Rapid molecular TB diagnosis: evidence, policy making and global implementation of Xpert MTB/RIF

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ABSTRACT If tuberculosis (TB) is to be eliminated as a global health problem in the foreseeable future, improved detection of patients, earlier diagnosis and timely identification of rifampicin resistance will be critical. New diagnostics released in recent years have improved this perspective but they require investments in laboratory infrastructure, biosafety and staff specialisation beyond the means of many resource-constrained settings where most patients live. Xpert MTB/RIF, a new assay employing automated nucleic acid amplification to detect *Mycobacterium tuberculosis*, as well as mutations that confer rifampicin resistance, holds the promise to largely overcome these operational challenges. In this article we position Xpert MTB/RIF in today's TB diagnostic landscape and describe its additional potential as an adjunct to surveillance and surveys, taking into account considerations of pricing and ethics. In what could serve as a model for the future formulation of new policy on diagnostics, we trace the unique process by which the World Health Organization consulted international expertise and systematically assessed published evidence and freshly emerging experience from the field ahead of its endorsement of the Xpert MTB/RIF technology in 2010, summarise subsequent research findings and guidance on who to test and how, and provide perspectives on scaling up the new technology.

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The most up-to-date evidence, policy-making and global implementation of the new diagnostic Xpert MTB/RIF http://ow.ly/ks9dN

This article has supplementary material available from www.erj.ersjournals.com

Received: Oct 04 2012 | Accepted after revision: Nov 08 2012 | First published online: Nov 22 2012

Introduction

With 8.7 million incident cases of tuberculosis (TB) and 1.4 million deaths estimated in 2011 [1], TB is a leading cause of morbidity and mortality worldwide. However, public health services globally reported only 5.8 million (66%) of the estimated TB cases in 2011. Moreover, less than 5% of notified TB cases were tested for drug resistance [1], which is often diagnosed after prolonged diagnostic delays [2–4]. Of the 310 000 notified new and re-retreatment cases with pulmonary TB estimated to have multidrug-resistant (MDR)-TB in 2011, just under 60 000 (19%) were reported to the World Health Organization (WHO) [1].

The main reasons for these gaps are inadequate diagnostic capacity and an over reliance on chest radiography and/or sputum smear microscopy as diagnostic tools. Patients with HIV-associated TB, those with sputum smear-negative and/or extrapulmonary disease, and drug-resistant TB patients are particularly affected by the failure of microscopy as a primary diagnostic tool. The ''classical'' diagnosis of HIV-associated and drug-resistant TB is complex, expensive, slow and technically demanding, relying on conventional culture and drug susceptibility testing (DST). The long delay (up to several weeks) required to obtain results has devastating consequences for patients who go undiagnosed (and therefore untreated or inappropriately treated), or are diagnosed too late [5].

Detecting more cases, detecting them early and rapidly identifying drug resistance are essential for improving individual patient health and avoiding transmission in the community. This requires universal access and early detection using contemporary tools and innovative strategies [1, 4, 5].

The past decade has seen unprecedented growth in the TB diagnostic pipeline and accelerated efforts to establish the necessary laboratory infrastructure [5]. Nevertheless, although recommended by WHO, the latest generation liquid culture diagnostics and molecular line probe assays for rapid detection of MDR-TB have not yet solved the diagnostic dilemma in most resource-limited settings, largely due to the need for expensive laboratory infrastructure, extensive biosafety precautions and specialised staff [5]. A new rapid test that overcomes many of the current operational difficulties was recommended for use by WHO in December 2010: the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is an automated, real-time nucleic acid amplification technology run on the multi-disease platform GeneXpert (Cepheid). The Xpert MTB/RIF assay represents a paradigm shift in the diagnosis of TB and drug-resistant TB by simultaneously detecting *Mycobacterium tuberculosis* and rifampicin resistance-conferring mutations in a closed system suitable for use outside conventional laboratory settings in less than 2 h, directly from sputum samples [6, 7].

Objectives

This article has three primary objectives. The first is to describe the dynamic process followed by WHO in policy development for TB diagnostics, using the example of Xpert MTB/RIF assay as a pathfinder. The second is to summarise subsequent evidence on the use of Xpert MTB/RIF, clarify common misconceptions about the technology, and provide perspectives on the role of the assay in improved case detection and care delivery. The third is to summarise the relevance of the technology for TB prevalence surveys and drug resistance surveillance, its impact on case and treatment outcome definitions, and discuss issues around affordability, sustainability, ethics and research priorities.

Methods

Existing policy and guidance documents on Xpert MTB/RIF are summarised to illustrate the WHO policy formulation process for new TB diagnostics. Outcomes are presented from a Global Consultation organised by WHO immediately prior to endorsement of the assay. Experiences shared by early implementers of the assay during two subsequent WHO global meetings are also summarised.

For additional evidence on the Xpert MTB/RIF assay since WHO endorsement, active scanning of the emerging literature was performed. PubMed and EMBASE results were searched to find articles dealing with the Xpert MTB/RIF test. Combinations of the following search terms were used: "tuberculosis", ''multidrug-resistant tuberculosis'', ''extensively drug-resistant tuberculosis'', ''Xpert MTB/RIF'' and ''rapid diagnosis". Although the search was not restricted to publications in English, articles not reporting an English summary were excluded.

Citations were independently screened by four investigators (K. Weyer, W. van Gemert, G.B. Migliori and R. Centis) by examining titles, abstracts and full articles to identify relevant studies, which are stored in the WHO database and regularly updated (last update September 21, 2012). Unpublished sources of data (multicentre laboratory validation and demonstration studies coordinated by FIND (Foundation for Innovative New Diagnostics; Geneva, Switzerland) and unpublished data from investigator-driven, single-centre studies) shared with WHO at the time of policy development were also included. Although this perspective article includes all available evidence on Xpert MTB/RIF, the formal criteria for a systematic review were not followed.

Brief overview of the Xpert MTB/RIF technology

The GeneXpert platform, launched in 2004, simplifies real-time PCR-based molecular testing by integrating and automating the key processes of sample preparation, amplification and detection. Core components of the system include the instrument, a personal computer, a barcode scanner and the software (fig. 1), together with disease-specific, single-use, disposable cartridges containing lyophilised reagents, buffers and washes. Target detection and characterisation is performed in real time using a six-colour laser detection device.

The Xpert MTB/RIF cartridge for the simultaneous detection of TB and rifampicin resistance was developed within 4 years, following a unique collaboration between academia and industry, brokered by FIND and financially supported by the US National Institutes of Health (Bethesda, MD, USA) and the Bill and Melinda Gates Foundation (Seattle, WA, USA) [8–13]. This collaboration serves as a blueprint for TB diagnostics development, consisting of clear end-user product specifications, adequate research funding, collaboration among academic partners on the core components of the technology, pooling of research resources, controlled clinical validation trials, large-scale field evaluations under well-designed operational research protocols, and a flexible response by industry engaging early on with FIND in negotiations on cost and preferential pricing.

The end-product was a fully automated, closed (and therefore safe) real-time PCR system, requiring basic not specialised laboratory infrastructure, operator skills or biosafety precautions [8–13]. The Xpert MTB/RIF assay employs five unique nucleic acid hybridisation probes (molecular beacons), each labelled with a coloured fluorophore responding to a specific target sequence within the *rpoB* gene of *M. tuberculosis*. More than 95% of mutations associated with rifampicin resistance occur in an 81-base pair core region of the rpoB (a bacterial RNA polymerase) gene and together these five molecular beacons encompass the entire core region. The generation of all five fluorescent colours during PCR amplification indicates the presence of rifampicin-susceptible *M. tuberculosis*, while any mutation in the core region prevents the binding of the respective molecular beacon, resulting in the absence of colour and indicating rifampicin resistance (fig. 2) [8–13].

Evidence-based policy development

In 2008, WHO adopted the international GRADE process (Grades of Recommendations Assessment, Development and Evaluation) for evidence synthesis and evaluation [14]. GRADE currently underpins all WHO recommendations and guidelines [15]. Recently refined for the evaluation of diagnostics [16], GRADE provides a systematic assessment of the quality of evidence underlying policy formulation, as well

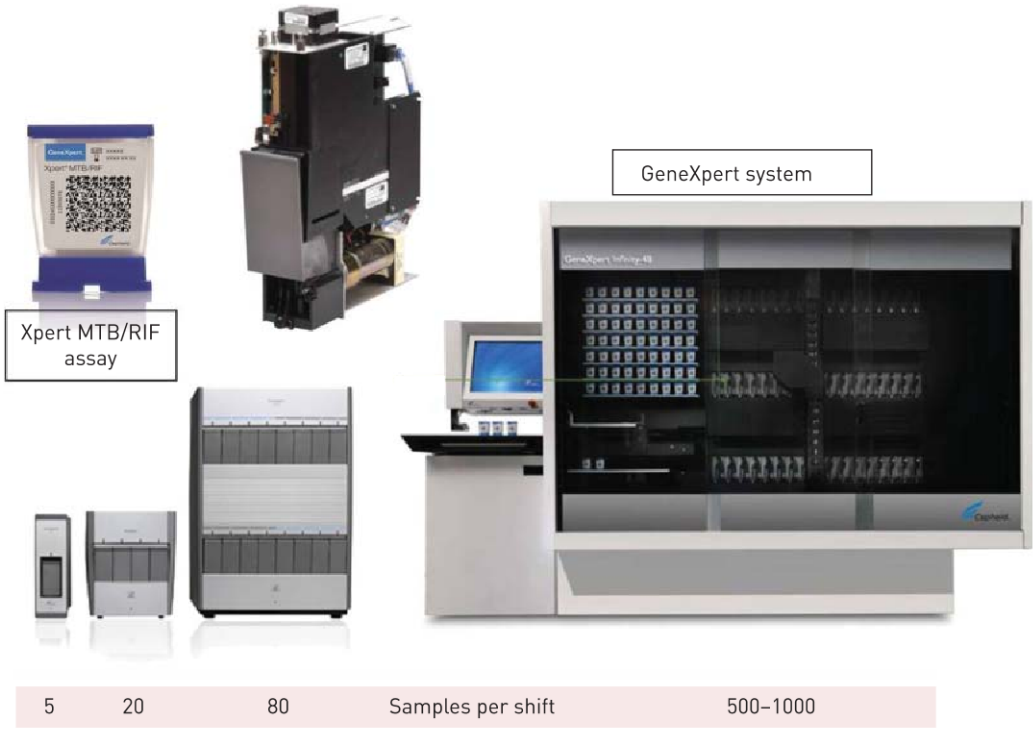

FIGURE 1 The GeneXpert system (personal communication; Foundation for Innovative New Diagnostics, Geneva, Switzerland).

DOI: 10.1183/09031936.00157212

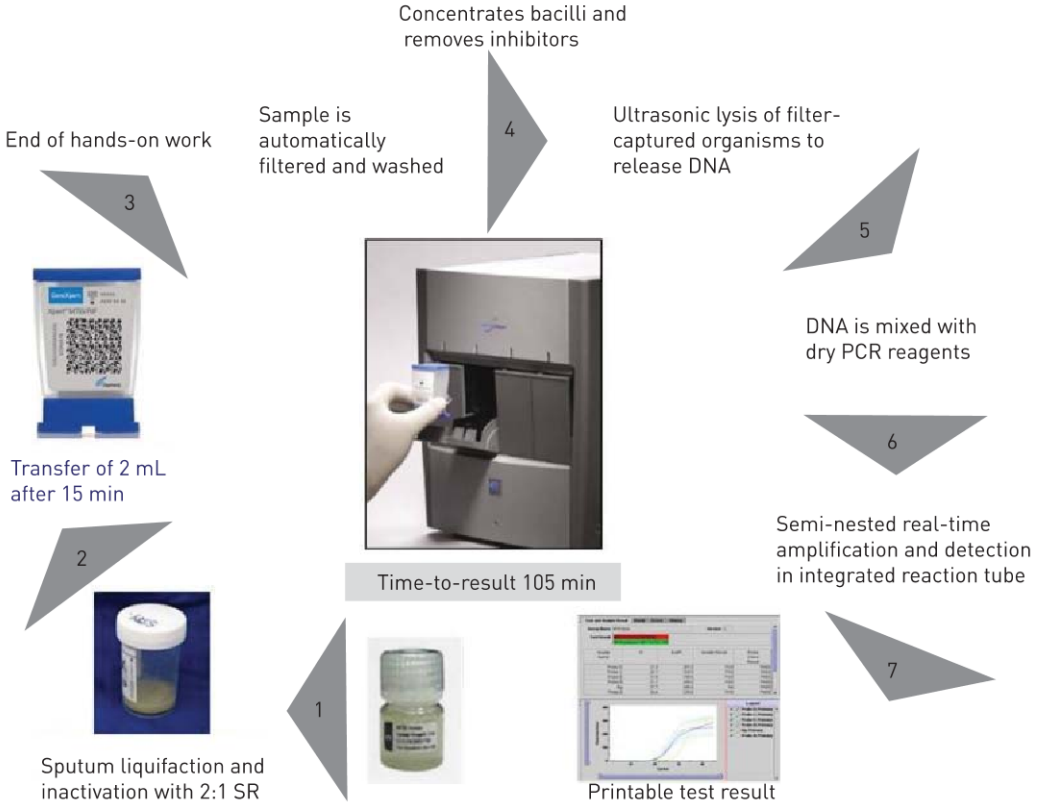

FIGURE 2 The step-by-step Xpert MTB/RIF assay process. SR: sterilising reagent. Reproduced from [6] with permission from the publisher.

as the strength of policy recommendations, aiming to achieve a balance between test performance, risks and benefits, and patient/public health impact [14, 16]. The process is overseen by the WHO Guidelines Review Committee, which was specifically established for this purpose [15].

