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Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test

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Rapid progress has been made in the development of new diagnostic assays for tuberculosis in recent years. New technologies have been developed and assessed, and are now being implemented. The Xpert MTB/RIF assay, which enables simultaneous detection of *Mycobacterium tuberculosis* (MTB) and rifampicin (RIF) resistance, was endorsed by WHO in December, 2010. This assay was specifically recommended for use as the initial diagnostic test for suspected drug-resistant or HIV-associated pulmonary tuberculosis. By June, 2012, two-thirds of countries with a high tuberculosis burden and half of countries with a high multidrug-resistant tuberculosis burden had incorporated the assay into their national tuberculosis programme guidelines. Although the development of the Xpert MTB/RIF assay is undoubtedly a landmark event, clinical and programmatic effects and cost-effectiveness remain to be defined. We review the rapidly growing body of scientific literature and discuss the advantages and challenges of using the Xpert MTB/RIF assay in areas where tuberculosis is endemic. We also review other prospects within the developmental pipeline. A rapid, accurate point-of-care diagnostic test that is affordable and can be readily implemented is urgently needed. Investment in the tuberculosis diagnostics pipeline should remain a major priority for funders and researchers.

Introduction

The global burden of tuberculosis is unacceptably high (panel 1)[1] and multidrug-resistant (MDR) tuberculosis is now a major health challenge worldwide. Of notified cases of pulmonary tuberculosis in 2011, an estimated 310 000 new cases were MDR, defined by active infection with *Mycobacterium tuberculosis* that is resistant to isoniazid and rifampicin.[1] To eliminate tuberculosis as a public health problem by 2050, incidence will have to fall by an average of 16% per year for the next 40 years.[2] Rates, however, are only declining at 2% per year.[1] The scale of the disease burden is compounded by the intersection of the HIV and tuberculosis epidemics and by the global spread of MDR tuberculosis and extensively drug-resistant (XDR) tuberculosis (panel 1). Despite major efforts to increase case detection, an estimated third of new tuberculosis cases are still being missed each year, and the unavailability of a rapid, low-cost, accurate diagnostic assay that can be used at the point of care is a major hindrance.

Low-income and middle-income countries, which bear most of the global burden of tuberculosis, rely heavily on outdated tuberculosis diagnostic tests, including sputum smear microscopy, solid culture, and chest radiography.

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Key messages

- The Xpert MTB/RIF assay is a landmark development in tuberculosis diagnostics and yet it does not fulfil requirements as a point-of-care assay
- One Xpert MTB/RIF test on sputum detects 90% of pulmonary tuberculosis (99% of smear-positive disease and about 75% of smear-negative disease)
- High sensitivity of Xpert MTB/RIF for rifampicin resistance is accompanied by some false-positive results (which might be reduced by the new G4 version of the assay) and confirmatory drug sensitivity testing is needed
- Despite substantial price discounting and relative simplicity of use, implementation of the Xpert MTB/RIF assay is hindered by several factors
- Studies of clinical and programmatic effects and associated cost-effectiveness of the Xpert MTB/RIF assay are needed
- Greater funding for research and development for a simple, low-cost, accurate point-of-care assay is needed

Panel 1: The estimated global burden of tuberculosis in 2011[1]

8·7 million incident cases
- 1·1 million (13%) cases in people living with HIV
- 490 000 cases in children younger than 15 years

1·4 million deaths
- 990 000 HIV-seronegative people
- 430 000 (31%) HIV-seropositive people
- 500 000 (36%) women
- 64 000 children younger than 15 years

Multidrug-resistant cases
- 630 000 prevalent cases
- 310 000 incident cases
- 3·7% of new incident cases
- 20% of previously treated incident cases
- 9% of multidrug-resistant cases are extensively drug-resistant

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These tests do not have sufficient sensitivity or specificity, are too slow, or are not available at the periphery of the health system where patients first seek care. Opportunities to intervene early in the disease are therefore lost. Global capacity for drug susceptibility testing (DST) is inadequate and only 9% of the estimated 630 000 prevalent cases of MDR tuberculosis worldwide in 2011 were diagnosed and notified.[1,3]

For the past 5 years, the development of diagnostics for tuberculosis has progressed rapidly (figure).[4] Old technologies have been reviewed and improved and new technologies have been developed, evaluated, and implemented. With a growing evidence base, WHO issued ten policy statements between 2007 and 2012 about tuberculosis diagnosis and diagnostic methods, which shows the progress that has been made. These policy statements address improvements in sputum smear microscopy,[5–8] use of commercial and non-commerical culture-based systems for diagnosis and DST,[9,10] and implementation of line-probe assays[11] for rapid molecular diagnosis of drug resistance. Negative recommendations were issued about the use of serodiagnostic tests and interferon-gamma release assays for diagnosis of tuberculosis or latent *M tuberculosis* infection in low-income and middle-income countries.[12,13] After initial endorsement in December, 2010, WHO issued a policy statement in 2011, on the Xpert MTB/RIF automated molecular assay for rapid diagnosis of tuberculosis and detection of rifampicin resistance.[14] Specifically, the assay was strongly recommended for use as the initial diagnostic test in individuals suspected of having MDR or HIV-associated tuberculosis.[15] The assay was also conditionally recommended as a follow-on test to microscopy in settings where MDR tuberculosis and HIV-associated tuberculosis are less of a concern.

The development of the Xpert MTB/RIF assay is a landmark event in tuberculosis research, and this article summarises what is known about this assay, its assessment in different settings, and its implementation. Despite many compelling attributes of this new diagnostic test, the Xpert MTB/RIF assay is by no means the ideal test. We discuss the challenges associated with its use in resource-limited settings and review other important developments and future prospects within the diagnostics developmental pipeline.

