

Using Xpert MTB/RIF

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Abstract: Xpert MTB/RIF is an automated real-time polymerase chain reaction test for simultaneous detection of tuberculosis and rifampicin resistance. Xpert MTB/RIF has demonstrated excellent accuracy in clinical evaluation studies, but has reduced sensitivity for detection of smear-negative tuberculosis. Since sample processing and detection are largely automated, Xpert MTB/RIF is potentially suitable for implementation in resource-limited settings. There are, however, a number of practical constraints to the use of Xpert at the point-of-care. Xpert remains a relatively costly test, and clear demonstration of cost-effectiveness will be needed to support efforts to scale up testing in high burden countries.

Keywords: Cost-effectiveness, detection, diagnosis, rifampicin, tuberculosis, Xpert MTB/RIF.

INTRODUCTION

Xpert MTB/RIF (Xpert) (Cepheid, Inc., Sunnyvale, CA, USA) is an automated, real-time PCR test incorporating integrated sample processing, for detection of tuberculosis as well as resistance to rifampicin. In 2010 the World Health Organization endorsed Xpert as a replacement test for smear microscopy in patients suspected of HIV-associated TB or multi-drug resistant TB (MDR-TB) [1]. This endorsement marked a significant shift in the TB diagnostic landscape. Smear microscopy has been the cornerstone of TB diagnosis for a century, but lacks sensitivity, particularly amongst HIV-infected individuals [2] and provides no information on drug resistance in the context of an emerging MDR-TB epidemic. While mycobacterial culture is more sensitive than microscopy and cultured isolates are available for drug susceptibility testing, these results are seldom available in a clinically relevant timeframe. Culture also requires significant infrastructure and highly trained staff. Xpert, a rapid, sensitive diagnostic test that is suitable for deployment at or close to the point of care [3], has broken the drought in the TB diagnostic pipeline.

Xpert is not the first commercially available nucleic acid amplification test (NAAT) for TB; indeed, the sensitivity of the assay is not substantially different from that of some competitor assays [3-5]. However, several important features distinguish Xpert from earlier tests. Firstly, nucleic acid extraction and purification is accomplished in a highly automated and operator-independent manner [6]. The only manual steps involve addition of the correct ratio of sample reagent (SR) to raw sputum, mixing for 15 minutes and transfer of a measured amount to the Xpert cartridge. This

represents a substantial advance over prior tests, which required decontaminated and concentrated sputum sediment and complex extraction protocols, and were therefore unsuitable for implementation in most TB-endemic settings. Secondly, Xpert utilizes real-time PCR, which does not require post-PCR manipulation of amplified mycobacterial DNA, and therefore reduces both complexity and the risk of cross-contamination by amplified DNA product. Finally, the simultaneous detection of rifampicin resistance, achieved by targeting the rifampicin-resistance determining region of the *rpoB* gene [7], identifies patients at highest risk of MDR-TB. This permits rapid targeted therapy as well as implementation of appropriate infection control measures.

PRE-CLINICAL STUDIES OF XPERT

Preclinical studies of Xpert demonstrated an analytical limit of detection of 4.5 (95% CI 3.3-9.7) genome copies per reaction, or 131 (95% CI 106-176) colony-forming units (CFU) of *M. tuberculosis* per ml of sputum [6]. This is higher than that of liquid mycobacterial culture (10-100 CFU/ml) but substantially lower than that of smear microscopy (10,000 CFU/ml) [8]. Specificity was demonstrated by failure of amplification of 20 non-tuberculous mycobacteria [6] as well as 89 different bacteria, fungi and viruses commonly found in the respiratory tract [9].

Importantly, incubation of SR with sputum was demonstrated to rapidly reduce viability of *M. tuberculosis*, with greater than 8 log reduction in viable bacilli within 15 minutes [6]. Further, bio-aerosols were not detected during the standard Xpert testing procedure, but were detected during preparation of smears for microscopy [10]. The biohazard risk associated with Xpert therefore appears to be very low, however it should be noted that SR does not completely sterilize sputum and that production of aerosols may differ in 'real-world' situations. It would therefore be prudent to consider the infectious risk as being similar to that

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of smear microscopy and apply appropriate infection control measures.