Figure 3 illustrates the structured approach to policy development on new TB diagnostics established in 2008 by the WHO Stop TB Department, while table 1 outlines the body of evidence required by WHO to proceed with policy formulation on TB diagnostics.

Dynamic policy development on Xpert MTB/RIF

In early September 2010, an Expert Group convened by WHO assessed the available data on Xpert MTB/RIF, including information from six published papers [8–13], two large multicentre laboratory validation and demonstration studies coordinated by FIND [18, 19], results from cost-effectiveness analyses [17] and unpublished data from 12 investigator-driven, single-centre studies (most of which were subsequently published) shared with WHO under nondisclosure agreements. The GRADE evaluation assessed assay performance, the feasibility and anticipated impact of programmatic implementation, cost-effectiveness, and issues to be addressed in future research.

Recommendations from the Expert Group were subsequently endorsed by STAG-TB (Strategic and Technical Advisory Group for Tuberculosis) in late September 2010, and WHO was advised to proceed immediately with policy guidance, develop a global strategy for rapid update, convene a Global Consultation on implementation considerations, and assist countries with technical support and planning [20].

WHO convened a Global Consultation in early December 2010, which was attended by approximately 120 institutional and country representatives. An agreement was reached on interim diagnostic algorithms and the positioning of Xpert MTB/RIF in defined risk groups (MDR-TB and HIV-associated TB) at different levels of health services. Consensus agreements were incorporated into a subsequent WHO Rapid Implementation document [7] supported by an Xpert MTB/RIF checklist [21] providing practical suggestions for systematic roll-out of the assay to optimise use and benefits of the technology while addressing key operational research aspects in more longitudinal efforts.

FIGURE 3 The World Health Organization (WHO) policy development process for tuberculosis (TB) diagnostics. GRADE: Grades of Recommendations Assessment, Development and Evaluation; QUADAS: Quality Assessment of Diagnostic Accuracy Studies; STAG-TB: Strategic and Technical Advisory Group for Tuberculosis.

In April 2011, WHO convened a meeting with early implementers of the Xpert MTB/RIF assay to refine the proposed diagnostic algorithms, develop a core set of variables to determine the impact of introducing the technology on laboratory workload, and clarify operational and logistical issues. A second WHO meeting of early implementers followed in April 2012 to share experiences during the introduction of the assay under routine TB control programme conditions.

Under its mandate to coordinate the global roll-out of Xpert MTB/RIF, WHO established a dedicated website and electronic data collection tool (http://who.int/tb/laboratory/mtbrifrollout/en/index.html), tracking country implementation and partner plans for scale-up in 145 countries eligible for preferential pricing of the assay, as well as to collect information from post-marketing surveillance of operational problems to guide scale-up of the technology under programmatic conditions.

Formal WHO policy recommendations on the use of Xpert MTB/RIF issued on December 8, 2010 arose from a solid GRADE assessment of the available evidence (table 2) [6].

Analytical studies

The Xpert MTB/RIF assay has analytic sensitivity of five genome copies of purified DNA, and 131 CFU·mL^{-1} of *M. tuberculosis* spiked into sputum. No cross-reactivity with non-tuberculous mycobacteria (NTM) was detected. TB and resistance to rifampicin were correctly detected when NTM DNA or mixed susceptible and resistant strains were present. The sample reagent, added in a 2:1 ratio to sputum, killed >6 $\log_{10}$ CFU·mL^{-1} of *M. tuberculosis* with 15 min of exposure and rendered >97% of sputum smear-positive samples negative by Lowenstein–Jensen culture. No infectious aerosols were detected following the Xpert MTB/RIF inoculation procedure and sample testing [8–13].

DOI: 10.1183/09031936.00157212

TABLE 1 Evidence required by the World Health Organization (WHO) to enable tuberculosis (TB) diagnostic policy development and policy update

Phase 1: research and development
- Typically consists of upstream research and development to define and validate a prototype, followed by laboratory validation under international standards that culminate in a design-locked product
- WHO interacts with developers if requested to discuss end-user requirements such as biosafety, assay robustness and intended setting of use

Phase 2: evaluation and demonstration
- The performance of the new diagnostic product should be evaluated in controlled trials at three to five trial sites in high-burden TB and HIV countries, ideally using pre-specified and-user product specifications. These data are often used for product registration with global and/or regulatory authorities such as FDA and/or CE marking.
- Product specifications and performance should subsequently be validated in uncontrolled trials under field conditions in five to 10 trial sites in high-burden TB and HIV countries, and include cost-effectiveness studies.

Phase 3: WHO evidence assessment using GRADE
- For new technologies or new indications for use of technologies already approved by WHO
  - Submission of dossier with phase 1 and 2 data to WHO
  - Structured evidence assessment process (described in figure 3)
- For fast-follower or generic versions of technologies already approved by WHO
  - Manufacture of the technology under ISO 13:485 standards; equivalent performance demonstrated in two to three independent supranational TB reference laboratories, to the reference technology already approved by WHO
  - Structured evidence assessment process (described in figure 3)
- WHO is not a regulatory authority and does not recommend technologies for individual country use

Phase 4: phased uptake and collection of evidence for scale-up
- New diagnostic successfully implemented in routine diagnostic services by early implementers in high-burden TB and HIV countries; systemic assessment of proposed algorithms, laboratory workload, operational constraints, country by cost-effectiveness. Lessons learnt by early implementers used for country adaptation.

Phase 5: scale-up and policy refinement
- Scale-up of the new diagnostic, with subsequent data used to inform and refine WHO policy guidance in a dynamic and ongoing process

FDA: Food and Drug Administration; CE: Communauté Européenne (European Community); GRADE: Grades of Recommendations Assessment, Development and Evaluation; ISO: International Organization for Standardization.

Controlled clinical validation trials

The performance of Xpert MTB/RIF was tested in 1730 patients suspected to be affected by drug-susceptible or pulmonary MDR-TB from Peru, Azerbaijan, South Africa and India. Among culture-positive patients, a single, direct Xpert MTB/RIF test identified 98.2% (551out of 561) of sputum smear-positive TB cases and 72.5% (124 out of 171) of those with sputum smear-negative TB. The test was specific in 604 (99.2%) out of 609 patients not affected by TB. A second Xpert MTB/RIF test among patients with sputum smear-negative, culture-positive TB increased sensitivity by 12.6% and a third by 5.1%, to reach 90.2%. When compared to phenotypic DST, the Xpert MTB/RIF assay identified correctly 97.6% (200 out of 205) of patients harbouring rifampicin-resistant strains and 98.1% (504 out of 514) of those with rifampicin-susceptible strains. Sequencing resolved all but two cases in favour of Xpert MTB/RIF [18].

TABLE 2 World Health Organization (WHO) policy recommendations on Xpert MTB/RIF

The GRADE process confirmed a solid evidence base to support widespread use of Xpert MTB/RIF for detection of TB and rifampicin resistance and resulted in the following main recommendations:
- Xpert MTB/RIF should be used as the initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB (strong recommendation)
- Xpert MTB/RIF may be considered as a follow-on test to microscopy in settings where MDR-TB or HIV is of lesser concern, especially in further testing of smear-negative specimens (conditional recommendation, acknowledging major resource implications)

Remarks
- These recommendations apply to the use of Xpert MTB/RIF in sputum specimens (including pellets from decontaminated specimens)
- Data on the utility of Xpert MTB/RIF in extrapulmonary specimens are still limited
- These recommendations support the use of one sputum specimen for diagnostic testing, acknowledging that multiple specimens increase the sensitivity of Xpert MTB/RIF but have major resource implications
- These recommendations also apply to children, based on the generalisation of data from adults and acknowledging the limitations of microbiological diagnosis of TB (including MDR-TB) in children
- Access to conventional microscopy, culture and DST is still needed for monitoring of therapy, prevalence surveys and/or surveillance and recovering isolates for DST other than rifampicin (including second-line anti-TB drugs)

GRADE: Grades of Recommendations Assessment, Development and Evaluation; MDR: multidrug resistant; DST: drug susceptibility testing. Data from [6].

Field demonstration studies

6648 individuals were prospectively enrolled in South Africa, Peru, India, Azerbaijan, the Philippines and Uganda, comparing Xpert MTB/RIF with microscopy in decentralised microscopy centres, and with culture and phenotypic DST in centralised laboratories. Xpert MTB/RIF detected 90.3% (933 out of 1033) of the culture-confirmed TB cases, compared with 67.1% (699 out of 1041) using microscopy. In sputum smear-negative, culture-positive TB patients Xpert MTB/RIF test sensitivity was 76.9% (296 out of 385) and specificity was 99.0% (2846 out of 2876). Sensitivity for rifampicin resistance was 94.4% (236 out of 250) and specificity was 98.3% (796 out of 810) [19].

While HIV co-infection substantially decreased the sensitivity of microscopy (to 47%), Xpert MTB/RIF performance was not significantly affected. The median time to detection of TB was 0 days (interquartile range (IQR) 0–1) using Xpert MTB/RIF, compared to 1 day (IQR 0–1) for microscopy, 30 days (IQR 23–43) for solid culture and 16 days (IQR 13–21) for liquid culture. The median time to detection of rifampicin resistance was 20 days (IQR 10–26) for line-probe assay *versus* 106 days (IQR 30–124) for phenotypic DST.

The Xpert MTB/RIF test reduced the median time to treatment for sputum smear-negative TB from 56 days (IQR 39–81) to 5 days (IQR 2–8). The indeterminate rate of Xpert MTB/RIF testing was 2.4% (126 out of 5321 samples) compared to 4.6% (441 out of 9690) for culture.

Unpublished, single-centre evaluation studies

Results from 12 studies with varying design and study populations reported Xpert MTB/RIF sensitivity in detecting TB ranging from 70% to 100% in culture-positive patients and ~60% in those with smear-negative disease. Specificity ranged from 91% to 100%. Pooled average sensitivity for TB detection was 92.5% and pooled average specificity was 98%. Average rifampicin sensitivity and specificity were ~98% and 99%, respectively.

Operational and logistical issues

The available evidence confirmed the robustness of the Xpert MTB/RIF assay under varying temperature and humidity conditions, the need for minimal staff training, basic biosafety requirements (as for sputum smear microscopy) and high levels of user satisfaction. Operational challenges included the requirement for an ambient temperature $<30^{\circ}C$ (necessitating air conditioning in hot climates), and uninterrupted and stable electrical power supply (requiring generators in several sites). Storage space and conditions ($<28^{\circ}C$) for cartridges, waste generated (considerably more than for microscopy), and the 12-month shelf-life of cartridges were listed as main operational challenges [6, 7].

Cost, affordability and cost-effectiveness analyses

Using Xpert MTB/RIF for the diagnosis of smear-negative pulmonary TB was deemed cost-effective compared with existing diagnostic strategies in India, South Africa and Uganda, and within WHO acceptable incremental cost effectiveness ratios [6, 7, 17].

The cost of achieving the diagnostic targets in the Global Plan to Stop TB, 2011–2015 [22] with and without use of Xpert MTB/RIF was appraised for three population groups, *i.e.* TB patients considered at risk of having MDR-TB, people living with HIV with TB signs and symptoms, and all people with TB signs and symptoms. Using the FIND negotiated price at the end of 2010 of US$16.86 per cartridge, there were four main findings. First, a diagnostic strategy using Xpert MTB/RIF with follow-on DST for rifampicin-positive cases was a lower cost approach for reaching the 2015 targets for diagnosis of MDR-TB, both globally and in all high TB and high MDR-burden countries, compared with reliance on conventional culture and DST only. Secondly, using Xpert MTB/RIF to diagnose TB in people living with HIV in high HIV-prevalence settings was of similar or lower cost, compared with the conventional diagnostic algorithm (based on culture and radiography) recommended by WHO, in most countries. Thirdly, the total cost of using Xpert MTB/RIF to diagnose MDR-TB and TB among people living with HIV was a small fraction ($<5\%$) of total spending on TB control in 2010. Finally, the cost of using Xpert MTB/RIF to test all people with TB signs and symptoms was much higher compared with conventional diagnosis based on smear microscopy and radiographs, but in middle-income countries would be relatively affordable compared with total spending on TB care and control.

Operational research informing policy on Xpert MTB/RIF

Operational research on the Xpert MTB/RIF assay has proliferated subsequent to WHO endorsement. Of particular programmatic relevance are several operational research studies addressing key research questions identified following the WHO Global Consultation. As of July 2012, at least 24 operational research projects in 16 countries were registered, covering multiple implementation aspects [1].

DOI: 10.1183/09031936.00157212

By the end of August 2012, more than70 peer-reviewed publications, commentaries, viewpoints and editorials had been published [8–13, 17–19, 23–90], including an updated systematic review of 18 studies involving 10 224 specimens, confirming the initial findings [23]. A single Xpert MTB/RIF test detected 90.4% of culture-confirmed pulmonary TB patients (98.7% of smear-positive specimens and 75.0% of smear-negative specimens). Similar accuracy was retained in specimens from HIV-infected patients, showing pooled values of 81.7% sensitivity (95% CI 77.0–85.8%) and 98.0% specificity (95% CI 96.6–98.9%). The accuracy of Xpert MTB/RIF in detecting paediatric TB was 74.3% (95% CI 62.4–84.0%). Accuracy in detecting extrapulmonary TB reached 70.7% sensitivity (95% CI 59.6–80.3%) and 82.6% specificity (95% CI 79.5–85.3%). Accuracy estimates for rifampicin resistance detection were equally similar to the initial data, with pooled sensitivity of 94.1% (95% CI 91.6–96.0%) and pooled specificity of 97.0% (95% CI 96.0–97.7%).