Development of the Xpert MTB/RIF assay

The GeneXpert diagnostic system was originally developed by Cepheid (Sunnyvale, CA, USA) for rapid detection of anthrax,[16] and was deployed for this purpose by the US Postal Service to permit rapid detection of mail contamination in sorting offices. It is a self-contained,
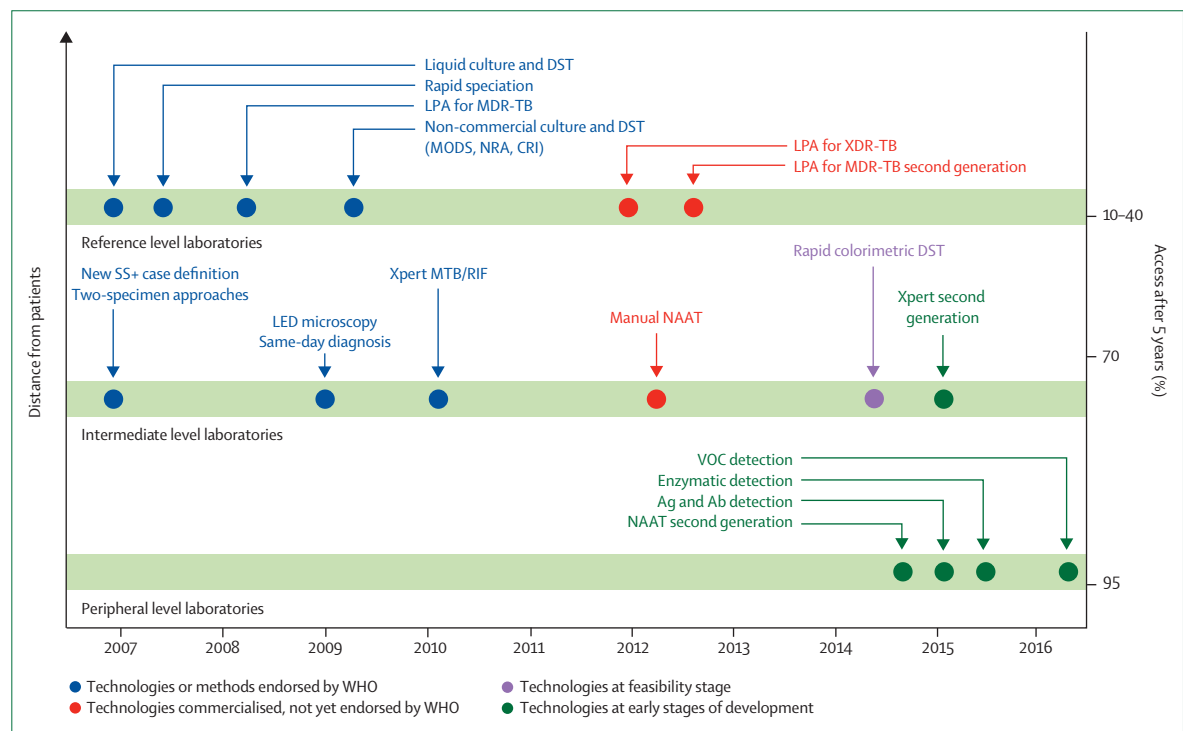
Figure: **Development pipeline for new tuberculosis diagnostics**
Reference level laboratories refer to national level facilities. Intermediate level laboratories refer to district and subdistrict level facilities. Peripheral level laboratories refer to community level facilities. Reproduced from WHO's global tuberculosis control report, 2012,[1] by permission of the World Health Organization. Ab=antibody. Ag=antigen. CRI=colorimetric redox indicator assay. DST=drug susceptibility test. LED=light emitting diode. LPA=line-probe assay. MDR-TB=multidrug-resistant tuberculosis. MODS=microscopic observation drug susceptibility. NAAT=nucleic acid amplification tests. NRA=nitrate reductase assay. SS+=sputum smear-positive. VOC=volatile organic compound. XDR-TB=extensively drug-resistant tuberculosis.

fully integrated, automated platform that can be used with minimal technical skills. The cartridge-based system incorporates microfluidics technology and fully automated nucleic acid analysis to purify, concentrate, detect, and identify targeted nucleic acid sequences from unprocessed clinical samples.[17] An expanding range of different organisms can be detected with pathogen-specific cartridges within the same test platform, including enteroviral meningitis, meticillin-resistant *Staphylococcus aureus*, group B streptococcus, and influenza. The test platform is modular, with each module independently processing one cartridge at a time. Machines with one, two, four, 16, and 48 modules are available, permitting several assays to be run concurrently and independently. A high-throughput machine is also available for centralised laboratories.

Rifampicin resistance is particularly amenable to rapid molecular detection because 95% of all rifampicin-resistant *M tuberculosis* strains contain mutations localised within the 81 bp core region of the bacterial RNA polymerase β subunit (*rpoB*) gene, which encodes the active site of the enzyme.[18] Moreover, mutations that occur in this region are highly predictive of rifampicin resistance[18,19] and the core region is flanked by *M tuberculosis* complex-specific DNA sequences. Thus, *M tuberculosis* and rifampicin resistance can be tested simultaneously by targeting one amplicon generated with PCR technology. Moreover, rifampicin resistance is strongly, although not invariably, indicative of MDR tuberculosis.

The Xpert MTB/RIF assay uses molecular beacon technology[20,21] to detect DNA sequences amplified in a heminested real-time-PCR assay. The assay uses single-use plastic cartridges with several chambers that are preloaded with liquid buffers and lyophilised reagent beads necessary for sample processing, DNA extraction, and PCR.[22,23] Sample reagent included in the assay is designed to reduce the viability of *M tuberculosis* in sputum and reduce the biohazard risk.[24] Subsequent processing is fully automated and results are available within 2 h with less than 20 min of hands-on time.

Preclinical laboratory-based assessment

A thorough preclinical assessment of analytic performance and biosafety of the Xpert MTB/RIF assay was done.[17] By spiking defined numbers of *M tuberculosis* bacilli into clinical sputum samples from patients without tuberculosis, the limit of detection (95% reliability for detection) of the assay was 131 colony forming units (cfu)/mL (95% CI 106–176) of sputum.[22] This result contrasts with the limit of detection of automated mycobacterial liquid culture, which is about 10–50 cfu/mL, and with that of smear microscopy, which is about 10 000 cfu/mL.[25] Thus, the Xpert MTB/RIF assay has a sensitivity that is roughly two orders of magnitude greater than that of smear microscopy, is similar to solid culture, but is not quite as sensitive as liquid culture. The assay correctly identified genomic DNA from 79 phylogenetically and geographically diverse strains of *M tuberculosis*[23] and no cross-reactivity occurred with a wide range of non-tuberculous mycobacteria or other organisms known to infect the respiratory tract.[22,23] Further experiments showed that false-positive reactions due to laboratory cross-contamination with amplicons from the GenoType MTBDR*plus* assay (Hain Lifescience, Nehren, Germany) was very unlikely.[23]