CLINICAL EVALUATION OF XPRT

A recent Cochrane review [11] has summarized the evidence base for the accuracy of Xpert for TB detection and rifampicin resistance detection in adults. The pooled sensitivity of a single Xpert test for detection of TB (15 studies, 7517 participants) was 88% (95% CI 83-92) and specificity 98% (95% CI 97-99). Sensitivity for smear-positive TB was 98% (95% CI 97-99) and for smear-negative TB 68% (95% CI 59-75). Amongst people living with HIV, the pooled sensitivity was 80% (95% CI 67-88) while it was 89% (95% CI 81-94) amongst those without HIV infection. In a single, large multicentre evaluation the incremental increase in sensitivity amongst smear-negative patients when Xpert testing was performed on second and third sputum specimens was 12.6% and 5.1% respectively [3]. While most of these studies were conducted in low and middle-income countries, a limitation of all but one of these studies is that Xpert testing was performed at reference laboratories, rather than in peripheral laboratories or health facilities [11].

Pooled estimates of sensitivity for detection of rifampicin resistance (11 studies, 2340 participants) were 94% (95% CI 87-97) and specificity 98% (95% CI 97-99%). It is relevant to note that the software algorithm used to determine rifampicin resistance was changed during the course of one study [12], and that the assay version assessed therefore differs between studies in this meta-analysis. This modification was made to improve specificity for detection of rifampicin resistance after the identification of false-rifampicin resistant calls. Subsequently, additional modifications were made to the assay (version G4, released December 2011), including a modification to fluidics and to the sequence of one of the fluorescent beacons, in order to further improve specificity and reduce assay error rates [13]. There are limited published data on the accuracy of the G4 assay for detection of rifampicin resistance, however routine data from the roll out of Xpert in Cape Town suggest high positive predictive value (164/165 [99.4%] rifampicin resistant cases identified by Xpert were confirmed by line probe assay testing) [14].

Several studies have examined accuracy of Xpert for TB detection in children [15-18]. Sensitivity is poorer than for adult TB, probably reflecting the paucibacillary nature of childhood disease and difficulties in obtaining suitable specimens for testing. Sensitivity of a single Xpert test for TB detection ranged from 59-90% [15-17] on sputum or induced sputum samples, depending on the number of samples cultured to establish the reference standard. For gastric lavage samples, sensitivity was 69% [18] and for nasopharyngeal aspirates 48% [15]. Specificity for all specimen types was similar to that for adult TB.

WHAT ARE THE IMPLICATIONS OF THESE PERFORMANCE DATA FOR CLINICAL PRACTICE?

Due to the moderate sensitivity outlined above, a single Xpert test cannot be used as a rule-out test for TB, particularly in persons living with HIV. Diagnostic algorithms for further assessment of Xpert-negative TB suspects have not been well evaluated and practice is largely determined by cost

constraints. Recent WHO recommendations for HIV-infected TB suspects with a negative Xpert test [19] include clinical assessment for extra-pulmonary TB or other illnesses, chest X-ray and treatment with antibiotics. If there is no response or partial response to antibiotic therapy, a second Xpert test is recommended. For seriously ill patients immediate repeat Xpert testing is recommended. However there is no clear evidence base for these recommendations. By contrast, in South Africa, where Xpert testing has largely replaced smear microscopy as the first-line test, the national algorithm currently recommends mycobacterial culture for all HIV-infected TB suspects with a negative Xpert test. This is a costly strategy and there is some evidence that a second Xpert test may be more cost-effective in these patients [20]. Further research is urgently needed to identify the most cost-effective testing strategy, which will likely vary depending on prevalence of TB and rates of HIV infection and MDR-TB.