Subsequent studies showed equally consistent results confirming the accuracy of the Xpert MTB/RIF assay in different settings and patient groups, with superior performance over microscopy. Most studies also confirmed the current operational limitations of the GeneXpert system: the sophisticated nature of the device requires care of handling, *i.e.* a stable and uninterrupted electrical supply to avoid interruption of the procedure and subsequent loss of results, an ambient temperature <30°C, security against theft, adequate storage space for the cartridges, and the need for sufficient staff to perform testing [6, 7, 21].

As with any other TB test, the positive predictive value (PPV) of Xpert MTB/RIF testing is adversely affected in low disease-prevalence settings or populations.

"Learning by doing": ongoing technological innovation and field experience

As of June 2012, 67 low- and middle-income countries had implemented the Xpert MTB/RIF assay, with 749 GeneXpert machines, 3602 cartridge modules and 1.1 million cartridges deployed or used (fig. 4).

Initial anxiety about errors and invalid results was reported during implementation of Xpert MTB/RIF in selected countries [24, 65, 70]. Recurring errors at particular sites were linked to improper procedures in specimen collection and/or preparation of samples, and faulty modules and cartridges. Since the evaluation and demonstration studies published in 2011, refinements to the reagents and software of the Xpert MTB/RIF assay have been made to decrease the frequency of false-positive rifampicin resistant results. Specifically, most of the false-positive results for rifampicin resistance were associated with a single probe; this has been redesigned to give more robust performance, with false-positive results for rifampicin resistance now rarely reported. The introduction of the latest generation Xpert MTB/RIF cartridge in December 2011 significantly reduced the number of signal loss (5011) errors, which had been the most commonly reported error [84].

The experience of the South African National Health Laboratory Service (NHLS) with over 300 000 tests found a decreasing error rate in the presence of adequate training and troubleshooting, with a current overall error rate of 2.2% (W. Stevens, NHLS, Johannesburg, South Africa; personal communication), which is very similar to unreadable microscopy results and much lower than acceptable culture contamination rates.

Changes have been made to minimise packaging, thus reducing waste and shipping costs [84]. Real-time stability studies are being performed by FIND and Cepheid to increase the current cartridge shelf-life of 12 months to 24 months. Stability data for 18-month shelf-life determination are expected in May 2013 and for 24 months in November 2013 (M. Perkins, FIND; personal communication). An on-site calibration kit

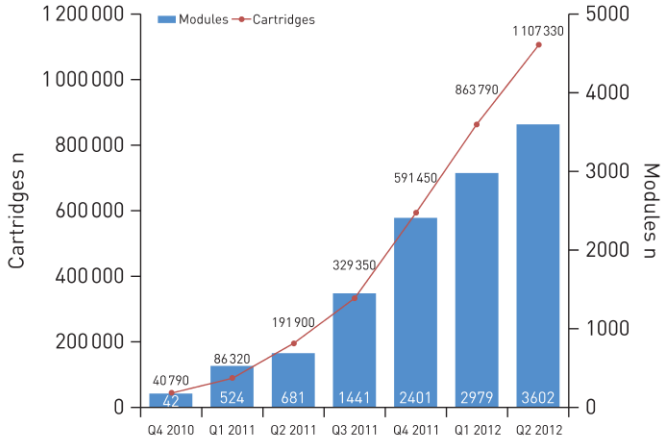

FIGURE 4 Global uptake of Xpert MTB/RIF up to June 30, 2012. As of June 30, 2012, a total of 749 GeneXpert instruments (comprising 3602 modules) and 1 107 330 Xpert MTB/RIF cartridges had been procured in the public sector in 67 of the 145 countries eligible for concessional pricing. Reproduced from [6] with permission from the publisher.

has been developed which allows users to recalibrate the optical system of the GeneXpert machine, verify the functioning thermal system, and conduct a series of system-level tests to ensure full system functionality within specifications, thus reducing the need for remote calibration of modules [84].

Experiences shared by early implementers showed that treatment of rifampicin-resistant TB cases diagnosed by Xpert MTB/RIF is a major, although controversial, concern [84]. While some argued for cautious roll-out in order to ensure treatment access, others felt that diagnosis in the absence of appropriate treatment allows for increased advocacy for scale-up of treatment and enables patients to make appropriate life decisions and protect the health of their families, while also facilitating interventions for reduced transmission of drug-resistant strains in healthcare facilities.

Early implementers reported high levels of user acceptance and satisfaction [84], describing the technology as fast, easy-to-use, modern, and much less cumbersome than conventional TB diagnostic techniques. They also indicated that the time and resources needed to develop and implement effective diagnostic as well as clinical management algorithms should not be underestimated, and stressed the need for training of doctors and nurses on interpretation of Xpert MTB/RIF results and clinical management of patients [84].

Several early implementers felt that adoption of Xpert MTB/RIF by the large private sector in many high-burden countries would be highly beneficial for increasing patient access to rapid and reliable diagnosis, while replacing poor technologies not endorsed by WHO [84]. Establishment of public–private collaborations was seen as mutually beneficial, allowing private providers to access concessional prices and national TB control programmes to ensure that patients detected in the private sector are duly reported and subsequently registered for appropriate treatment [84].

Cost, affordability and cost-effectiveness

While the Xpert MTB/RIF assay allows decentralised testing, cost and affordability of the assay are often cited as a barrier to wide-scale implementation. Price negotiations by FIND prior to launching the assay resulted in a significant upfront cost reduction (up to 85%) and preferential pricing of both the GeneXpert instrument and Xpert MTB/RIF cartridges for the public sector in 145 low- and middle-income countries [7]. A further major price reduction in cartridge cost (from US$16.86 to US$9.98) was recently achieved following a novel financing agreement between the manufacturer and the Bill and Melinda Gates Foundation, the US Agency for International Development (USAID), the Office of the United States Global AIDS Coordinator (OGAC) and UNITAID [91]. Detailed analyses using the price of US$9.98 per Xpert MTB/RIF cartridge to assess cost and affordability of diagnostic strategies confirmed and strengthened the findings of the analyses performed for the Global Consultation [85].

Studies of the cost and cost-effectiveness of Xpert MTB/RIF are currently limited to three countries: India, South Africa and Uganda [19, 46, 75–78, 85]. The data show that Xpert MTB/RIF is cost-effective compared with conventional diagnostic strategies, especially when the test is used as recommended by WHO, *i.e.* in persons suspected of MDR and/or HIV-associated TB. Use of the assay has also been found to be cost-effective in pre-antiretroviral treatment (ART) screening and in reducing early mortality during the first 6 months of ART [77, 78]. In South Africa, a diagnostic strategy of combining microscopy and Xpert MTB/RIF was found to have produced the highest accuracy and lowest cost [17].

Placement of Xpert MTB/RIF in tiered laboratory services

Currently recommended TB diagnostics require different levels of laboratory sophistication due to technical complexity and biosafety concerns. To date, technologies to diagnose drug-resistant TB and smear-negative TB have been suitable for use only at the apex of tiered laboratory services, *i.e.* reference laboratories at central or regional level (table 3). A distinct advantage of Xpert MTB/RIF is its suitability for use at the district and sub-district level and the technology should therefore not be restricted to central/reference laboratory level only [6, 7].

Countries already using high-throughput liquid culture and DST systems or molecular line probe assay (LPA) for rapid diagnosis of rifampicin resistance at central/reference laboratory level should introduce Xpert MTB/RIF at lower health service levels. Selection of sites for placement of Xpert MTB/RIF testing should be guided by: 1) the prevalence of MDR or HIV-associated TB; 2) the current or estimated workload of the facility; 3) availability of adequate infrastructure; 4) availability of staff; and 5) availability and capacity for appropriate treatment [6, 7, 21].

None of the existing TB diagnostic tools are mutually exclusive and implementation (in various combinations in country screening and diagnostic algorithms) is highly setting and resource specific [92]. One size no longer fits all, and expert laboratory input is needed to define the most cost-effective and

DOI: 10.1183/09031936.00157212

TABLE 3 Summary of tuberculosis (TB) diagnostics evaluated by the World Health Organization 2007–2012

2007: Commercial liquid culture and DST systems
- Automated and manual commercial systems for liquid culture and DST recommended for use at central/reference laboratory level
- Phased implementation recommended within the context of comprehensive country plans for strengthening TB laboratory capacity
- Currently regarded as the reference standard for conventional culture and DST and recommended as a stand-alone diagnostic test for TB and drug-resistance detection

2007: Rapid speciation strip technology
- Rapid chromatographic strip speciation recommended for distinguishing *Mycobacterium tuberculosis* from non-tuberculous mycobacteria
- Recommended for use in combination with conventional culture and DST systems, at central/reference laboratory level
- Recommended as a stand-alone speciation test for *Mycobacterium tuberculosis* isolates

2008: Molecular line probe assay for first-line anti-TB drugs
- Commercial line probe assays recommended for rapid detection of rifampicin alone or in combination with isoniazid resistance detection in smear-positive sputum specimens and *Mycobacterium tuberculosis* isolates grown from culture, for use at central/reference laboratory level
- Phased implementation recommended within the context of national plans for MDR-TB diagnosis, including development of country-specific screening/diagnostic algorithms
- Can be used as a stand-alone diagnostic test for rifampicin resistance (but no other resistance) once laboratory proficiency and equivalence with commercial liquid culture systems have been validated
- Need for conventional culture (for smear-negative sputum specimens and treatment monitoring) as well as phenotypic DST capacity remains

2010: LED microscopy
- Recommended as immediate replacement for conventional fluorochrome microscopy and as gradual replacement for conventional light Ziehl–Neelsen microscopy
- Suitable for use at peripheral microscopy, as well as higher laboratory levels

2010: Selected non-commercial DST methods: MODS, NRA, CRI
- Recommended as interim solutions for rapid rifampicin testing in resource-constrained settings, at central/reference laboratory level
- Phased implementation under strict laboratory protocols and quality assurance recommended within the context of national plans for MDR-TB diagnosis, including development of country-specific screening/diagnostic algorithms
- Phased implementation recommended within the context of national plans for MDR-TB diagnosis, including development of country-specific screening/diagnostic algorithms
- Can be used as stand-alone diagnostic tests for rifampicin resistance (but no other resistance) once laboratory proficiency and equivalence with conventional culture systems have been validated
- Need for conventional culture (for smear-negative sputum specimens and treatment monitoring) as well as DST capacity remains. MODS and NRA are suitable for direct testing on smear-positive sputum specimens and indirect testing on *Mycobacterium tuberculosis* isolates grown from culture
- CRI is suitable for indirect testing on *Mycobacterium tuberculosis* isolates only

2011: Automated real-time nucleic acid amplification technology: Xpert MTB/RIF system
- Recommended as rapid diagnostic test for TB and rifampicin resistance at peripheral microscopy, as well as higher laboratory levels
- Can be used as stand-alone diagnostic test for TB detection in all settings (including HIV co-infected patients) and for rifampicin resistance in patients at risk of drug-resistant disease
- Phased implementation and rapid scale-up recommended within the context of national TB and MDR-TB plans, including development of country-specific screening/diagnostic algorithms
- Need for conventional microscopy and culture remains to monitor treatment and to conduct additional DST

Evaluated but not yet approved due to lack of adequate evidence
- Sputum concentration and decontamination methods (evaluated 2008)
- Phage-plaque technology for rapid rifampicin resistance detection (evaluated 2008)
- Thin-layer agar methods for rapid culture and DST (evaluated 2010)
- Molecular line probe assays for second-line anti-TB drugs (evaluated 2012)
- Loop-mediated isothermal amplification test kit for tuberculosis (evaluated 2012)

Not approved for use
- Commercial serodiagnostic tests for TB diagnosis (evaluated 2011)
- IFN-γ release assays for detection of active TB in all settings (evaluated 2011)
- IFN-γ release assays as replacement for TST to detect latent TB in low- and middle-income (typically high TB and/or HIV burden) settings (evaluated 2011)

DST: drug susceptibility testing; MDR: multidrug resistant; LED: light-emitting diode; MODS: microscopic observation of drug susceptibility; NRA: nitrate reductase assay; CRI: colorimetric redox indicator; IFN: interferon; TST: tuberculin skin test.

efficient algorithms in individual countries, guided by WHO standards and procedures, and within the context of overall, integrated laboratory strengthening activities [92].

Targeting risk groups for testing

Maximum benefit from the Xpert MTB/RIF assay is obtained by targeted testing of individuals considered at risk of drug-resistant TB and/or smear-negative TB, such as those co-infected with HIV. Risk groups for drug-resistant TB include all re-treatment categories (*i.e.* failure, relapse and default cases), as well as those

described in WHO guidelines [93, 94] or national policies, including those with HIV infection [95]. These individuals should receive an Xpert MTB/RIF test as a primary diagnostic test, *i.e.* subsequent confirmation of the diagnosis is not required and appropriate treatment should be started on the basis of the Xpert MTB/RIF result.

Published studies have shown significant increases in TB case detection when Xpert MTB/RIF is used as an add-on or replacement test for microscopy, especially in settings with high HIV prevalence [18, 19, 34, 35, 50, 61]. Both diagnostic delay and treatment initiation can be significantly shortened compared to conventional approaches [92], reducing premature death and on-going transmission. The 2012 WHO Global Report [1] also notes the still insufficient screening of HIV patients for TB and the low proportion of patients started on isoniazid preventive therapy.

HIV testing should be routinely offered to all persons suspected of having TB, based on WHO recommendations [95], ideally before investigation with Xpert MTB/RIF. Up to 25% of patients accessing HIV services may have active TB, the vast majority of which would be missed using conventional microscopy as a primary diagnostic tool [96]. The systematic introduction of Xpert MTB/RIF in HIV services would, therefore, make a major contribution to intensified TB case finding efforts and increased uptake of isoniazid preventive therapy.