Genomic DNA from several rifampicin-susceptible and rifampicin-resistant *M tuberculosis* isolates with diverse *rpoB* mutations were tested, and excellent accuracy for rifampicin resistance was reported.[22,23] Further experiments were done in which DNA from resistant and susceptible strains were mixed in varying ratios to assess how this affected detection of rifampicin resistance.[23] To enable detection, 65–100% of the DNA from the rifampicin-resistant isolate had to be present, depending on the mutation.[23] Overall, this finding suggests that in patients with mixed infections, the Xpert MTB/RIF assay might only detect the resistant strain if this strain is predominant. Moreover, subsequent selection of resistant strains during the course of standard tuberculosis treatment might lead to an apparent switch from a susceptible to a resistant phenotype when baseline testing is compared with repeat testing during treatment.

To assess biosafety requirements for the Xpert MTB/RIF assay, bioaerosol generation and bacterial viability studies were done. The viability of *M tuberculosis* was reduced by more than 8 logs within 15 min of incubation of sputum in sample reagent.[22] Viable bioaerosols were not generated during the manual sputum processing with sample reagent or during automated processing with the Xpert MTB/RIF assay, whereas infectious bioaerosols were generated during routine preparation of smears.[24] These data therefore suggest that the Xpert MTB/RIF assay poses a substantially smaller biohazard risk compared with direct smear microscopy and, given adequate room ventilation, might reasonably be done without the need for special equipment such as biosafety cabinets, which are absent in most resource-limited settings.

Diagnostic accuracy of the Xpert MTB/RIF assay for pulmonary tuberculosis

Many studies in both high-income and resource-limited settings of the diagnostic accuracy of the Xpert MTB/RIF assay for pulmonary tuberculosis have been published.[17,26] The multicountry assessment done by the Foundation for Innovative and New Diagnostics (FIND), published in 2010,[27] enrolled 1730 patients suspected of having drug-sensitive or drug-resistant tuberculosis at five study sites in South Africa, Peru, Azerbaijan, and India. One direct test on sputum detected 551 (98·2%) of 561 patients with smear-positive tuberculosis and 124 (72·5%) of 171 patients with smear-negative tuber-culosis.[27] The test was specific in 604 (99·2%) of

609 patients without tuberculosis. In patients with smear-negative tuberculosis, processing one, two, or three samples was associated with sensitivities of 72·5%, 85·1%, and 90·2%, respectively. These data formed a substantial part of the evidence base that led to the endorsement of the assay by WHO in 2010.[15]

A systematic review of studies published up to October, 2011, identified 18 studies containing 10 224 patients.[26] 15 reported on diagnosis of pulmonary tuberculosis, and the meta-analysis provided an overall pooled sensitivity of 90·4% (95% CI 89·2–91·4) and a pooled specificity of 98·4% (98·0–98·7). The pooled sensitivities for sputum smear-negative and smear-positive disease were 75·0% and 98·7%, respectively. Data published after this date have broadly similar findings. A modified G4 version of the cartridge was launched in December, 2011, and independent data on the diagnostic accuracy of this version are needed.

Data about the effect of implementation of Xpert MTB/RIF on clinical outcomes of patients investigated for tuberculosis are scarce. FIND did a multicentre assessment of implementation in South Africa, Uganda, Peru, India, Azerbaijan, and the Philippines.[28] In all centres, the GeneXpert machines were located within laboratories at health facilities where smear microscopy was being done. The assay greatly accelerated the time to diagnosis, with a median time of 0 days compared with 1 day for smear microscopy, 16 days with liquid culture, and 20 days with solid culture.[28] For patients with smear-negative tuberculosis, the Xpert MTB/RIF assay reduced the median time to start of treatment from 56 days (IQR 39–81) to 5 days (2–8). Rates of untreated smear-negative culture-positive tuberculosis decreased from 39·3% without the Xpert MTB/RIF assay to 14·7% with the assay. Assay performance for detection of rifampicin resistance was also excellent, with a median time to

	Country	TB reference standard diagnoses (samples)	TB not diagnosed (samples)	Main sample types testing positive for TB (samples)	Reference standard for TB diagnosis	Xpert MTB/RIF assay sensitivity for TB, % (95% CI)	Xpert MTB/RIF assay specificity, % (95% CI)
Testing of various extrapulmonary samples for TB							
Armand et al, 2011[31]	France	32	NA	Lymph nodes (16), pleural fluid (7), bone (5)	Culture (solid and liquid media)	53·1% (34·7–70·9)	NA
Causse et al, 2011[32]	Spain	41	299	Tissue biopsy samples (18), CSF (6), gastric aspirates (8), pleural fluid (4), purulent exudates (5)	Culture (solid and liquid media)	95·1% (83·5–99·4)	100% (98·8–100)
Friedrich et al, 2011[33]	South Africa	20	5	Pleural fluid (25)	Culture (liquid media)	25·0% (8·7–49·1)	100% (47·8–100)
Hillemann et al, 2011[34]	Germany	45	476	Tissue (30), gastric aspirate (8), urine (5)	Culture (solid and liquid media)	77·3% (60·5–87·1)	98·2% (96·0–98·9)
Ligthelm et al, 2011[35]	South Africa	30	18	Fine needle aspiration lymph node biopsy	Composite standard: positive cytology + acid-fast bacilli and/or culture of TB	96·6% (86·6–100)	88·9% (69·6–100)
Moure et al, 2012[36]	Spain	108	41	All smear-negative, pleural fluid (26), lymph nodes (34), abscess aspirates (17), tissues (12)	Culture (solid and liquid media)	58·3% (48·5–67·8)	100% (91·4–100)
Vadwai et al, 2011[37]	India	283	250	Tissue biopsy samples (105), pus (98), body fluids (24)	Composite of smear, culture, clinical, radiology, and histology	80·6% (75·5–85·0)	99·6% (97·8–100)
Zeka et al, 2011[38]	Turkey	48	128	Pleural fluid, lymph node biopsy, CSF, urine, skin biopsy samples, pericardial fluid	Culture (solid and liquid) or suggestive clinical features, radiology or histology	54·2% (40·3–67·4)	100% (97·2–100)
Tortoli et al, 2012[39]	Italy	268	1206	Tissues biopsies or fine needle aspirates (94), pleural fluid (18), gastric aspirates (61), pus (55), CSF (14), urine (16), peritoneal, synovial, or pericardial fluid (10)	Culture (solid and liquid) or suggestive radiology or histology with documented positive response to TB treatment	81·3% (76·2–85·8)	99·8% (99·4–100)
Testing of urine samples from patients infected with HIV with culture-positive pulmonary TB							
Lawn et al, 2012[40]	South Africa	84 outpatients screened before antiretroviral therapy	NA	2·0 mL of urine	Sputum liquid culture	Overall: 19·0% (11·3–29·1); CD4 <50: 44·4%; CD4 50–150: 25·0%; CD4 >150: 2·7%	NA
Peter et al, 2012[41]	South Africa	113 inpatients	62	1·0–10·0 mL of urine (+/– centrifugation)	Liquid culture of sputum or extrapulmonary sample	Overall: 47·8% (38·8–56·9); CD4 <200: 53·8%; CD4 >200: 30·8%	98% (95–100)