The approach to the patient with rifampicin resistant TB on Xpert testing is informed by the prevalence of rifampicin resistance amongst the TB suspect population. As an example, assuming specificity of 98% for identification of rifampicin resistance for the current G4 assay version, if the prevalence of rifampicin resistance amongst TB cases is 5%, then 2 out of every 7 cases of rifampicin resistant TB detected by Xpert will be false-resistant calls. In contrast, amongst high-risk TB suspects, e.g., patients experiencing treatment failure, the positive predictive value of a rifampicin resistant Xpert result is much higher. The decision as to whether confirmatory testing is required and whether to treat for RR TB immediately on receipt of a rifampicin resistant Xpert result therefore depends on the pre-test likelihood of rifampicin resistance in the particular patient or epidemiological setting [21].

EXPERIENCE FROM THE ROLLOUT OF XPRT IN SOUTH AFRICA

Following the 2010 WHO recommendation, the South African Ministry of Health made a decision to commence rollout of Xpert as a replacement test for smear microscopy in South Africa. Testing commenced in March 2011, and was rapidly scaled up, so that as of 31 January 2013, 966,033 tests had been performed and 203 Xpert instruments placed within the National Health Laboratory Service, which provides pathology services to the state sector in South Africa (Personal communication, Wendy Stevens, Head of National Priority Programmes, NHLS). Problems encountered included high assay failure rate (which was resolved when the G4 assay version became available), the need to develop connectivity to the laboratory information service for reporting and surveillance purposes, lack of external quality assurance panels and intermittent cartridge supply problems. A novel verification panel using dried *M. tuberculosis* culture spots was developed and used at time of implementation. This approach may be a suitable matrix for future external quality assurance panels [22].

One of the most challenging aspects of the implementation was the implementation of a revised national TB testing algorithm. The diagnostic approach to patients with TB has become entrenched through lack of advance over past decades. This was compounded by the phased implementation of Xpert in South Africa, where two

different diagnostic algorithms, one based on Xpert, the other on smear microscopy, were simultaneously operational, leading to confusion and inequitable access. TB control programmes are typically algorithm driven, since concepts such as the predictive value of a positive or negative test are not easily translated to treatment decisions at the primary health care level. There is a need for clear, simple, unambiguous and feasible algorithms to be developed and piloted well in advance of Xpert implementation so that adequate training of laboratory and clinical staff can be ensured.

The development of a rational algorithm for patients identified as having RR TB by Xpert posed a particular challenge. Despite the estimated relatively modest positive predictive value of Xpert for RR in South Africa (with test specificity of 98% and RR prevalence of 6%, PPV would be 75%), the Ministry of Health made a decision to treat for MDR-TB whilst waiting for confirmatory testing. Whilst this decision will result in some inappropriate treatment for MDR-TB, this is balanced by more rapid initiation of therapy for MDR-TB patients and an associated reduction in risk of transmission [23]. A second difficulty is that there is typically considerable delay in identification of resistance to isoniazid and second-line TB drugs following a rifampicin resistant Xpert result, as no isolate of *M. tuberculosis* is available for susceptibility testing. Line probe assay testing directly on sputum samples from smear-positive, Xpert rifampicin resistant cases [24, 25] permits rapid confirmation of rifampicin resistance and identification of isoniazid resistance. However, the sensitivity of line probe assay testing for isoniazid resistance is suboptimal [25, 26] and this test requires well-trained staff and suitably equipped laboratories [27]. WHO has not yet endorsed more recent versions of the line probe assay, which may be suitable for use on smear-negative specimens [28], although this may change as more data become available.