The distinct advantage of Xpert MTB/RIF in providing a rapid, simultaneous diagnosis of both TB and rifampicin resistance has also given rise to continuing debate and concerns about the implications of positive results in different epidemiological and resource settings [24, 42, 43, 79, 80]. It is therefore important to distinguish the performance characteristics and treatment implications of the assay for: 1) TB detection and; 2) rifampicin resistance detection.

In many settings, the vast majority of persons suspected of having TB will not have risk factors for drug resistance or be HIV-positive. Careful consideration should be given in these circumstances to the resource implications of offering routine Xpert MTB/RIF testing [6, 7] and the low PPV of the assay for detecting rifampicin resistance at a low underlying prevalence (tables 4 and 5). Where resources are limited, national TB control programmes will have to prioritise specific groups for testing, decide whether Xpert MTB/RIF is performed as an initial diagnostic test or as a follow-on test after sputum smear microscopy, and consider the use of chest radiography as a first screening tool.

Predictive values of Xpert MTB/RIF for TB case detection

In the GRADE framework, diagnostic test accuracy can be interpreted as proxy measures for patient-important outcomes based on the relative importance/impact of false-positive and false-negative results [16]. Poor sensitivity would result in false-negative results with adverse consequences for patient morbidity and mortality and ongoing disease transmission. Poor specificity would result in false-positive results exposing patients to unnecessary treatment while the underlying cause of disease remains undiagnosed.

Test accuracy is also dependent on underlying disease prevalence. Typically, between 10% and 20% of persons with respiratory symptoms may have confirmed TB in high-burden settings. Table 4 presents the predictive values for TB detection using Xpert MTB/RIF (compared to conventional culture) in settings or populations with varying TB prevalence. The negative predictive value (NPV) is over 99% in settings with both low and high prevalence of TB, *i.e.* a negative result reliably excludes TB. Table 4 shows that the vast majority of patients with a negative Xpert MTB/RIF result in such settings will not have TB and very few false-positive results will occur. Even with a low PPV the absolute number of false-positives will usually be very low and the proportion of overall true results (positive and negative combined) far outweigh the proportion of overall false results.

Predictive values of Xpert MTB/RIF for rifampicin resistance detection

Table 5 presents PPV and NPV for rifampicin resistance detection using Xpert MTB/RIF in settings or populations with varying prevalence of rifampicin resistance. The NPV is over 99% in settings with both low and high prevalence of rifampicin resistance, *i.e.* a negative result reliably excludes resistance and no further testing to confirm negative results is required.

The PPV for rifampicin resistance using Xpert MTB/RIF exceeds 90% in settings or patient groups where the underlying prevalence of rifampicin resistance is >15% (table 5). In settings or patient groups where rifampicin resistance is rare, the PPV of Xpert MTB/RIF (and any other test) is adversely affected, significantly diminishing when rifampicin resistance prevalence falls below 5%.

The PPV for rifampicin resistance using Xpert MTB/RIF (or any other test) can be substantially improved by careful risk assessment in individual patients and targeted testing of risk groups: drug resistance surveillance data from 114 countries showed that the weighted proportion of MDR among previously

DOI: 10.1183/09031936.00157212

TABLE 4 False positive, false negative and predictive values for tuberculosis (TB) detection using Xpert MTB/RIF[#]

TB prevalence %	PPV %	NPV%	True positive[¶]	False negative[¶]	False positive[¶]	True negative[¶]
1	48	100	9.1	0.9	9.9	980.1
2	65	100	18.2	1.8	9.8	970.2
3	74	100	27.3	2.7	9.7	960.3
4	79	100	36.4	3.6	9.6	950.4
5	83	100	45.5	4.5	9.5	940.5
6	85	99	54.6	5.4	9.4	930.6
7	87	99	63.7	6.3	9.3	920.7
8	89	99	72.8	7.2	9.2	910.8
9	90	99	81.9	8.1	9.1	900.9
10	91	99	91	9	9	891
11	92	99	100.1	9.9	8.9	881.1
12	93	99	109.2	10.8	8.8	871.2
13	93	99	118.3	11.7	8.7	861.3
14	94	99	127.4	12.6	8.6	851.4
15	94	98	136.5	13.5	8.5	841.5
20	96	98	182	18	8	792
25	97	97	227.5	22.5	7.5	742.5

PPV: positive predictive value; NPV: negative predictive value. [#]: according to varying TB prevalences in a sample population of 1000 individuals; [¶]: sensitivity (91%) and specificity (99%) for Xpert MTB/RIF TB detection, compared with reference method (culture).

treated cases is 19.8% (95% CI 14.4–25.1), which is several times higher than the proportion of new TB cases with MDR (3.4%; 95% CI 1.9–5.0) [3]. Therefore, even in low MDR-TB prevalence settings, testing previously treated patients should result in high PPV for rifampicin resistance, allowing treatment to be initiated based on the Xpert MTB/RIF result. Testing new TB cases not at risk of MDR-TB in low MDR-TB prevalence settings will, however, result in low PPV, requiring confirmation of rifampicin resistance by phenotypic DST or LPA (and not by a second Xpert MTB/RIF test) prior to treatment initiation.

The performance of Xpert MTB/RIF has been evaluated against existing reference standards, *i.e.* microscopy and culture for TB testing and phenotypic DST for rifampicin resistance testing. None of the currently available microbiological reference methods is 100% accurate, a well-recognised constraint in TB diagnostic test development and evaluation. Emerging data seem to suggest that low-level but potentially clinically relevant rifampicin resistance linked to infrequent *rpoB* mutations may be missed by standard growth-based

TABLE 5 False positive, false negative and predictive values for rifampicin resistance using Xpert MTB/RIF[#]

Rifampicin resistance prevalence %	PPV %	NPV %	True positive[¶]	False negative[¶]	False positive[¶]	True negative[¶]
1	32.4	99.9	9.5	0.5	19.8	970.2
2	49.2	99.9	19	1	19.6	960.4
3	59.5	99.8	28.5	1.5	19.4	950.6
4	66.4	99.8	38	2	19.2	940.8
5	71.4	99.7	47.5	2.5	19	931
6	75.2	99.7	57	3	18.8	921.2
7	78.1	99.6	66.5	3.5	18.6	911.4
8	80.5	99.6	76	4	18.4	901.6
9	82.4	99.5	85.5	4.5	18.2	891.8
10	84.1	99.4	95	5	18	882
11	85.4	99.4	104.5	5.5	17.8	872.2
12	86.6	99.3	114	6	17.6	862.4
13	87.7	99.2	123.5	6.5	17.4	852.6
14	88.5	99.2	133	7	17.2	842.8
15	89.3	99.1	142.5	7.5	17	833
20	92.2	98.7	190	10	16	784
25	94.1	98.3	237.5	12.5	15	735

PPV: positive predictive value; NPV: negative predictive value. [#]: according to varying prevalences of rifampicin resistance in a sample population of 1000 individuals; [¶]: sensitivity (95%) and specificity (98%) for Xpert MTB/RIF rifampicin resistance, compared with reference method (culture).

methods, particularly the automated broth-based systems [97]. Sequencing, albeit limited, has largely resolved discordant results in favour of Xpert MTB/RIF, although a few truly false-positive results have been reported [17, 18, 84]. Additional data on mutation sequencing of *M. tuberculosis* strains and the clinical outcomes of patients with rifampicin resistance detected by Xpert MTB/RIF are therefore highly desirable.

Use of Xpert MTB/RIF in diagnosis of paediatric TB

Laboratory diagnosis of TB in children remains a real challenge due to the low sensitivity of sputum smear microscopy, the difficulty in collecting sufficient and high-quality specimens, and a substantial proportion of paediatric cases with extrapulmonary involvement.

Current WHO policy recommendations on the use of Xpert MTB/RIF in children are extrapolated from data on adults [6, 7], given the well-known limitations of microbiological methods in diagnosing paediatric TB. Subsequent studies have shown a significant improvement in diagnosing TB in children using the Xpert MTB/RIF assay.

Both high sensitivity (86.9%) and specificity (99.7%) of Xpert MTB/RIF in extrapulmonary paediatric samples (n=344, mainly gastric aspirates and biopsies) were reported by TORTOLI *et al.* [72], using positive culture and/or therapeutic response as a composite standard. In a large study on young South African children (including 24% with HIV co-infection), NICOL *et al.* [50] showed a sensitivity of 74.3%. Using Xpert MTB/RIF on two induced sputum specimens detected twice as many cases (75.9%) compared to sputum smear (38%) resulting in an overall Xpert specificity of 98.8%. Similar results were obtained by RACHOW *et al.* [71] using Xpert MTB/RIF for diagnosis of pulmonary TB in 164 (51.2%) older children in a high HIV prevalence setting.

Use of Xpert MTB/RIF in diagnosis of extrapulmonary TB

The diagnosis of extrapulmonary TB poses a serious challenge due to the pleomorphic presentation of the disease. Samples collected for microbiological diagnosis are often paucibacillary, resulting in a low sensitivity of smear microscopy and earlier nucleic acid amplification tests.

Several studies have now assessed the performance of Xpert MTB/RIF in the diagnosis of extrapulmonary TB [23, 27–31, 38, 39, 53–56, 59–62]. The sensitivity and specificity ranged between 77% and 95% for biopsy, urine and pus while it was lower than 50% for cavitary fluids [23]. The specificity in these specimens ranged from 97% to 100% [23].

Management of patients detected by Xpert MTB/RIF

TB patients identified by Xpert MTB/RIF without rifampicin resistance should receive appropriate first-line anti-TB treatment immediately. HIV co-infected patients detected by Xpert MTB/RIF should be managed according to WHO guidelines, including HIV clinical staging, immunological staging with CD4 count, initiation of co-trimoxazole preventive therapy and initiation of antiretroviral therapy irrespective of CD4 count [95].

Rapid DST for rifampicin is recommended by WHO [92–94]. Patients at risk of drug resistance in whom rifampicin resistance is detected by Xpert MTB/RIF should be placed on an appropriate MDR-TB regimen immediately and isoniazid added until the DST result for isoniazid is available. These patients should provide an additional sputum specimen for conventional culture and DST against other first- and second-line drugs according to WHO recommendations [93, 94], and their treatment adjusted accordingly.

Molecular tests, including Xpert MTB/RIF, are not suitable for patient monitoring as these tests detect DNA from both viable and non-viable bacilli. Conventional laboratory capacity is, therefore, required to monitor treatment response of patients detected by Xpert MTB/RIF and to conduct additional DST in patients with rifampicin resistance.

Patients whose diagnosis of TB is confirmed by Xpert MTB/RIF and who have rifampicin-susceptible TB disease should be monitored during treatment with sputum smear microscopy. No additional sputum smear microscopy examination needs to be performed for establishing baseline status. Sputum smear microscopy should be performed at completion of the intensive phase of treatment, 5 months into treatment and at the end of treatment as per WHO guidelines [98].

Treatment outcomes for patients with a positive smear culture or Xpert MTB/RIF result at the start of treatment should be categorised according to current WHO guidelines [7, 98]. Current treatment outcome definitions apply, including the outcome "cured", *i.e.* a patient with a positive Xpert MTB/RIF test (only) at baseline can be declared cured if negative smear results during and at the end of the treatment were recorded.

DOI: 10.1183/09031936.00157212

Patients placed on MDR-TB treatment should be monitored by sputum culture as per current WHO guidelines. If resources permit, monthly culture throughout treatment is recommended given that this has shown the highest benefit to detect failures [99].

Use of Xpert MTB/RIF *vis à vis* other tests

From a purely technical perspective, no test for TB is perfect. Xpert MTB/RIF limitations have been described previously. Microscopy, conventional culture and DST (both phenotypic and genotypic) all have shortcomings and limitations related to accuracy and effectiveness, operator dependency, training and resource requirements, and biosafety. WHO policy guidance on new TB diagnostics takes all these aspects into careful consideration [92], balancing test accuracy with potential harms and benefits, operational considerations, resource implications and anticipated public health impact.

As outlined previously, Xpert MTB/RIF efficiency is maximised and cost minimised by targeted testing of individuals at risk of drug resistance and/or HIV co-infection. In these patient groups, Xpert MTB/RIF clearly outperforms microscopy and should be used as the initial diagnostic test.

Xpert MTB/RIF is currently the only DST technology suitable beyond central/reference laboratory level and should be the first point of testing when MDR-TB is suspected. It is a relatively low throughput technology (maximum of 20 specimens per day in the four-module GeneXpert machine). Settings with higher patient loads should consider bigger capacity machines or the referral of specimens to central/national laboratory levels for first-line LPA or phenotypic DST (both high through-put technologies).

All DST methods currently recommended by WHO show similar accuracy for rifampicin resistance detection. All tests have poor PPV in settings with low levels of MDR-TB and good PPV in settings with high levels. In settings where rifampicin/MDR resistance is rare, resistance by any test therefore needs to be confirmed by an alternative WHO-recommended DST method. Specimens from confirmed MDR-TB patients need to undergo phenotypic DST against fluoroquinolones and kanamycin, amikacin and capreomycin to check for extensively drug-resistant-TB [93, 94].

Strategies for Xpert MTB/RIF testing of all persons suspected of having TB will be strongly dependent on available resources and the screening and diagnostic algorithms at country level. TB screening as per national guidelines should take place and pre-test screening strategies, including chest radiography, should be considered to optimise Xpert MTB/RIF efficiency and cost. Individuals with sputum smear-positive microscopy results do not need to be retested with Xpert MTB/RIF unless they belong to the risk groups for drug resistance, as described previously.

In summary, Xpert MTB/RIF does not eliminate the need for traditional bacteriological methods (direct examination, culture and DST) and for other rapid molecular methods. National programmes need to develop setting-specific, evidence-based and cost-effective algorithms designed to ensure universal access to quality diagnosis for all TB cases.