Only studies with at least 20 reference standard diagnoses of extrapulmonary tuberculosis were included. TB=tuberculosis. MTB=*Mycobacterium tuberculosis*. RIF=rifampicin. NA=not applicable.

Table 1: Summary of studies of the diagnostic accuracy of the Xpert MTB/RIF assay for extrapulmonary tuberculosis

detection of 1 day (IQR 0–1) compared with 20 days (10–26) with a line-probe assay and 106 days (30–124) for phenotypic DST. Despite these promising results, they only come from one multicentre study. Furthermore, published data on the effect of the Xpert MTB/RIF assay on clinical outcomes are not available. Operational research on the outcomes and effects of programmatic implementation efforts are urgently needed.

Molecular techniques that detect DNA from *M tuberculosis* detect both live and dead organisms and so might test positive by PCR despite being culture negative.[29] A positive Xpert MTB/RIF assay result therefore does not imply viability of the organism and thus cannot be used to monitor response to treatment, treatment success, treatment failure, or relapse. Attempts are being made to develop a protocol whereby sputum samples are pretreated to prevent the DNA in non-viable organisms being amplified during PCR.[30] However, even if such an approach proved successful, it would be complex to implement. Alternative approaches being assessed include detection of RNA expression.

Diagnostic accuracy for extrapulmonary tuberculosis

The Xpert MTB/RIF assay was developed, optimised, assessed, and endorsed specifically for the detection of pulmonary tuberculosis using sputum. More recently, however, assessments of the assay have extended to various non-respiratory clinical samples from patients with extrapulmonary tuberculosis. Investigation for use in extrapulmonary tuberculosis is far more complex because of the diversity of clinical sample types, difficulties in obtaining adequate tissue for analyses, the challenge of providing a rigorous reference standard for comparison, and the range of ways to process samples before analysis.

Table 1 summarises data from studies of the diagnostic accuracy of the Xpert MTB/RIF assay that included a wide range of different samples from extrapulmonary sites. The reported sensitivity of the assay for extrapulmonary tuberculosis was highly heterogeneous, ranging from 25·0% to 96·6%, but exceeded 50·0% in all but one study (table 1). The heterogeneity between studies might be a result of the differences between patient populations, patient selection, type of extrapulmonary tuberculosis, the quality of samples, differences in sample processing, and the diagnostic reference standard used. The median sensitivity of these nine studies was 77·3% (range 25·0–96·6), consistent with a meta-analysis of the few studies published before October, 2011, which reported a pooled sensitivity of 80·4% (95% CI 75·0–85·1).[26]

The two largest studies in table 1 reported that the sensitivity of the Xpert MTB/RIF assay was much higher for smear-positive than for smear-negative extrapulmonary tuberculosis samples.[37,39] Overall, sensitivities exceeded 70% for tissue biopsy samples, fine needle aspirates, pus samples, gastric aspirates, and urine.

However, reported sensitivities on small numbers of CSF samples differed substantially.[37,39] Lower sensitivity has been noted when testing pleural, pericardial, peritoneal, and synovial fluid samples.[33,39] Increasing evidence from diagnostic accuracy studies might, in the future, open the possibility for international recommendations for use of the assay for diagnosis of extrapulmonary tuberculosis.

Diagnostic accuracy in children

Microbiological confirmation of tuberculosis is possible only in a small minority of the children treated for the disease, and the time to diagnosis by culture is often prolonged. Table 2 summarises data from five studies on the use of the Xpert MTB/RIF assay to diagnose pulmonary and extrapulmonary tuberculosis in children. Using culture as the reference standard, four of these studies reported that the sensitivity of the Xpert MTB/RIF assay for pulmonary tuberculosis was about two to three times higher than that of smear microscopy when testing induced sputum, nasopharyngeal aspirates, and gastric aspirate lavages.[42–45] Sensitivity ranged between 65·1% and 75·9% and specificity was 98·8–100%. Two of the studies reported a high incremental yield (27·8%[42] and 20%[43]) from testing a second sample. When analysing samples from a wide range of extrapulmonary sites from children, the Xpert MTB/RIF assay generated a substantial diagnostic yield (table 2).[39] Thus, although most disease in children is still clinically diagnosed, the Xpert MTB/RIF assay increases the proportion with laboratory confirmation compared with smear microscopy