Mycobacterial culture is required for further susceptibility testing of smear-negative cases as well as for identification of resistance to second line agents, most notably injectable drugs and fluoroquinolones (required to detect extensively drug-resistant, or XDR-TB). There have been a number of important recent advances in this area, most notably the availability of a line probe assay for identification of mutations associated with resistance to fluoroquinolones, injectables and ethambutol [29]; however there is geographic variability in the distribution of specific resistance mutations [30], and the sensitivity of this assay for detection of XDR-TB varies considerably from region to region [31-33]. Whilst these assays may be used to rule-in a diagnosis of XDR-TB, they cannot replace conventional susceptibility testing [34]. There is thus an urgent need for sensitive and specific genotypic tests that will allow for more detailed and rapid resistance characterization in patients identified as having rifampicin resistance TB by Xpert.

THE ROLE OF XPERT IN DIAGNOSING EXTRA-PULMONARY TUBERCULOSIS (EPTB)

While most studies have focused on use of Xpert for diagnosis of pulmonary TB, there is now a substantial literature on use of Xpert for diagnosis of EPTB. There is significant heterogeneity between studies, with differences in

patient population, specimen type, processing methods as well as the reference standard applied. Reported sensitivities range from as low as 25% (pleural fluid) [35] to as high as 96.7% (lymph node aspirates) [36]. Specificity was high (>98%) in all studies, apart from one small study of lymph node aspirates [36].

In a large Italian study evaluating different sample types (1493 samples from 1068 patients) [37], results were compared to both culture and a composite reference standard incorporating clinical diagnosis. The sensitivity of Xpert overall, using the composite reference standard, was 88.3% (95% CI 82-95%) with specificity 100%. These results are comparable to most other studies [38]. However Xpert testing of pleural fluid (n=292) and other cavitory fluid (n=87) yielded sensitivities of 33.3% (95% CI 9-57%) and 50% (95% CI 15-85%) respectively. This is similar to the sensitivity of 25% achieved in one study of 20 pleural fluid samples [35]. By contrast, the sensitivity of Xpert on adult CSF samples in the Italian study (n=86) was 100% (95% CI 100-100%), while in a study performed in India [39], the sensitivity of Xpert on CSF samples (n=23) was only 29% (95% CI 8-65%).

A number of issues related to the use of Xpert for EPTB still need to be addressed. Firstly, more work needs to be done to properly describe the performance of the assay in different specimen types. Secondly, optimal specimen processing methodologies (for example use of concentration techniques prior to Xpert) need to be identified. Finally, consideration needs to be given to the most cost-effective approach for diagnosis of EPTB, particularly given that culture may be required in parallel for most sample types.

COST-EFFECTIVENESS OF XPERT

Whilst Xpert represents a major advance over smear microscopy, costs are a major obstacle to widespread rollout. There has, however, been a rapid decline in cartridge costs (at present US\$9.98 for high burden countries). Instrument costs remain prohibitive for many low-income countries and are dependent on the number of modules ranging from US\$15,700 for a 4-module instrument to US\$65,500 for a 16-module instrument (prices as procured in South Africa). By contrast, smear microscopy is inexpensive (e.g., US\$1.63 for fluorescence microscopy [40]). Uptake of Xpert will be contingent on clear data demonstrating cost-effectiveness. Several studies have confirmed reduced time to diagnosis and treatment [41-43], however these data alone are unlikely to be persuasive. Reduced mortality, decreased costs associated with hospitalization and, perhaps most importantly, reduced transmission of TB (particularly drug-resistant TB) are potential drivers of efficiency which need to be addressed in carefully designed studies. Whilst direct empirical evidence is lacking, several investigators have modeled the potential impact of Xpert on the TB epidemic [23, 44, 45]. In Southern Africa, implementation of Xpert in place of smear microscopy is projected to reduce prevalence of TB by 28% by 2022 (although the impact on incidence is more modest), with an estimated cost-effectiveness of US\$959 per disability-adjusted life-year averted over the same period [23]. The absolute number of MDR-TB cases was projected to be 25% lower with implementation of Xpert. In a separate analysis the incremental cost

effectiveness ratio of Xpert as replacement for smear microscopy was estimated to range between US\$52 and US\$138 per disability-adjusted life-year averted [44]. Much of the additional cost in the first analysis related to antiretroviral therapy for TB/HIV co-infected patients who survived longer due to improved diagnosis and treatment of TB. Input parameters in published models differ widely, and most have not accounted for empiric treatment for TB (in the absence of microbiological confirmation) or extra-pulmonary TB, both particularly important in the context of HIV-associated TB.