Use of Xpert MTB/RIF in prevalence surveys and drug resistance surveillance

Upgrading laboratory infrastructure and strengthening capacity for culture and DST are among the most important indirect benefits of implementing a drug resistance survey [100] as many laboratories need considerable refurbishment and/or upgrade, training of staff, and procurement of equipment and consumables before starting a survey [101]. Although not a complete surrogate for MDR-TB, particularly in settings with low resistance levels [102], rifampicin resistance is the most important indicator of MDR-TB, with serious clinical implications for affected patients.

At least two groups of countries could benefit considerably from the use of Xpert MTB/RIF as a screening tool in drug resistance surveys. The first group is countries in which laboratories would struggle to cope with the huge workload generated by a survey while managing their routine work and maintaining high-quality standards. The second group is countries where there is no capacity to perform culture and DST. In these settings, instead of relying entirely on testing abroad, usually at a TB Supranational Reference Laboratory, with increased logistics and operational costs, Xpert MTB/RIF could be used to screen specimens and identify those requiring further testing in a specialised laboratory.

Given that most patients enrolled in drug resistance surveys are newly diagnosed with TB and at low risk of rifampicin resistance, the PPV of any test will not be adequate to identify true positives but the NPV will be sufficiently high to accurately identify true negatives. Xpert MTB/RIF could, therefore, be used as screening tool to identify those with no resistance to rifampicin, while patients with rifampicin resistance undergo further confirmatory testing with a second WHO-approved technology.

In a recent TB prevalence study, DORMAN *et al.* [86] suggested the potential use of the Xpert MTB/RIF as a single testing strategy: the diagnostic yield of *M. tuberculosis* was 2.7% (187 out of 6893) for liquid culture, 2.1% (144 out of 6893) for Xpert MTB/RIF and 1.3% (91 out of 6893) for smear microscopy. Agreement of Xpert MTB/RIF with liquid culture was 98.5% (95% CI 98.2–98.8%) and respective test failure rates (noninterpretable results) were 0.3% for Xpert MTB/RIF and 3.6% for liquid culture. Overall Xpert MTB/RIF sensitivity was 62.6% (95% CI 55.2–69.5%), specificity was 99.6% (95% CI 99.4–99.7%), PPV was 81.3% (95% CI 3.9–87.3%), and NPV was 98.9% (95% CI 98.6–99.2%) [86]. While these results are encouraging, more evidence is needed on the use of Xpert MTB/RIF in the context of prevalence surveys and other case-finding strategies in which TB prevalence and the pre-test probability of TB disease are relatively low.

Aligning diagnostic and treatment capacity

Lack of diagnostic capacity has been a longstanding and major barrier to scaling up MDR-TB care. The advent of new TB diagnostics, and Xpert MTB/RIF in particular, allows this constraint to be largely overcome. Early implementers have reported a 30–40% increase in the number of drug-susceptible TB patients being detected after roll-out of Xpert MTB/RIF, while MDR-TB cases have increased two- to three-fold in many settings [17, 18, 71].

Providing a definitive diagnosis for the large proportion of drug susceptible TB cases currently being reported without laboratory confirmation would allow treatment to be shifted away from those who do not need it to those who do. For the $\geqslant$80% of MDR-TB patients estimated to arise each year but remaining undiagnosed, prompt and appropriate treatment would prevent premature death, reduce the risk for aggravating drug resistance, and curtail disease transmission. Introduction of Xpert MTB/RIF should also allow for more robust and reliable forecasting of patient numbers, one of the most pressing constraints in securing adequate availability of second-line drugs. This in turn could stimulate more investment into second-line drugs and drive down the exorbitant cost of MDR-TB treatment.

An unintended consequence of scaling up diagnostics is the risk that patients diagnosed with MDR-TB cannot access the complex, second-line drug treatment required. This raises the question of ethics, human rights and public health. Should a diagnosis not lead to appropriate treatment? Concerns about the ethics of rolling out Xpert MTB/RIF in developing countries in the presumed absence of treatment for MDR-TB have been raised [42, 87], as have the counterpoint on the ethics of not rolling out the assay in low-income countries[43, 88].

Public health, ethics and human rights should be balanced when addressing the cost, risks and benefits, and technical limitations of any new, transformational intervention. Systematic roll-out of the Xpert MTB/RIF assay complies fully with WHO guidance on ethics of TB prevention, care and control [103], as well as the WHO-endorsed "progressive realisation" approach which states that "while countries are in the process of scaling up treatment, the use of DST can be appropriate as an interim measure even when no second-line drug treatment is available, or when the only available treatment is substandard"[43]. Virtually all low-income countries have ratified the International Covenant on Economic, Social, and Cultural Rights (ICESR) governing the WHO-endorsed strategy of progressive realisation. We therefore agree with leading global ethicists that public health, ethics and human rights obligations apply equally to high TB burden low-income countries as they do to resource-rich countries and that the public health potential of the Xpert MTB/RIF assay should be considered despite cost and operational considerations [43, 103].

Research needs

As countries start to implement Xpert MTB/RIF they will be facing operational and logistical challenges related to changes in screening and diagnostic algorithms, shifts in laboratory organisation and workload, and requirements for improved supply chain management. In addition, country-specific adaptation of the diagnostic algorithms (*e.g.* prioritisation of patient groups to be tested) may be dictated by the availability of resources. WHO has therefore recommended that roll-out of Xpert MTB/RIF be addressed in a systematic and coordinated approach to optimise the usefulness of the technology under routine programme conditions and to ensure maximum efficiency [6, 7]. In addition, WHO has recommended ongoing operational research to refine and inform future policy, in line with the requirement for dynamic policy guidance by the WHO Guidelines Review Committee [16].

More studies merely evaluating the performance of the Xpert MTB/RIF assay against conventional diagnostics in detecting pulmonary TB are not expected to challenge or change the existing evidence base. The assay has been shown repeatedly to be highly accurate, particularly if used in targeted testing as recommended by WHO. Implementation research should therefore now focus on the "how" and "when" of Xpert MTB/RIF implementation and scale-up, informed by appropriately designed studies (and using real

DOI: 10.1183/09031936.00157212

data) that evaluate the test's impact and cost-effectiveness when used in different algorithms and with other screening and diagnostic tests. Policy refinement will also benefit from additional data on the use of the assay in extrapulmonary and paediatric TB, in prevalence surveys and drug resistance surveillance, and in active case finding.

Xpert MTB/RIF MTB/RIF roll-out can, and should, serve as a pathfinder for implementation of future TB tests by providing national TB control programmes with data to develop long-term TB diagnostic strategies. Experiences and lessons learnt from programmatic roll-out (*i.e.* evidence for scaling up) will inform and facilitate eventual country-wide scale-up and assist other countries intending to embark on the same process.

Cost and potential disruption of health services are characteristic consequences of introducing any new public health intervention tool [88]. Rather than blocking or slowing down the introduction of new technologies [87] or waiting until ideal operational conditions are in place [89], scientific debate should focus on whether patient and public health benefits warrant implementation, even of a so-called "disruptive" intervention. Although not expected to show overall cost disadvantages, in-depth, cost-effectiveness studies on the impact of Xpert MTB/RIF in different settings would therefore be advantageous, especially since the assay will be used in varied diagnostic algorithms and underlying TB and MDR-TB epidemiology.

On a more fundamental research level, second-generation Xpert MTB/RIF tests with probes to detection resistance other than rifampicin will be most advantageous. In addition, the development of competing technologies with comparable performance and ease-of-use to the Xpert MTB/RIF assay is strongly encouraged to generate increased demand and market competition. Most pressing is the need for a robust, low-cost and safe point-of-care diagnostic for TB and drug-resistant TB. This will require dramatic increases in research investment to identify appropriate biomarkers and capitalise on technological breakthroughs to create innovative test platforms [90]. The experiences in HIV test and drug development have shown the advantages gained from innovation and solid investment in research [104], strikingly different from the TB research world that remains woefully under-funded [105].

A summary of the current evidence available on Xpert MTB/RIF [8–13, 18, 19, 27–41, 50–74, 86, 106–112] is available in the supplementary material.

Conclusion

In the mid-1990s, when WHO declared TB a global emergency and subsequently introduced the DOTS (directly observed treatment, short course) strategy, the impact of the HIV epidemic on the dynamics of TB control (especially in Africa) was not fully realised, and no information on the public health impact of the growing problem of TB drug resistance was available. Under the assumption that MDR-TB was a rare event, good microscopy services were deemed sufficient to control TB in most settings. Indeed, many national programmes witnessed annual decreases in TB case rates following the wide implementation of microscopy services linked to the use of short-course chemotherapy under close supervision. Before the end of the 20th century, however, three events suggested that microscopy would become inadequate. The first was the magnitude of the HIV pandemic and its extraordinary impact on susceptibility to TB. The second was the growing burden and geographical spread of MDR-TB. Thirdly was the very slow decline in TB incidence in countries implementing DOTS, even when the prevalence of MDR-TB and HIV co-infection was low.

In 2006, WHO introduced the Stop TB Strategy which, in addition to the essential elements of the DOTS strategy, included measures specifically targeting (amongst others) proper care of HIV-associated TB and MDR-TB. As a result, in 2009, the World Health Assembly called for universal access to culture and DST, marking a dramatic shift in strategy. The updated Global Plan to Stop TB, 2011–2015 [22], underpinned by the Stop TB Strategy, called for massive investment in laboratory services to achieve screening for MDR in at least 20% of new TB cases and 100% of those previously treated by 2015. It also called for >50% of all smear-negative cases to be tested with molecular or culture-based methods. Data reported to WHO in 2012 clearly show that these targets are not on track. Reasons include huge gaps in funding to establish the required laboratory infrastructure, slow diagnostic policy reform at country level, and critical shortages of specialised laboratory staff.

WHO policy guidance on Xpert MTB/RIF recognises its potential in addressing some of the most pressing barriers to rapid diagnosis of TB and drug-resistant TB, and has attempted to provide the necessary information and support to enable countries to make appropriate decisions on its utilisation. The WHO guidance also explicitly highlights the resource implications of rolling out the technology, as well as the need to ensure appropriate treatment of those patients detected; however, increased demand for testing, the difficulties in providing care for drug-resistant patients, and concerns about affordability should not be the prime drivers delaying roll-out of new and innovative interventions.

Evidence on ''where'' to locate Xpert MTB/RIF (peripheral *versus* central laboratories) and ''whom'' to test (targeted *versus* general use) is growing, allowing rational and sustainable roll-out of the technology even in resource-constrained settings.

The experience of HIV testing despite inadequate treatment facilities provides a solid precedent for TB to follow: in the HIV world, moral pressure has been put on drug and diagnostics manufacturers to lower the prices of their products and to develop novel ones. Increased demand for drugs as a result of improved case detection has created scale and ultimately lowered prices, thus facilitating increased access.

As countries adjust their diagnostic algorithms to accommodate Xpert MTB/RIF roll-out, diagnostic paradigms for HIV-associated and drug-resistant TB are expected to shift significantly, away from highly centralised, complex diagnostic algorithms and referral systems (with inevitable long delays) towards simplified diagnostic approaches for at-risk patients at decentralised levels of the health system. These should be accompanied by more focused use of screening methods to increase the pre-test probability of TB prior to Xpert MTB/RIF testing, accelerated implementation of WHO screening policies for TB-HIV using Xpert MTB/RIF as the initial diagnostic test, and more focused identification of patients suspected of having to suffer from MDR-TB, using Xpert MTB/RIF as a rapid test rather than waiting for patients to fail first-line therapy before proceeding with culture and DST.

Stagnation in TB control and MDR-TB care delivery has severe consequences for TB patients, who often belong to the most vulnerable and neglected sector of society. Scientific breakthroughs such as the Xpert MTB/RIF assay (and hopefully additional new diagnostics, drugs and vaccines coming to use in the next few years) should not be withheld from these marginalised groups but deployed without undue delay, optimising patient and public health benefits.