	Country	Summary of findings
Nicol et al, 2011[42]	South Africa	Prospective study of inpatients (n=452) with median age 19 months (maximum 15 years) and suspected TB: from two induced sputum samples, the Xpert MTB/RIF assay diagnosed 75·9% (44 of 58) of culture-positive cases (specificity 98·8%) compared with 37·9% using smear microscopy; in smear-negative cases, the incremental yield of the second Xpert MTB/RIF test was 27·8%
Rachow et al, 2012[43]	Tanzania	Prospective study of 164 children aged <14 years (median 5·8 years): of 28 microbiologically confirmed cases, the Xpert MTB/RIF assay diagnosed 100% (7 of 7) smear-positive cases and 66·6% (14 of 21) smear-negative cases with 100% specificity; the incremental yields of testing second and third samples were 20% and 16%, respectively
Zar et al 2012[44]	South Africa	Prospective study of inpatients (n=535) with median age 19 months (maximum 15 years) and suspected TB: the yield of two Xpert MTB/RIF assay tests on nasopharyngeal aspirates from culture-confirmed cases was 65% (41 of 63) compared with 33% (21 of 63) by smear microscopy
Bates et al, 2013[45]	Zambia	Prospective study of inpatients (n=930) with median age 24 months (maximum 15 years) and suspected TB: in culture-positive cases (n=58), the Xpert MTB/RIF assay was more sensitive than smear microscopy when testing sputum samples (90·0% vs 30·0%) or gastric lavage aspirates (68·8% vs 25·0%) and specificity was 99·3%
Tortoli et al, 2012[39]	Italy	Study of the diagnosis of extrapulmonary TB in adults and children with a wide range of different sample types (tissue biopsies, pleural fluid, gastric aspirates, pus, CSF, and urine) that used a composite reference standard of culture, radiology, histology, and treatment response: the sensitivity in samples from children (86·9%) tended to be higher than that in samples from adults (77·6%), possibly as a result of the types of clinical samples in each group

TB=tuberculosis. MTB=*Mycobacterium tuberculosis*. RIF=rifampicin.

Table 2: Studies of the Xpert MTB/RIF assay for diagnosis of tuberculosis in children

and greatly accelerates diagnosis compared with culture. Studies in progress are assessing the use of the Xpert MTB/RIF assay on non-respiratory samples such as stool,[46] urine, and CSF. The WHO and Global Laboratory Initiative is planning to revise policy for use of the Xpert MTB/RIF assay for childhood tuberculosis diagnosis (and diagnosis of extrapulmonary tuberculosis).

Diagnostic accuracy in people living with HIV

Diagnosis of HIV-associated tuberculosis is a huge challenge.[47,48] Table 3 summarises seven studies of patients infected with HIV, comparing the sensitivity of sputum microscopy and the Xpert MTB/RIF assay with culture as the reference standard. The median sensitivity of smear microscopy was 52·8% (range 22·2–68·9) compared with 84·0% (58·3–91·7) with the Xpert MTB/RIF assay. In all seven studies, the sensitivity of the Xpert MTB/RIF assay exceeded that of microscopy with a median increment of 30·0% (range 17·4–37·8). The overall sensitivity of the Xpert MTB/RIF assay for HIV-associated tuberculosis was very heterogeneous (range 58·3–91·7%) and is likely to be a result of patient selection. The lowest sensitivity was in a study in which patients were actively screened for tuberculosis, irrespective of symptoms, and subanalysis showed that the sensitivity was very high in patients who had a cough for 2 weeks or longer.[51] Overall, sensitivities were higher in studies of outpatients with chronic symptoms and higher still in studies of patients admitted to hospital (table 3). Thus, the sensitivity of the Xpert MTB/RIF assay relates to severity of symptoms, which in turn might reflect mycobacterial load.

Although the sensitivity of smear microscopy is substantially lower in patients with HIV than in uninfected patients,[47,48] such an association is unclear for the Xpert MTB/RIF assay. Of the three studies with relevant comparative data[28,49,52] (table 3), two[28,49] studied outpatients and reported that sensitivity was roughly 10% lower in patients with HIV than in those without HIV. However, results of the third study,[52] of inpatients, showed the converse. Importantly, the subset of patients with HIV with culture-positive but Xpert MTB/RIF assay-negative disease have far more favourable prognostic characteristics and a lower risk of death than do those testing positive with the Xpert MTB/RIF assay.[55]

Two studies describe the usefulness of the Xpert MTB/RIF assay to diagnose HIV-associated tuberculosis through urine sample testing (table 1).[40,41] Despite only small volumes of urine being tested, the yield of HIV-associated tuberculosis was substantial, with positive results in samples from 44·4% of outpatients with culture-positive pulmonary tuberculosis and CD4 cell counts of fewer than 50 cells/μL and 47·8% of inpatients with tuberculosis. Lower CD4 cell counts were strongly associated with higher yield[40] and yield was also increased

	Country	Clinical population	Patient selection	Sensitivity of smear microscopy, % (95% CI)	Sensitivity of single Xpert MTB/RIF assay test, % (95% CI)
Studies of outpatients					
Boehme et al, 2011[28]	South Africa, Uganda, India, Peru, Azerbaijan, Philippines	Outpatients (HIV+ and HIV−)	Presentation with suspected TB with cough ≥2 weeks	HIV+: 44·6% (37·7–51·6); HIV−: 68·6% (63·5–73·3); p<0·001	HIV+: 82·4% (76·7–86·9); HIV−: 90·7% (87·2–93·4); p=0·08
Theron et al, 2011[49]	South Africa	Outpatients (HIV+ and HIV−)	Presentation with suspected TB	HIV+: 50·0% (36·1–63·9); HIV−: 73·2% (62·7–81·6); p=0·01	HIV+: 69·6% (55·2–80·1); HIV−: 82·9% (73·4–89·6); p=0·09
Scott et al, 2011[50]	South Africa	Outpatients (mostly HIV+) with suspected TB with cough for ≥2 weeks	Presentation with suspected TB with cough ≥2 weeks	HIV+: 54% (38–69)	HIV+: 84% (69–93)
Lawn et al, 2011[51]	South Africa	Outpatients (HIV+) enrolling in an antiretroviral treatment clinic	Unselected patients screened for TB irrespective of symptoms before antiretroviral therapy	HIV+: 22·2% (13·3–33·6)	HIV+: 58·3% (46·1–69·8)
Studies of hospital inpatients					
O'Grady et al, 2012[52]	Zambia	Hospital medical inpatient admissions (HIV+ and HIV−)	All who could produce sputum samples	HIV+: 52·8% (45·1–60·4); HIV−: 48·6% (33·0–64·4); p=0·71	HIV+: 88·2% (81·9–92·6); HIV−: 74·3% (56·4–87·0); p=0·033
Balcells et al, 2012[53]	Chile	Hospital medical inpatients (HIV+)	Admission with suspected TB and symptoms >10 days	HIV+: 66·7% (39·1–86·2)	HIV+: 91·7% (64·6–98·5)
Carriquiry et al, 2012[54]	Peru	Hospital medical inpatients (HIV+)	Admission with suspected TB and cough >10 days plus abnormal chest radiograph plus additional symptoms	HIV+: 68·9% (54·3–80·6)	HIV+: 86·3% (74·3–93·2)

TB=tuberculosis. MTB=*Mycobacterium tuberculosis*. RIF=rifampicin.