THE ROLE OF XPERT AS A SCREENING TEST FOR HIV-INFECTED PATIENTS

Several studies have identified high rates of prevalent TB in HIV-infected patients enrolling in or receiving antiretroviral therapy [46]. Xpert increased case detection by 45%, compared with smear microscopy in patients enrolling in antiretroviral therapy in South Africa [47]. The sensitivity of Xpert was lower in this patient population (58.3%, 95% CI 46.1-69.8 for one test and 72.2%, 95% CI 60.4-82.1 for two tests), when compared with a more typical TB suspect population (in the multicenter evaluation study 92.2%, 95% CI 90.0-93.9 for a single test [3]). Given the relative lack of sensitivity, screening for prevalent TB by Xpert in unselected patients accessing antiretroviral therapy is a relatively costly strategy even in settings with very high rates of prevalent TB; at 22% TB prevalence, the incremental cost effectiveness ratio is estimated at US\$2800 per year of life saved for smear microscopy and \$5100 per year of life saved for two Xpert tests [48]. Studies are needed to identify ways of selecting patients at high risk of TB or TB-related mortality for Xpert screening. There is also a need to determine the frequency at which repeat Xpert screening is needed in this patient population.

THE ROLE OF XPERT IN POINT-OF-CARE TESTING FOR TB

The platform for the Xpert assay is the Cepheid GeneXpert modular system, available in 4, 16, 48 and 80 module instruments. A major advantage is the ability to load each module independently, so that there is no need for batch processing of samples [8]. The 4-module (GX4) instrument is relatively robust, portable, and requires minimal training to operate and maintain [12]. It may therefore be potentially suitable for point-of-care testing (POC) for TB, where a POC test is defined as a test which is performed at the facility where treatment is instituted, and where results can be delivered to inform patient management during the same treatment episode.

There are several limitations to the use of Xpert as a POC test. It requires a stable, uninterrupted power supply, an operating temperature range of 15 to 30°C, trained staff, regular maintenance and annual calibration of modules (which can now be performed remotely, through an internet portal) [49]. If these conditions are met, then consideration may be given to deploying Xpert at the POC. However, in many real-world situations, turnover of trained staff, lack of attention by busy clinical staff to instrument maintenance and testing protocols, and, most compellingly, the reduced

economies of scale associated with low test volumes [50], raise concerns regarding the sustainability of deploying Xpert outside of laboratory services.

A further constraint to true POC use is test turn-around time. Since economies of scale are likely to restrict use to busier health care facilities, and on-instrument assay time is approximately 2 hours, same-visit delivery of results is difficult to achieve. In one study, a GX4 instrument was not able to deliver 16-test capacity during a working day [51] and, in another, substantial additional resources were needed to ensure same-day treatment [51].

SUMMARY

Xpert is an important new tool for the diagnosis of TB, which has the potential to impact not only on individual patient outcomes, but also on the course of the TB epidemic in high burden countries. Perhaps most importantly, Xpert has established the principle that nucleic acid amplification tests for TB can be sensitive, specific and feasible for implementation in poorly resourced settings. It is likely that this will now be an area of rapid progress, with the entrance of competitor systems into the market.

However, there is much work to be done to better understand how Xpert can be utilized in a programmatic setting to maximize its potential impact and cost-effectiveness. Key issues to be addressed are the level of instrument placement within the health services and the development of rational algorithms for screening of HIV-infected individuals, particularly those with a single negative Xpert test.

Finally, the diagnosis of TB is one component of the overall control strategy; without effective linkage into care and strong systems for delivery of treatment and patient retention, there will be little impact on the TB epidemic.

CONFLICT OF INTEREST

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