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Ref N.	Year	Setting	Main Findings	Main Conclusions
8	1998	USA	A set of overlapping molecular beacons was used to analyze an 81-bp region of the MTB rpoB gene for mutations that confer resistance to the antibiotic R. In a blinded study of 52 R-resistant and 23 R-susceptible clinical isolates, this method correctly detected mutations in all of the resistant strains and in none of the susceptible strains.	The assay was carried out entirely in sealed PCR tubes and was simple to perform and interpret. This approach can be used to analyze any DNA sequence of moderate length with single base pair accuracy
9	2000	Spain and USA	A rapid closed-tube PCR assay using fluorogenic reporter molecules called molecular beacons to detect reportedly common MTB mutations associated with resistance to H and R was developed. The overall SENS and SPEC of the assay for H resistance were 85 and 100%, respectively, and those for R resistance were 98 and 100%, respectively. R resistance mutations were detected equally well in isolates from both study populations; however, H resistance mutations were detected in 94% of the isolates from Madrid but in only 76% of the isolates from New York (P = 0.02). In New York, H resistance mutations were significantly more common in the MDR isolates (94%) than in single-drug-resistant isolates (44%; P < 0.001). No association between previously described mutations in the kasA gene and H resistance was found. The first mutations that cause H resistance may often occur in sequences that have not been commonly associated with H resistance, possibly in other as yet uncharacterized genes	The molecular beacon assay was simple, rapid, and highly sensitive for the detection of R-resistant MTB isolates and for the detection of H resistance in MDR isolates.
10	2001	USA	The study reports on a highly SENS PCR assay that takes less than 3 h and reliably identifies R-resistant MTB in DNA extracted directly from SS. When 148 MTB clinical isolates of known susceptibility to R were tested, mutations associated with R resistance were detected in 63 of the 65 R-resistant isolates, and no mutations were found in any of the 83 R-susceptible isolates. When DNA extracted directly from the SS of 11 patients infected with R-resistant TB was tested, mutations were detected in all of the samples.	The use of this rapid assay should enable early detection and treatment of drug-resistant TB in clinical settings.
11	2010	Vietnam and Uganda	Analytic tests of MTB DNA demonstrated a limit of detection (LOD) of 4.5 genomes per reaction. Studies using sputum spiked with known numbers of MTB CFU predicted a clinical LOD of 131 CFU/ml. Killing studies showed that the assay's buffer decreased MTB viability by at least 8 logs, substantially reducing biohazards. Tests of 23 different commonly occurring R resistance mutations demonstrated that all 23 (100%) would be identified as R-resistant. An analysis of 20 NTM species confirmed high assay specificity. A small clinical validation study of 107 clinical sputum samples from suspected TB cases in Vietnam detected 29/29 (100%) SS+ C+ cases and 33/39 (84.6%) or 38/53 (71.7%) SS-,C+ cases, as determined by growth on solid medium or on both solid and liquid media, respectively. MTB was not detected in 25/25 (100%) of the C- samples. A study of 64 SS- smear-C+ sputa from retreatment TB cases in Uganda detected 63/64 (98.4%) C+ - cases and 9/9 (100%) cases of R resistance. R resistance was excluded in 54/55 (98.2%) susceptible cases. SPEC rose to 100% after correcting for a conventional susceptibility test error.	This highly sensitive and simple-to-use system can detect MTB directly from SS in less than 2 h.
12	2010	USA	79 MTB isolates and 89 NTM isolates were studied .The Xpert®MTB/RIF assay correctly identified all 79 MTB isolates and correctly excluded all 89 NTM isolates. R resistance was correctly identified in all 37 resistant isolates and in none of the 42 susceptible isolates. Dynamic range was assessed by adding 102 to 107 Colony Forming Units (CFU) of MTB into MTB-negative sputum samples. The assay showed a log-linear relationship between cycle threshold and input CFU over the entire concentration range. Resistance detection in the presence of different mixtures of R-resistant and R-susceptible DNA was assessed. Resistance detection was dependent on the particular mutation and required between 65% and 100% mutant DNA to be present in the sample for 95% certainty of resistance detection	The Xpert®MTB/RIF assay was relatively resistant to contamination by MTBDRplus amplicons and could safely be used in the same laboratory environment. This was the result of inefficient amplification of the MTBDRplus amplicon, which has only limited overlap with the outer priming regions of the Xpert®MTB/RIF assay. The Xpert®MTB/RIF itself contains amplicons within the sealed cartridge, reducing or eliminating the need for the precautions typically associated with other nucleic acid amplification tests.
13	2010	USA	The bioaerosols generated by the Xpert®MTB/RIF assay was compared to AFB microscope slide SS preparation. The Xpert®MTB/RIF assay sample treatment reagent (SR) was also studied for its sterilizing capacity, stability, and effect on assay SENS after prolonged treatment. Neither the sample preparation steps for the Xpert®MTB/RIF assay nor its automated processing produced any culturable bioaerosols. In testing of SR sterilizing capacity, clinical SS samples from strongly SS+ TB patients treated with SR at a 2:1 ratio eliminated MTB growth in all but 1/39 or 3/45 samples cultured on solid or liquid medium, respectively. These few unsterilized samples had a mean 13.1-day delay in the time to C+. SR treatment at a 3:1 ratio eliminated growth in all samples. SR retained a greater than 6-log-unit	These results suggest that benchtop use of the Xpert®MTB/RIF assay limits infection risk to the user.

			killing capacity despite storage at temperatures spanning 4 to 45°C for at least 3 months.	
17	2010	Peru, Azerbaijan, South Africa, and India	Among C+ patients, a single, direct Xpert®MTB/RIF test identified 551/561 patients with SS+ TB (98.2%) and 124 of 171 with SS- TB (72.5%). The test was specific in 604 of 609 patients without TB (99.2%). Among patients with SS- C+ TB, the addition of a second Xpert®MTB/RIF test increased SENS by 12.6% and a third by 5.1%, to a total of 90.2%. As compared with phenotypic DST, Xpert®MTB/RIF MTB/RIF testing correctly identified 200/205 patients (97.6%) with R-resistant bacteria and 504/514 (98.1%) with R-susceptible bacteria. Sequencing resolved all but two cases in favour of the Xpert®MTB/RIF assay.	The Xpert®MTB/RIF test provided sensitive detection of TB and R resistance directly from untreated SS in less than 2 hours with minimal hands-on time.
18	2011	Multicentre study in South Africa, Peru, India, Azerbaijan, Philippines, and Uganda	6648 participants were enrolled. Once-off Xpert®MTB/RIF testing detected 933 (90·3%) of 1033 C-confirmed cases of TB, compared with 699 (67·1%) of 1041 for microscopy. Xpert®MTB/RIF test SENS was 76·9% in SS-, C+ patients (296 of 385 samples), and 99·0% specific (2846 of 2876 non-tuberculosis samples). Xpert®MTB/RIF test SENS for R resistance was 94·4% (236 of 250) and SPEC was 98·3% (796 of 810). Unlike microscopy, Xpert®MTB/RIF test SENS was not significantly lower in patients with HIV co-infection. Median time to detection of TB for the Xpert®MTB/RIF was 0 days (IQR 0—1), compared with 1 day (0—1) for microscopy, 30 days (23—43) for solid C, and 16 days (13—21) for liquid C. Median time to detection of resistance was 20 days (10—26) for line-probe assay and 106 days (30—124) for conventional DST. Use of the Xpert®MTB/RIF test reduced median time to treatment for SS- TB from 56 days (39—81) to 5 days (2—8). The indeterminate rate of Xpert®MTB/RIF testing was 2·4% (126 of 5321 samples) compared with 4·6% (441 of 9690) for C.	The Xpert®MTB/RIF can effectively be used in low-resource settings to simplify patients' access to early and accurate diagnosis, thereby potentially decreasing morbidity associated with diagnostic delay, dropout and mistreatment.
27	2011	Turkey	253 pulmonary and 176 extrapulmonary specimens obtained from 429 patients were included in the study. 110 (89 C+ and 21 C- for MTB) of the 429 patients were considered to have TB. In pulmonary specimens, SENS of Xpert®MTB/RIF were 100% (27/27) and 68.6% (24/35) for SS+ and SS- specimens respectively. It had lower SENS in extrapulmonary specimens; for SS+ 100% (4/4) and SS- 47.7% (21/44). The test accurately detected the absence of TB in all 319 patients studied. The Xpert®MTB/RIF assay also detected one R-resistant and 88 R-susceptible specimens which were confirmed by phenotypic DST.	The Xpert®MTB/RIF test was a simple method and routine staff with minimal training could use the system. The test appeared to be as SENS as C with SS+ specimens but less SENS with SS- pulmonary and extrapulmonary specimens that included low number of bacilli.
28	2011	Kuwait	Clinical specimens included 206 pulmonary and 29 extra-pulmonary samples .72 (60 pulmonary and 12 extra-pulmonary) samples yielded MTB by C, while 56 (78%) C+ samples (46 pulmonary and 10 extra-pulmonary) were also SS+. Xpert®MTB/RIF showed98% agreement for SS+, C+ samples and 69% agreement for SS-, C+ samples for detection of MTB. Overall concordance with C was 92%.There was 98% and 64% agreement for SS+ and SS- pulmonary specimens, respectively. Xpert®MTB/RIF showed 100% agreement with C for both SS+, C+ and SS-, C+ extra-pulmonary specimens.	The rapidity and simplicity of the closed cartridge Xpert®MTB/RIF test made it a good TB diagnostic test for routine use in reference laboratories of countries with low to intermediate incidence of TB, and may also help in reducing further transmission of infection in such settings
29	2011	Athens, Greece.	The Xpert®MTB/RIF assay was evaluated with microscopically negative and -positive pulmonary- and extrapulmonary specimens. For the pulmonary samples, SENS, SPEC, PPV and NPV were 90.6%, 94.3%, 93.5%, and 91.7%, and for the extrapulmonary samples, these were 100%, 91.6%, 50%, and 100%, respectively. For microscopically negative specimens, the respective values were 86.3%, 93%, 79%, and 95.6%. The assay correctly detected R resistance in all but one specimen, which harboured a mixed population.	The Xpert®MTB/RIF assay was highly effective for TB diagnosis and identification of R resistant strains in smear-negative samples.
30	2011	Singapore	162 respiratory and non-respiratory specimens were compared for the Xpert®MTB/RIF and the Amplified Mycobacterium Tuberculosis Direct (MTD) assay. Based on C as the gold standard, the overall SENS and SPEC for all sample types for the Xpert®MTB/RIF was 90.9 and 89%, respectively, whilst for the MTD assay, the overall SENS and SPEC was 97.3 and 87.1 %, respectively. A higher proportion of total equivocal results were obtained for the MTD assay at 10.5% (17/162) whilst the Xpert®MTB/RIF assay generated 5.5% (9/162) of invalid reads. The limited number of R-resistant isolates (n=4 present in this study hindered a proper assessment of the efficacy of Xpert®MTB/RIF in detecting R resistance.	In the specific laboratory context, the MTD test has a similar performance to the Xpert assay. The MTD test is however a fully manual test. In contrast, the Xpert MTB/RIF is a self-contained, integrated test that offers minimal hands-on time with low potential for PCR contamination. The concurrent detection for R associated mutations is also an added advantage

31	2011	North Carolina, USA	The Cepheid Xpert MTB/RIF research-use-only (RUO) assay and a laboratory-developed test (LDT) targeting IS6110 were evaluated and compared to mycobacterial C as the gold standard in112 specimens from 90 patients, including 89 pulmonary specimens and 23 extrapulmonary specimens. Of the specimens tested, 37 (33%) were C+ for MTB complex; 29 were pulmonary, and 8 were extrapulmonary. Of the C+ specimens, 83% of the pulmonary specimens and 50% of the extrapulmonary specimens were SS+. There was complete concordance between the SS+ C+ specimens, independent of the anatomical site (100% SENS). The SENS of the MTB/RIF RUO assay for SS- specimens was 60% for pulmonary and 75% for extrapulmonary specimens, while the IS6110 LDT SENS were 40% and 0%, respectively. There was also complete concordance among the C- specimens tested. Both assays showed 95% SPEC, with four C- specimens testing as positive. A review of patient records indicated that there was a high likelihood of the presence of MTB complex DNA in the false-positive specimens. Biosafety analysis was performed and showed an acceptable reduction in organism viability using the processing methods described above	Both molecular assays are suitable for the detection of MTB isolates in SS+ pulmonary and extrapulmonary specimens, while SENS of the detection of MTB isolates in SS- specimens was variable.
32	2011	Multisite clinical trial, USA	2,008 samples were tested. Decreasing MTB Ct was associated with increasing SS microscopy grade for smears of concentrated sputum pellets (rs = −0.77) and directly from SS (rs = −0.71). A Ct cut off of approximately 27.7 best predicted SS+ status. The association between MTB Ct and time-to-detection in liquid C (rs = 0.68) and semi-quantitative colony counts (rs = −0.56) was weaker than SS. Tests of paired same-patient SS showed that high-viscosity SS samples contained ×32 more MTB than non-viscous samples. Comparisons between the grade of the acid-fast bacilli SS and Xpert®MTB/RIF quantitative data across study sites enabled the identification a site outlier in microscopy.	Xpert®MTB/RIF quantitation offers a new, standardized approach to measuring bacterial burden in the sputum of TB patients.
33	2011	Primary health care clinic, Johannesburg, South Africa	Consecutive adults with suspected TB attending a primary health care clinic were prospectively enrolled and evaluated for TB according to national guidelines, including assessment for SS- TB by chest X-ray, clinical evaluation, and HIV testing. A single SS sample underwent routine decontamination, AFB SS microscopy, liquid C, and phenotypic DST. Residual sample was batched for molecular testing. 311 participants, HIV prevalence 70% (n = 215), with 120 (38.5%) C+ TB cases. Compared to liquid C, SENS of all the test methodologies, determined with a limited and potentially underpowered sample size (n = 177), were 59% (47%–71%) for SS microscopy, 76% (64%–85%) for MTBDRplus, 76% (64%–85%) for LCTB, and 86% (76%–93%) for , Xpert®MTB/RIF with specificities all >97%. Among HIV+ individuals, SENS of the Xpert®MTB/RIF assay was 84% (69%–93%), while the other molecular tests had SENS reduced by 6%. TB detection among SS-, C+ samples was 28% (5/18) for MTBDRplus, 22% (4/18) for LCTB, and 61% (11/18) for Xpert®MTB/RIF. A few (n = 5) R-resistant cases were detected using phenotypic DST. Xpert®MTB/RIF detected four of these five cases (fifth case not tested) and two additional phenotypically susceptible cases.	The Xpert®MTB/RIF test has superior performance for rapid diagnosis of MTB over existing AFB SS microscopy and other molecular methodologies in an HIV- and TB-endemic region. Its place in the clinical diagnostic algorithm in national health programs needs exploration.
34	2011	South Africa	Xpert®MTB/RIF was evaluated using single archived spot-SS samples from 496 South African patients with suspected TB. Overall, Xpert®MTB/RIF detected 95% (95% CI, 88–98%; 89 of 94) of SS+ C+ cases and SPEC was 94% (91–96%; 320 of 339). SENS in SS- cases was 55% (35–73%; 12 of 22) when the analysis was restricted to 1 ml of unprocessed SS and C time-to-positivity of less than or equal to 28 days. Compared with SS microscopy (n = 94), Xpert®MTB/RIF detected an additional 17 cases (n = 111) representing an 18% (11–27%; 111 vs. 94) relative increase in TB case detection. Compared with SS microscopy, the inclusion of Xpert®MTB/RIF -positive C- TB cases (ruled-in by an alternative diagnostic method) resulted in the detection of a further 16 cases (n = 127), thus significantly increasing the TB case detection rate to 35% (95% CI, 26–45%; 94 to 111 vs. 94 to 127; P < 0.01), the overall SPEC to 99.1% (97–100%; 320 of 323; P < 0.001), and SENS in SS- TB to 60% (P = 0.12). Performance was strongly correlated with SS status and C time-to-positivity. In patients infected with HIV compared with patients uninfected with HIV, Xpert®MTB/RIF showed a trend to reduced SENS (P = 0.09) and significantly reduced NPV (P = 0.01). The NPV for RMP resistance was 99.4%.	Xpert®MTB/RIF outperformed SS microscopy, established a diagnosis in a significant proportion of patients with SS- TB, detected many highly likely TB cases missed by C, and accurately ruled out R-resistant TB. Sample-specific factors had limited impact on performance. Performance in patients infected with HIV, especially those with advanced immunosuppression, warrants further study.