Table 3: Studies assessing the diagnostic accuracy of the Xpert MTB/RIF assay compared with culture in patients with HIV investigated for pulmonary tuberculosis

when larger volumes of urine were concentrated by centrifugation.[41] This might represent an important alternative diagnostic modality for the sickest patients with HIV-associated tuberculosis, especially those who cannot produce sputum samples. Studies are needed to assess the effect of the new diagnostic on different populations, including those in which treatment is frequently started presumptively on the basis of clinical assessment.

Xpert MTB/RIF assay for active pulmonary tuberculosis case finding

In addition to screening for tuberculosis before anti-retroviral therapy,[51] use of the Xpert MTB/RIF assay for active case finding is being explored in other clinical populations. This assay might enable active tuberculosis screening to be done within antenatal clinics in high tuberculosis burden settings,[56] for example, although data are awaited. If this assay was done at point-of-care, screening could be much more readily integrated into the antenatal care pathway. The Xpert MTB/RIF assay has also been used successfully in a small pilot study of active case finding in household contacts of smear-positive index cases in Tanzania.[57] In a large tuberculosis prevalence survey in a South African gold mine,[58] the sensitivity was substantially higher than that of smear microscopy, but much lower (62·6%, 95% CI 55·2–69·5) than that of the liquid culture reference standard, which is consistent with the findings of active case finding in an antiretroviral treatment clinic.[51] Disadvantages of the Xpert MTB/RIF assay in the prevalence survey were that it tested positive in a subset of patients currently or previously treated for tuberculosis and was also more expensive per test than smear microscopy and liquid culture combined, although this excess cost might be offset by recent cartridge price reductions and by the advantage of test simplicity.[58]

Rifampicin resistance

Despite the first large-scale multicountry assessment of the Xpert MTB/RIF assay by FIND reporting high specificity for detection of rifampicin resistance,[27] several subsequent studies have reported cases of confirmed false-positive rifampicin resistance detected with the original version of the assay.[50,51,59–61] Although absolute numbers of such cases have been quite small, this drawback is a substantial problem for clinical decision making in settings where the prevalence of rifampicin resistance is low and the positive predictive value for rifampicin resistance is therefore poor. Where resistance is present in more than 15% of isolates, the positive predictive value is estimated to be more than 90%, but where the prevalence is under 5%, the positive predictive value might be less than 70%.[62] Moreover, although the Xpert MTB/RIF assay seems to provide a high sensitivity initial screen for MDR tuberculosis, data from 14 supranational tuberculosis reference laboratories show that 0·5–11·6% of rifampicin-resistant strains are sensitive to isoniazid, with marked regional variation.[63] WHO has recommended that patients with rifampicin-resistant results should receive an MDR tuberculosis treatment regimen pending additional culture-based investigation and DST for first-line and second-line drugs.[62]

In addition to false-positive rifampicin resistance results, a few studies reported a high rate of inconclusive results. In 2011, the manufacturers did a root cause analysis of these problems, and software and reagent changes have subsequently been made to the cartridges, including the redesign of probe B. The new software and cartridge combination, called G4, has undergone analytic laboratory assessment, and was launched in December, 2011.[64] Early reports from South Africa suggest that the concordance with the rifampicin resistance results of line-probe assays is improved and that inconclusive results are decreased using the G4 cartridges,[65] but more solid evidence is awaited.

Costs and cost-effectiveness

The high cost of this technology (similar to that of liquid culture, but far exceeding that of smear microscopy) is seen as a key hurdle to implementation.[66,67] FIND negotiated a discounted pricing structure applicable to 145 high burden and developing countries.[68] A four module GeneXpert platform and linked computer costs about US$17 000 (more than 60% lower than elsewhere). Compared with cartridge costs of roughly $65 in the European Union, discounted costs were initially $18·68 per cartridge when first endorsed by WHO.[69] Costs have since fallen, and with funding from the President's Emergency Plan For AIDS Relief, US Agency for International Development, UNITAID, and the Bill & Melinda Gates Foundation, the cost per cartridge was set at $9·98 from Aug 6, 2012, for the next 10 years.[68]

Initial analyses of the use of the Xpert MTB/RIF assay in countries with a high burden of tuberculosis suggest that this technology is likely to be a highly cost-effective method of tuberculosis diagnosis,[70–72] although this will of course be setting specific. Cost-effectiveness does not denote affordability, however, and in the poorest countries of the world with a high tuberculosis burden, the total yearly expenditure per head on health might be little more than $10–20. Moreover, neither the true costs of implementation nor the overall benefits are known. In South Africa, for example, the national scale-up of the Xpert MTB/RIF assay is estimated to be associated with a 53–57% increase in the yearly cost of the tuberculosis diagnostic programme.[73] These costs would also vary depending on whether GeneXpert machines were placed only in existing microscopy laboratories or were extended to all facilities providing tuberculosis treatment, which could increase the budget by more than 50%.[74] Moreover, increased overall case detection and diagnosis of MDR tuberculosis are estimated to increase the treatment programme costs by 34–37%.[73] Conversely, the potential benefits from reduced morbidity, mortality, and disease

transmission associated with appropriate delivery of tuberculosis treatment and lower rates of inappropriate therapy have yet to be defined. In South Africa, only about half of notified tuberculosis cases are micro-biologically confirmed, and whether implementation of the Xpert MTB/RIF assay will increase the overall number of tuberculosis diagnoses or simply increase the proportion of cases with microbiological confirmation is unknown. Further cost-effectiveness analyses using data generated during scale-up in the field will be essential.

