Isoniazid resistance is a key precursor to MDR-TB [12, 13], which indicates the need for improved detection and appropriate treatment of non-MDR isoniazid resistant strains that are not detected by Xpert MTB/RIF or Ultra. Few countries have laboratory capacity to assess isoniazid resistance [14], which the new assay has excellent capacity to detect; sensitivity 98.3% and specificity 95% compared to phenotypic DST [3]. This is important given that WHO recommends a modified treatment regimen for these patients to improve treatment outcomes and to limit MDR-TB generation [6]. However, with placement as a “reflex test” this ability will not be utilised and it would have been of greater value if isoniazid and rifampicin resistance testing was combined in a frontline diagnostic. It should be noted that isoniazid resistance (including high-level resistance) can be conferred by mutations in genes not targeted by the Xpert MTB/XDR, such as \( \text{aphC}, \text{ndh}, \text{mshA} \) and \( \text{mymA} \). These mutations are uncommon, but their geographic distribution has not been assessed. Furthermore, their frequency may increase with diagnostic selection, a phenomena observed in Swaziland where rifampicin resistance mediated by mutation outside the resistance hotspot has become common [15].

Ethionamide has chemical similarity to isoniazid and shares a final common pathway targeting mycolic acid biosynthesis. Ethionamide resistance is evaluated through identification of mutations in the \( \text{inhA} \) promoter region known to confer co-resistance to isoniazid (low level) and ethionamide (high level) [16, 17]. However, there are additional genes (\( \text{ethA}, \text{ethR}, \text{ndh} \) and \( \text{mshA} \)) and unknown mechanisms that also confer resistance [18]. Since phenotypic ethionamide DST is lacking in most settings [19], genotypic approaches are valuable for identification of ethionamide resistance. Although new WHO guidelines classify ethionamide as a group C drug that is only considered when newer/re-purposed agents are not available [6], it remains a potent agent to consider and knowledge of \( \text{inhA} \) promoter mutations also guide potential use of high dose isoniazid [20].

Given evidence for improved treatment outcomes and reduced adverse effects, new RR/MDR-TB treatment guidelines prioritise the use of oral agents (e.g. bedaquiline, linezolid, clofazimine, cycloserine) [6, 21]. Therefore, the detection of resistance to injectable agents has become less relevant. However, knowledge of susceptibility to injectable drugs remains useful in instances where an effective all-oral treatment regimen cannot be assembled and if adequate measures to monitor adverse effects are in place [22]. With positioning as a “reflex test”, minimal sample volume to permit follow-on testing and conditions for sample storage will have to be critically evaluated to streamline logistics and maintain good performance.

Future in the field
As with the implementation of Xpert MTB/RIF and Ultra, future studies should assess the performance of Xpert MTB/XDR in different geographical settings and with different clinical specimens. This is particularly important in people living with HIV and in children, in whom extra-pulmonary and paucibacillary disease are more common [23, 24]. Samples such as fine needle aspiration biopsies may deliver excellent yields, as for Xpert MTB/RIF [25], while reduced sensitivity is expected in cerebrospinal fluid, although a positive test will have major clinical significance [26]. The new assay demonstrated comparable sensitivity to Xpert MTB/RIF in pulmonary samples, but its performance using extra-pulmonary samples has not been evaluated [3, 27]. The limit of detection for \( \text{Mycobacterium tuberculosis} \) by Xpert MTB/XDR (71.9 CFU·mL\(^{-1}\)) is comparable to MTB/RIF (86.9 CFU·mL\(^{-1}\)), but not as low as Ultra (15.6 CFU·mL\(^{-1}\)). How to deal with Xpert Ultra “trace” calls will have to be discussed by an independent WHO-convened guideline development group, who will consider different scenarios for incorporating the new assay into existing diagnostic algorithms.

As WHO looks to expand the capacity for detection of DR-TB, new rapid molecular diagnostic technologies are critical to improve TB control and progress towards ultimate TB elimination [28]. The new assay will improve rapid diagnosis of patients with second-line drug resistance, enabling early initiation of appropriate treatment and optimal person-centred care. However, the assay only targets a limited number of resistance variants recognised as “hot spots” in particular target genes, which may lead to variable sensitivity in different settings. The detection of isoniazid resistance should ideally be included with a front-line TB detection test, not as an add-on after rifampicin resistance has been detected. The main value of the assay is the rapid detection of fluoroquinolone resistance to guide all-oral MDR-TB treatment, but a caveat is the fact that it does not detect resistance against newer/re-purposed drugs.
included in the regimen. Routine whole genome sequencing (WGS) overcomes this shortcoming by interrogating the entire genetic repertoire, but this is currently only feasible in high resource settings and a full understanding of the genetic determinants of resistance against newer/re-purposed drugs is lacking. Moreover, in most instances WGS still requires initial culture of the specimen, which introduces potential strain biases and critical delays in patient management.

A key benefit of the Xpert MTB/XDR assay is the robustness of the Xpert platform, its minimal biosafety requirements and the fact that TB programmes are already familiar with its use. Its launch is timely, given the urgent need for rapid fluoroquinolone resistance determination to guide the use of new all-oral short-course RR/MDR-TB regimens, and especially to protect bedaquiline against the rapid emergence of drug resistance. However, strategies that integrate the new assay into existing algorithms require careful consideration of how to extract optimal value and establish appropriate monitoring processes in a variety of settings. While molecular targets chosen by the manufacturers several years prior may no longer be as contemporary as desired, this new assay successfully demonstrates rapid multiplex technology suitable for near patient testing that is potentially amenable to inclusion of alternate target mutations.

Conflict of interest: None declared.

References