35	2011	HIV clinic, South Africa	The accuracy of the X Xpert®MTB/RIF assay for diagnosing TB and drug resistance was assessed in comparison with other tests, including fluorescence SS microscopy and automated liquid C (gold standard) and DST. Of 515 patients enrolled, 468 patients (median CD4 cell count, 171 cells/µl; IQR 102–236) produced at least one SS sample, yielding complete sets of results from 839 samples. MTB was cultured from 81 patients (TB prevalence, 17.3%). The overall SENS of the Xpert®MTB/RIF assay for C+ TB was 73.3% (SPE, 99.2%) compared to 28.0% (SPE, 100%) using SS microscopy. All SS+, C+- disease was detected by Xpert®MTB/RIF from a single sample (SENS, 100%), whereas SENS for SS-, C+ TB was 43.4% from one SS sample and 62.3% from two samples. Xpert®MTB/RIF correctly identified R resistance in all four cases of MDR-TB but incorrectly identified resistance in three other patients whose disease was confirmed to be drug sensitive by gene sequencing (SPEC, 94.1%; PPV, 57%).	In this population of individuals at high risk of TB, intensive screening using the Xpert®MTB/RIF assay increased case detection by 45% compared with SS microscopy, strongly supporting replacement of microscopy for this indication. However, despite the ability of the assay to rapidly detect R-resistant disease, SPEC for drug-resistant TB was sub-optimal.
36	2011	University Center for Chronic Diseases, Groesbeek, The Netherlands	The Authors analysed 89 unprocessed clinical samples (86 SS samples, 1 pleural fluid,1 gastric fluid, 1 bronchial washing). 26 samples were obtained fresh, in clinical routine, 63 samples previously stored at −70°C. SENS for detecting MTB in C+ samples was 93.8% (60/64) and exceeded SS microscopy (40/64, 62.5%). SPEC for detecting MTB was 92.0% (23/25) and for R resistance 100% (8/8). In the 40 SS+ samples that grew MTB, SENS was 100%. One SS+,C- gastric fluid sample was positive with Xpert®MTB/RIF;In 48 SS- samples, SENS was 83.3% (20/24), and SPEC was 95.8% (23/24).The combined SENS for detecting MTB in SS- and SS+ sputum samples was 93.8% (60/64), and SPEC was 92.0% (23/25). The sample set included 8 samples from R-resistant TB patients (4 fresh, 4 frozen; 6 MDR-TB,2 R-monoresistant); all were recognised as R-resistant by the Xpert®MTB/RIF assay. No false positives or -negatives were noted; SENS and SPEC for detecting R-resistance was 100%. No significant differences in SENS and SPEC in fresh (SENS 100%, SPEC 90.1%, n = 26) vs. frozen samples (SENS 91.8%, SPEC 92.9%,n = 63) were detected.	The test was technically simple to conduct and required neither PCR facilities nor biosafety precautions other than those of routine SS handling. These characteristics render it a promising close-to-patient test for TB in various settings. The Xpert®MTB/RIF thus combines all the characteristics required of a close-to-patient test. The analysis was rapid, simple, safe and required hardly any training. This platform is highly promising for close to-patient TB diagnostics.
37	2011	Tanzania	In 292 samples, the diagnostic performance of Xpert®MTB/RIF was compared to standard SS microscopy and C. Xpert®MTB/RIF achieved 88.4% (95%CI = 78.4% to 94.9%) SENS among patients with C+ and 99% (95%CI = 94.7% to 100.0%) SPEC in patients who had no TB. HIV status did not affect test performance in 172 HIV-infected patients (58.9% of all participants). Seven additional cases (9.1% of 77) were detected by Xpert®MTB/RIF among the group of patients with clinical TB who were C- . In 45 sputum specimens that grew NTM the assay's SPEC was 97.8% (95%CI = 88.2% to 99.9%).	The Xpert®MTB/RIF assay was a highly sensitive, specific and rapid method for diagnosing TB which has potential to complement the current reference standard of TB diagnostics and increase its overall sensitivity. Its usefulness in detecting SS and C- patients needs further study. Further evaluation in high burden TB and HIV areas under programmatic health care settings to ascertain applicability, cost-effectiveness, robustness and local acceptance are required.
38	2011	France	The Authors evaluated 117 clinical specimens (97 C+ and 20 C- for MTBC) frozen in sediment. The 97 clinical specimens included 60 respiratory and 37 non respiratory specimens, of which 36 were SS+ and 61 were SS-.Among the 97 C+ specimens, 4 had R-resistant isolates. Both methods were highly SPEC and exhibited excellent SENS (100%) with SS+ specimens. SENS of the Xpert®MTB/RIF test with the whole SS- specimens was more reduced than that of the IS6110-TaqMan assay (48 versus 69%, P = 0.005). Both methods exhibited similar SENS with SS- respiratory specimens, but the Xpert®MTB/RIF test had lower SENS with SS- non respiratory specimens than the IS6110-TaqMan assay (37 versus 71%, P = 0.013). Finally, SENS of the Xpert®MTB/RIF test and the IS6110-TaqMan assay were 79% and 84%, respectively with respiratory specimens, and 53% and 78% respectively (P = 0.013), with non-respiratory specimens. The Xpert®MTB/RIF test correctly detected R resistance in SS+ specimens but not in the one SS- specimen.	The Xpert®MTB/RIF test was a simple rapid method well adapted to a routine laboratory that appeared to be as SENS as the IS6110-TaqMan assay with respiratory specimens but less SENS with paucibacillary specimens, such as SS- non respiratory specimens.
39	2011	University hospital Caen, France	91 respiratory and 89 non-respiratory samples were evaluated. Overall, 31 (17.2%) of the 180 samples, including 17 respiratory and 14 non-respiratory (respectively 17 and 12 PCR-positive), yielded MTB on C. SENS and SPEC of PCR were respectively 100% and 100%, and 85.7% and 97.3% for respiratory and non-respiratory samples.	Although the Xpert®MTB/RIF testis validated only for respiratory samples, findings suggested that it could be useful for the diagnosis of extra-pulmonary TB.

40	2011	Western United States	A total of 217 specimens were submitted to evaluate the GeneXpert MTB/RIF assay (for research use only).Overall agreement compared to C was 89% (98% for SS+ and 72% for SS-) for detection of MTB.	Overall, the GeneXpert assay was simple, fast, accurate, and cost-comparative to other commercially available PCR assays for the direct detection of MTB.
41	2011	Spain	64 of 85 (75.3%) SS- respiratory (n = 78) and non respiratory (n = 7) samples with C+ MTB complex (MTC) were detected by the GeneXpert (GX) system using the Xpert®MTB/RIF assay. In addition, GX found rpoB mutations in all six of the R-resistant strains detected. The test was negative in 20 C- and 20 NTM C+ samples (100% SPEC).	The global data obtained in the present study indicate that GX has a high SENS, since all samples analyzed had a low mycobacterial load. The relatively high cost of GX was an important issue that TB control programs should consider prior to implementation of this assay. Its clinical and epidemiological advantages should be weighed against the resources available in each setting. In summary, the GX technique demonstrated a high capacity for detecting MTC and for predicting MDR in SS- clinical samples. Moreover, its rapidity, simplicity, and low laboriousness make the technique a good candidate for routine use in many clinical laboratories whenever the clinical criteria for its application are met.
50	2011	South Africa	452 children (median age 19·4 months, IQR 11·1—46·2) had at least one induced sputum specimen; 108 children (24%) had HIV infection. 27 children (6%) had a positive SS result, 70 (16%) had a positive C result, and 58 (13%) had a positive Xpert®MTB/RIF test result. With mycobacterial C as the reference standard, Xpert®MTB/RIF tests when done on two induced sputum samples detected twice as many cases (75.9%, 95% CI 64.5—87.2) as did SS microscopy (37.9%, 25·1—50·8), detecting all of 22 SS+ cases and 22 of 36 (61·1%, 44.4—77.8) SS- cases. For SS- cases, the incremental increase in SENS from testing a second specimen was 27.8% for Xpert®MTB/RIF , compared with 13·8% for C. SPEC of Xpert®MTB/RIF was 98.8% (97.6—99.9). Xpert®MTB/RIF results were available in median 1 day (IQR 0—4) compared with median 12 days (9—17) for C (p<0·0001).	Xpert®MTB/RIF testing of two induced sputum specimens was warranted as the first-line diagnostic test for children with suspected pulmonary TB.
51	2011	South Africa	The Authors compared the turn-around-time, detection-threshold, dynamic range, reproducibility, relative discriminative ability, of 4 mycobacterial load determination techniques: automated liquid culture (BACTEC-MGIT-960), [3H]-uracil incorporation assays, luciferase-reporter construct bioluminescence, and quantitative PCR(Xpert -MTB/RIF) using serial dilutions of Mycobacterium bovis and Mycobacterium tuberculosis H37RV. Mycobacterial colony-forming-units(CFU) using 7H10-Middlebrook solid media served as the reference standard. All 4 assays correlated well with the reference standard, however, bioluminescence and uracil assays had a detection threshold ≥1×103 organisms. By contrast, BACTEC-MGIT-960 liquid C, although only providing results in days, was user-friendly, had the lowest detection threshold (<10 organisms), the greatest discriminative ability (1 vs. 10 organisms; p = 0.02), and the best reproducibility (coefficient of variance of 2% vs. 38% compared to uracil incorporation; p = 0.02). Xpert®MTB/RIF correlated well with mycobacterial load, had a rapid turn-around-time (<2 hours), was user friendly, but had a detection limit of ~100 organisms.	Choosing a technique to quantify mycobacterial burden for laboratory or clinical research depends on availability of resources and the question being addressed. Automated liquid culture had good discriminative ability and low detection threshold but results were only obtained in days. Xpert®MTB/RIF provided rapid quantification of mycobacterial burden, but had a poorer discrimination and detection threshold.
52	2011	South Africa	20 cases with confirmed TB pleural effusion. Xpert®MTB/RIF SENS and SPEC in pleural fluid was 25% and 100%, respectively. All positive Xpert®MTB/RIF results were also pleural fluid C+.	Xpert®MTB/RIF testing in pleural fluid samples is feasible, but of low SENS and linked to a positive pleural fluid C. There is an indication for high SPEC, which must be verified with larger studies including more patients with a pleural effusion due to other causes than TB. Before this is attempted the methods for collection, storage and preparation of pleural fluid samples need to be optimized in order to increase Xpert®MTB/RIF SENS on pleural fluid.