Implementation and scale-up of the Xpert MTB/RIF assay

WHO policy guidance on the Xpert MTB/RIF assay has been incorporated into national guidelines by a third of reporting countries.[1] Two-thirds of high tuberculosis burden countries and a half of high MDR tuberculosis burden countries have already incorporated the assay into their revised diagnostic policies. Up to the end of June, 2012, 1·1 million test cartridges were procured by 67 (46%) of the 145 countries eligible to purchase them at FIND-negotiated concessional prices.[1,4] Scale-up is expected to be substantially accelerated by the reduction in cartridge costs announced in August, 2012.[68]

WHO endorsement of the Xpert MTB/RIF assay has resulted in rapid donor and partner-driven infusions of GeneXpert machines and cartridges into countries. Although this unprecedented support of tuberculosis diagnostic implementation should be encouraged, maximising the effects and long-term sustainability of the Xpert MTB/RIF assay will probably prove to be dependent on national ministry of health leadership, strategic planning, coordination of technical partners and donors, and continuous monitoring and assessment. Large-scale implementation will invariably need revision of national algorithms, policies, registers, request forms, and monitoring and assessment methods. Thus, ministries of health are encouraged to take a step-wise approach to introduction and scale-up, beginning with the establishment of an in-country coordination mechanism, such as an Xpert MTB/RIF assay technical working group or advisory team. Such working groups should include representation from all key stakeholders, including national tuberculosis and AIDS control programmes, national public health laboratories and supranational tuberculosis reference laboratories, implementing partners, and donors and should be tasked with leading the strategic planning, implementation, and assessment processes. Implementation plans should consider the local epidemiology, available diagnostic services and laboratory systems, and first-line and second-line drug treatment capacity. Moreover, implementation should be in line with relevant strategic plans (eg, national tuberculosis and AIDS control programmes and national laboratory strategic plans). Furthermore, implementation should be closely linked to monitoring and assessment of clearly defined outcome measures to inform revisions in procedures, policies, and plans.

South Africa has led the way with national implementation of the Xpert MTB/RIF assay. The South African Ministry of Health has recommended replacement of smear microscopy as the initial diagnostic test for tuberculosis. This step is unlikely to be taken by other countries in the region in the foreseeable future because of cost and logistical constraints. As of June, 2012, South Africa accounted for 37% of the modules and 53% of the cartridges procured globally.[1] In March, 2011, the National Department of Health announced the plan to achieve national scale-up over 2–3 years. The South African National Health Laboratory Service launched a pilot programme, placing GeneXpert platforms in 25 smear microscopy centres across the country with throughputs ranging from 16 to more than 400 tests per day.[73] Following this successful pilot, machines are now being placed in all existing smear microscopy laboratories, fully replacing smear microscopy for diagnosis of pulmonary tuberculosis in South Africa.[73]

Panel 2: Key strengths and weaknesses of the Xpert MTB/RIF assay

Strengths

- Robust
- Good accuracy for tuberculosis diagnosis
- Simple to use
- Rapid (2 h) compared with existing tests
- Detects both *Mycobacterium tuberculosis* complex and rifampicin resistance
- Better sensitivity and specificity than smear microscopy
- Does not need advanced biosafety equipment
- Closed system with low risk of cross-contamination
- Could potentially be used to test a broad range of samples from extrapulmonary sites—eg, lymph node aspirates, gastric lavage, urine, and CSF (not yet endorsed by WHO; more data awaited)
- GeneXpert platform is multifunctional and could be used for other diagnostics such as HIV viral load
- Modular platform permits capacity to match demand in a given facility
- Operators do not need formal laboratory training

Weaknesses

- Expensive
- Sophisticated hardware needing calibration and maintenance and linkage to a computer and secured premises
- Operators need training in basic computer skills
- Needs continuous electrical power supply and air conditioning
- Storage of samples at room temperature restricted to 3 days
- Relatively short shelf life of reagent cartridges needing good procurement systems
- Need for cartridge storage at 2–28°C and system for disposal after use
- Although comparatively rapid, the turnaround time is a challenge for same-day diagnosis and treatment in overcrowded health facilities
- False-positive rifampicin resistance results
- Cannot differentiate between live and dead *M tuberculosis*, thus cannot be used to monitor treatment success or failure, or relapse
- Cannot differentiate between *M tuberculosis, M bovis,* and BCG vaccine
- Use with extrapulmonary samples is not yet fully defined

Embedded research studies and monitoring and assessment are likely to yield invaluable data that will increase the understanding of how best to implement this assay.

Challenges associated with implementation

Panel 2 summarises the key strengths and weaknesses of the Xpert MTB/RIF assay and panel 3 summarises the potential benefits as well as the challenges of Xpert MTB/RIF assay implementation for routine use in resource-limited settings.[66,75] Increased diagnosis of drug-sensitive tuberculosis and MDR tuberculosis should be matched by expanded capacity to effectively treat these cases, including a scale-up in quality MDR tuberculosis treatment facilities and trained staff. Rigorous quality assessment programmes will also be needed, following, for example, a model developed in South Africa that used dried culture spots of inactivated *M tuberculosis* on filter paper.[76] This is essential to ensure that results are accurate.

Despite being relatively simple, implementation of the Xpert MTB/RIF assay in resource-limited settings has needed investments in training of operators and laboratory staff. The computer interface has been more challenging than expected for operators in some countries and additional training has been needed. This issue should be taken into consideration by continuing efforts aimed at development of nucleic acid amplification-based platforms for implementation in more decentralised facilities. Cartridges have to be stored at 2–28°C, which might be difficult in hot climates where transportation is difficult and lengthy and where a cold chain is not available.

A further challenge is the feasibility of deploying the assay at the point-of-care. Centralised location has been associated with failures to link results to patients to inform treatment in a timely manner, undermining outcomes.[77] By contrast, use of the assay at the district and subdistrict levels resulted in a substantial increase in treatment uptake in the FIND implementation study.[28] Location and use within tuberculosis treatment facilities adds further challenges. In South Africa, for example, laboratory placement would need 274 instruments, whereas location at points of treatment would require 4020 instruments with a 51% increase in cost ($107 million per year).[74] Moreover, results of operational research into point-of-care implementation showed that the turnaround time for sample processing was often more than 2 h and that failure to link results to patients on the same day was an unforeseen difficulty.[78] A faster assay would be a substantial advantage in this respect. Taking a patient through the whole process of obtaining samples, running the Xpert MTB/RIF assay test, linking results back to the patient, and starting tuberculosis treatment on the same day needed a substantial increase in human resource requirements in the clinic, such that the equivalent of an additional 2·5 staff were needed to manage 16 patients per day suspected of tuberculosis.[78] Use of the assay in the clinic was also associated with

increased management responsibilities. Nevertheless, this was offset by increased case detection with same-day treatment initiation in more than 80% of new cases, a corresponding increase in enthusiasm and morale of clinic staff, and reduced laboratory requirements.

Other advances in tuberculosis diagnostic tests

Further developments in nucleic acid amplification test (NAAT) technology are promising.[4] A simplified manual NAAT using loop-mediated isothermal amplification with a simple visual colorimetric read-out is being assessed for use in peripheral laboratory facilities in resource-limited settings.[4,79] However, fully automated systems that use isothermal amplification and operate at lower temperatures could potentially be used outside the laboratory environment.[4,80] Hand-held systems the size of a smartphone produce PCR product more rapidly and have much lower power needs than does GeneXpert, permitting battery operation. Identification of resistance mutations to several key drugs with multiplexed assays might greatly reduce the need for follow-on DST. Thus, several fully

Panel 3: Use of Xpert MTB/RIF assay as the initial diagnostic test for tuberculosis in resource-limited settings

Anticipated benefits
- Increase in tuberculosis case detection, especially of smear-negative disease
- Reduction in time to diagnosis and treatment
- Reduced patient default during investigation for tuberculosis, increasing uptake of tuberculosis treatment
- Reduced morbidity, mortality, and tuberculosis transmission
- Increased detection and treatment of multidrug-resistant tuberculosis
- Increased morale of health-care workers in tuberculosis services
- Reduced need for culture
- Reduced biohazard
- Reduced presumptive prescribing of tuberculosis treatment

Challenges
- Increase in budget needed for tuberculosis diagnostics
- Additional testing for drug resistance needed for those testing positive for rifampicin resistance
- Use of the assay in centralised laboratories might blunt the potential effect of this near-patient technology
- Use of outside laboratories might be associated with increased human resource needs and administrative responsibilities in clinics
- Rapid diagnosis has to be translated into more rapid treatment initiation, which is challenging in some settings
- Increased diagnostic capacity should be matched by increases in treatment capacity for drug sensitive and multidrug-resistant tuberculosis
- Diagnostic algorithms, notification systems, and methods for monitoring and assessment need to be redefined
- Restricted operating temperature range
- Need for a stable electricity supply
- Instruments and associated computers might break down or be stolen
- Need for external quality assurance and yearly calibration of instruments
- Need for robust supply chains and storage facilities for bulky cartridges with short shelf-life

automated assays that compete with the Xpert MTB/RIF assay and that will be more applicable for point-of-care are likely to be developed in the future.[4] However, how the donor assistance that has heavily subsidised the implementation of Xpert MTB/RIF in resource-limited settings will affect the development and entry of newer diagnostic assays to the marketplace is not clear. Commercially, funding is not a level playing field. Moreover, the up-front costs of doing field assessment trials needed to gain regulatory approval and WHO endorsement are substantial and might be prohibitive for small companies. Furthermore, the prospect of the emergence of cheaper rapid tests more applicable at the periphery (community level) poses an interesting dilemma as to whether investment should be made in current more costly technology, or whether it might be better to wait for the next generation of tests to become available.

A promising development is a point-of-care immuno-chromatographic (dip-stick) assay that detects myco-bacterial lipoarabinomannan in urine.[81–83] The specific niche for this assay seems limited to the diagnosis of HIV-associated tuberculosis in patients with advanced immunodeficiency (CD4 cell counts <200 cells/μL), such as those being screened in antiretroviral treatment clinics or medical inpatients with HIV.[81,84–86] Studies from South Africa have reported that in patients with the lowest CD4 cell counts, the assay can potentially diagnose around two-thirds of cases with high specificity within 30 min.[83] Patients whose tuberculosis is detected by this assay are the subset who are likely to have disseminated tuberculosis, have the highest mortality risk,[87,88] and are most likely to benefit from same-day initiation of tuberculosis treatment. More data on standardisation of test production by the manufacturer and on diagnostic accuracy in well conducted studies in different settings are needed, as are studies of clinical effect.

Other systems such as breathalysers that detect *M tuberculosis*-specific antigens[89] and so-called electronic noses that detect volatile biomarkers using chemical sensors and pattern recognition systems[90,91] are being explored. Meanwhile, in the present postgenomics era, the diagnostics developmental pathway should continue to be fuelled by basic research and development to identify biomarkers that can serve as new specific targets for diagnostic assays.[92]

Future prospects for point-of-care diagnosis

The ideal test for tuberculosis will be a true point-of-care assay that enables accurate diagnosis of tuberculosis and detection of drug resistance within the time of a clinic consultation, and one that can be implemented at all levels of the health system for adults and children, with and without HIV.[93] Although the Xpert MTB/RIF assay undoubtedly represents an important breakthrough and step forward towards this ideal, its high relative cost, sophisticated hardware, and constraints for point-of-care use will undoubtedly restrict its implementation. Future

advances in molecular diagnostics should build on this success and tackle these remaining challenges.[94] Despite recent developments in nucleic acid amplification-based diagnostics and related technological platforms, the tuberculosis diagnostic pipeline is nevertheless weak and should be strengthened. The need for a better test for paediatric tuberculosis diagnosis is largely unmet because there is no evidence to suggest that the Xpert MTB/RIF assay will have a major effect on tuberculosis diagnosis in this group.

Conclusions

The emergence of the Xpert MTB/RIF assay represents a major step forward in tuberculosis diagnostics. Although this assay is not perfect, the advantages offered in settings with high disease burdens and high rates of drug-resistant and HIV-associated tuberculosis convinced a country such as South Africa to adopt this technology as the initial diagnostic test for pulmonary tuberculosis. More wide-scale implementation of the Xpert MTB/RIF assay will provide data on clinical effect and programmatic outcomes so that the true cost-effectiveness of the assay can be assessed. Rapid developments in nucleic acid amplification technology are fuelling the emergence of further fully automated systems that might be more readily implementable at the point of care. However, a rapid, accurate, and affordable diagnostic test for tuberculosis that can be easily implemented is urgently needed. Greater investment in the developmental pipeline for tuberculosis diagnostics remains a priority for funders and developers.

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