53	2011	Spain	340 non-respiratory samples were processed using two real-time PCR assay kits: Xpert®MTB/RIF and Cobas TaqMan MTB. SENS and SPEC of the Xpert assay were 95% and 100%, respectively, compared to 78% and 98% for the Cobas assay.	Both molecular techniques represent an important contribution to the detection of MTB, since they can provide results in a matter of hours, whereas the reference C method takes days. Real-time PCR techniques afford greater SENS and SPEC and a much-reduced response time,as well as enabling visualization of amplification curves. One limitation of these techniques is that, in detecting MTB DNA, they cannot distinguish between viable and nonviable microorganisms. For that reason, although these assays are semi-quantitative, they should not be used for monitoring patient progress or treatment efficacy
54	2011	South Africa	To determine the diagnostic utility of the Xpert® 52 MTB/RIF, FNAB (fine needle aspiration biopsy) were collected from 50 consenting patients by aspirating TB lymphadenitis..Aspirates underwent C (MGIT 960), genotypic DST (Genotype MTBDR*plus* assay) and Xpert MTB/RIF. Compared to the reference standard, Xpert MTB/RIF correctly identified 29 out of 30 TB cases (sensitivity 96.7%, 95%CI, 86.6-100). The possible "false negative" result had a prolonged transit interval of 9 days before Xpert MTB/RIF testing, which may have affected the result. Xpert MTB/RIF was positive in two cases with negative cytomorphology and culture (specificity 88.9%, 95%CI, 69.6-100). The Xpert MTB/RIF test was positive in all 6 smear negative culture positive cases and correctly identified the 1 of the 2 R resistant cases. The average time to result for microbiological C was 18.5 days (range 9-55 days), while the Xpert MTB/RIF test result was available within 2 hours of commencing the test.	This study demonstrated the excellent diagnostic accuracy of the Xpert® MTB/RIF test in patients with TB lymphadenitis.
55	2011	India	547 extrapulmonary specimens were split and processed simultaneously for both C (solid and liquid) and Xpert®MTB/RIF testing. For culture, SENS was low, 53% (150/283 specimens). Xpert®MTB/RIF SENS and SPEC results were assessed in comparison to a composite reference standard made up of SS and C results and clinical, radiological, and histological findings. SENS of the Xpert®MTB/RIF assay was 81% (228/283 specimens) (64% [89/138] for SS- cases and 96% [139/145] for SS+ cases), with 99.6% SPEC. SENS was found to be high for the majority of specimen types (63 to 100%) except for cerebrospinal fluid, with SENS of 29% (2/7 specimens). The Xpert®MTB/RIF correctly identified 98% of phenotypic R--resistant cases and 94% of phenotypic R-susceptible cases. Sequencing of the 6 discrepant samples resolved 3 of them, resulting in an increased specificity of 98%.	The results of this study suggested that the Xpert®MTB/RIF test also showed good potential for the diagnosis of extrapulmonary TB and that its ease of use made it applicable for countries where TB is endemic.
56	2011	Germany	521 non-respiratory specimens were comparatively investigated with the Xpert®MTB/RIF assay and conventional liquid and solid C methods. 20 (3.8%) of the 521 specimens gave no interpretable result. Whereas SENS of the X Xpert®MTB/RIF with tissue specimens was 69.0% (20 out of 29 C+ cases detected), 100% SENS was found with urine and stool specimens. The combined SENS and SPEC of the Xpert®MTB/RIF assay were calculated to be 77.3% and 98.2%, respectively.	The Xpert®MTB/RIF assay could be applied to extrapulmonary specimens with a high SENS and SPEC, which, coupled with its speed and simplicity, made this technique a very useful tool for the diagnosis of extrapulmonary TB.
57	2011	CapeTown, South Africa	In this cost-analysis study the Authors assessed the accuracy and/or laboratory-associated cost-of-diagnosis of SS microscopy,chest-radiography, and interferon-γ-release assays (IGRAs; T-SPOT-TB and QFT-GIT), combined with a single Xpert-MTB/RIF, for the diagnosis of TB in 480 suspects. When conducted prior to Xpert®MTB/RIF testing: (i) SS-microscopy followed by Xpert®MTB/RIF (if SS-) had the lowest cost-of-diagnosis of any strategy investigated; (ii) a combination of SS-microscopy, chest-radiography (if SS-) and Xpert®MTB/RIF (if imaging compatible with active-TB) did not further reduce the cost per TB case diagnosed; (iii) a normal chest radiograph ruled-out TB in 18% of suspects [57/324; NPV 100%(57/57)]. When downstream adjunct tests were applied to Xpert®MTB/RIF -negative individuals: (i) radiology ruled-out TB in 24% [56/234; NPV 100%(56/56)]; (ii) SS-microscopy ruled-in TB in 21%(7/24) of C+ individuals; (iii) IGRAs were not useful in either context.	In resource-poor settings, SS-microscopy combined with Xpert®MTB/RIF had the highest accuracy and lowest cost-of-diagnosis compared to either technique alone. In Xpert®MTB/RIF-negative individuals, chest radiography had poor rule-in value but couldreliably rule-out TB in ~1 in 4 of such cases. These data informed the programmatic utility of Xpert®MTB/RIF in high burden settings. Detailed cost-effectiveness analyses are required.
58	2011	South Africa	A pilot program using dried culture spots (DCS) of inactivated MTB is described. Of 274 DCS results received, 2.19% generated errors; the remaining yielded 100% correct MTB detection. Probe A cycle threshold (Ct) variability of three DCS batches ≤3.47Ct. Longer-term DCS stability is on-going.	This study provided preliminary demonstration through the use of inactivated MTB coupled with easier transportation of DCS material that an EQA program can be safely provided. Future design of an

				Xpert®MTB/RIF EQA program could be similarly based on line probe assay programs using one pan-susceptible strain, one R-monoresistant strain with a common rpoB mutation, one MDR strain, one NTM strain and a negative control each placed on a DCS card and distributed 3/6 monthly.
59	2012	Italy	Review of the findings by Tortoli et al (Ref 72) done by a per-sample analysis of 268 diagnoses of extrapulmonary TB (EPTB) at a range of anatomic sites (SENS: 81.3%; 95% CI: 76.2–85.8) and data for 1206 samples in which EPTB was excluded (SPEC: 99.8%; 95% CI: 99.4–100).	The AA conclude that this study (Ref 72) was an important addition to the growing body of literature demonstrating the utility of Xpert®MTB/RIF for EPTB diagnosis when applied to diverse types of clinical samples.
60	2012	USA	The Authors determined the lower limit of detection (LOD) of the GeneXpert MTB/RIF assay with non-respiratory specimens and investigated the utility of flotation procedures for concentrating the bacilli. Clinical specimens (9 CSF, 13 gastric aspirate, 8 tissue, and 17 stool) were spiked with single-celled MTB and the LOD of the GeneXpert was determined. Flotation studies were conducted with sucrose and NaCl and the cycle thresholds of the MTB/RIF assay were compared between treated and untreated samples.There was no significant difference between the LOD of the Xpert®MTB/RIF with saline (median 33 CFU/ml) and CSF (median 25 CFU/ml) ($P > 0.05$) or gastric aspirate samples (median 58 CFU/ml) ($P > 0.05$). The LOD with spiked tissue (median 1,525 CFU/ml) and stool samples (median 6,800 CFU/ml) was significantly elevated compared to saline ($P \leq 0.05$ and ≤ 0.0005, respectively). Flotation studies with sucrose or NaCl did not consistently result in lowered cycle thresholds in stool or gastric aspirates but >10 cycle reduction was achieved in two of the three pooled CSF samples.	Unlike with tissue and stool samples, there was no significant PCR inhibition in the Xpert®MTB/RIF assay with CSF and gastric aspirates. Although pre-concentration of CSF samples with sucrose and NaCl may enhance detection of MTB by PCR, further advances are needed to concentrate the bacilli and eliminate PCR inhibitors in paucibacillary non-respiratory samples.
61	2012	South Africa	The Authors determined the diagnostic yield of the Xpert MTB/ RIF assay for TB when testing small volumes of urine from ambulatory HIV-infected patients before starting ART therapy in South Africa. Among 602 patients recruited, 535 produced at least 1 sputum sample and a specimen of urine. Sputum C results were available from 516 patients and these yielded 85 diagnoses of C+ TB. The remainder (n = 431) were sputum C negative.Compared with a gold standard of sputum culture, SENS of urine Xpert®MTB/RIF among those with CD4 cell counts of <50, 50–100, and >100 cells per microliter were 44.4%, 25.0%, and 2.7% ($P = 0.001$), respectively.	Urine Xpert®MTB/RIF testing provided a means of rapid TB diagnosis in patients with advanced immunodeficiency and poor prognosis. These data were indicative of high rates of TB dissemination and renal involvement in this clinical population.
62	2012	Spain	Among 108 SS- extrapulmonary samples showing a C+ for MTB complex (43 body fluids and 65 non liquid specimens), 63 (58.3%) were positive with the Xpert®MTB/RIF assay . SENS was quite low for samples from sterile locations (especially for pleural fluids: 26.9%) but high for some non-liquid samples, like abscess aspirates (76.5%).	Xpert®MTB/RIF may be a useful tool to be considered for extrapulmonary TB diagnosis.
63	2012	University Teaching Hospital Lusaka, Zambia	C+ TB was found in 201/881 patients (22.8%). Xpert®MTB/RIF SPEC was 95.0% (95% CI, 92.4%–96.8%); SENS was 86.1% (95% CI, 80.3%–90.4%). In SS-, C+ cases, the assay had 74.7% SENS (95% CI, 64.6%–82.8%), identifying 71 additional TB cases that were not detected by SS. 18/111 patients with TB who were tested (16.2%) had MDR- TB. SENS and SPEC of Xpert®MTB/RIF for detecting C+, Resistant TB was 81.3% (95% CI, 53.7%–95.0%) and 97.5% (95% CI, 90.4%–99.6%), respectively.	Xpert®MTB/RIF performed better than SS in an inpatient setting in a country where TB and HIV infection are highly endemic. Assessment of its usefulness and cost-effectiveness for increased detection of TB cases missed by SS and for concomitant screening for MDR-TB among adult inpatients attending tertiary care referral centres in other countries with a high burden of TB and HIV infection is warranted.
64	2012	Different Hospitals, sub-Saharan Africa	42% (116/242) of patients had C+ TB. 18% (20/54) were SS scarce. In SS-scarce patients, SENS of urine Xpert®MTB/RIF MTB/RIF and LAM ELISA test was 40% (95%CI: 22–61) and 60% (95%CI: 39–78), respectively. Urine Xpert®MTB/RIF SPEC was 98% (95%CI: 95–100). Combined SENS of urine LAM ELISA and Xpert®MTB/RIF was better than Xpert®MTB/RIF alone [Xpert®MTB/RIF and LAM: 70% (95%CI: 48–85) vs. Xpert®MTB/RIF: 40% (95%CI: 22–61), $p = 0.03$]. Significant predictors of urine Xpert®MTB/RIF positivity were CD4<50 cells/ml ($p = 0.001$), elevated protein-to-creatinine ratio ($p<0.001$) and LAM ELISA positivity ($p<0.001$). Urine centrifugation and pelleting significantly increased the SENS of Xpert®MTB/RIF over unprocessed urine in paired samples [42% (95%CI: 26–58) vs. 8% (95%CI: 0–16), $p<0.001$]. Urine Xpert®MTB/RIF -generated CT (cycle-threshold)values correlated poorly with markers of bacillary burden (SS grade and time-to-positivity).	This preliminary study indicates that urine-based Xpert®MTB/RIF , alone or in combination with LAM antigen detection, maypotentially aid the diagnosis of TB in HIV-infected patients with advanced immunosuppression when SS-based diagnosis is not possible. Concentration of urine prior to Xpert®MTB/RIF testing significantly improves SENS.

65	2012	City Hospital Auckland, New Zealand	All AFB SS+ respiratory specimens, were processed and analysed by Xpert® MTB/RIF assay using C (MGIT 960 system) as the gold standard, A total of 169 specimens (89 SS+ respiratory specimens; 9 smear-positive extra-pulmonary specimens and 71 positive MGIT liquid C vials) from 169 patients were analysed. With the use of culture as the "gold standard", the overall SENS and SPE of the MTB/RIF assay for the detection of MTB were 100% (141/141) and 100% (28/28), respectively. The MTB/RIF assay detected R resistance in 13/169 (7.7%) specimens. However, using standard phenotypic methods, R resistance was detected in only 7/13 (54%) isolates. In 2 of the remaining 6 isolates, amplification and sequencing of the rpoB gene revealed mutations associated with increased but low-level R resistance.	Similar to previous studies, the Authors found the MTB/RIF assay highly SENS and SPE for the detection of MTB, when used for both smear-positive pulmonary and extrapulmonary specimens as well as for isolates in liquid C media. However, the Authors found that the assay was less reliable for the detection of R resistance, producing false-positive results in 4/13 (31%) specimens. Further work is therefore required to evaluate the performance of the MTB/RIF assay for the detection of R resistance in a range of clinical settings and on a range of specimen types.
66	2012	Supra-national Reference Laboratory, Milan, Italy	An innovative approach allowed selective amplification of DNA derived from viable MTB in clinical specimens, which was useful for monitoring mycobacterial load in pulmonary TB patients during anti-TB treatment. The protocol was based on pre-treatment of samples with propidium monoazide (PMA).PMA did not significantly affect PCR yield of specimens collected at time zero, confirming that the SS positivity of these samples was mostly due to highly damaged bacteria. Moreover, Delta Ct (difference in amplification yield between samples with and without PMA pre-treatment) calculated between t0 and t1 in PMA-untreated samples was found to be too low to represent a real decrease in bacterial load due to therapy. All patients were successfully treated and cured at the end of therapy, consistent with the reduction of live bacteria detected by the PMA assay.	Quantitative molecular techniques combined with the PMA method could be an alternative to SS and C for monitoring early treatment response and for preliminary evaluation of personalised regimens. The use of this assay can allow earlier evaluation of treatment efficacy, showing a clear decrease in the vital mycobacterial load. Absence of the response to therapy might also be promptly identified by the test allowing a regimen change and limiting the spread of infection and further resistance development
67	2012	HIV service, South Africa	523/602 patients screened had ≥1 Xpert®MTB/RIF and C result, yielding 89 C+ TB diagnoses. Of these, 37 (42%) of TB patients were Xpert®MTB/RIF -negative when a single SS sample was tested, compared with 25 (28%) when 2 samples were tested. Compared to patients with Xpert®MTB/RIF -positive TB, those with Xpert®MTB/RIF -negative TB (using either definition) had substantially higher CD4 cell counts, lower plasma viral loads, higher hemoglobin concentrations, and higher body mass index. Their TB was also less advanced, with a lower frequency of prolonged cough (≥2 weeks), less extensive radiographic abnormalities, and a lower frequency of detectable lipoarabinomannan antigenuria and mycobacteriuria. Xpert®MTB/RIF -negative cases were all SS- with prolonged time to C positivity (median, 21 days). Despite greater delays in starting TB treatment, Xpert®MTB/RIF -negative patients were less likely to die during follow-up.	Compared to patients with Xpert positive TB diagnosed during pre-ART screening, Xpert negative cases had less advanced immunosuppression and less advanced TB and did not have adverse outcomes despite substantial delays in starting TB treatment.
68	2012	Primary care clinic, Cape Town, South Africa	Lawn and colleagues described the clinical characteristics of patients with pulmonary TB who tested negative by Xpert®MTB/RIF but had radiographically less advanced TB and fewer adverse outcomes compared with those who tested positive, prior to commencing antiretroviral therapy in Cape Town, South Africa (ref 67). In a primary care clinic based in the same city, and using archived samples from a prospectively recruited cohort, Theron et al. demonstrated Xpert®MTB/RIF –negative TB patients to have a significantly diminished SS bacillary load compared with those who tested positive. They also described the use of different tests, including chest radiography, when further investigating individuals. Theron's et al data imply that, contrary to what was suggested by Lawn and colleagues, Xpert®MTB/RIF was more likely to miss HIV-infected individuals with advanced immunosuppression. Given the small sample sizes of both studies, and the conflicting findings, the impact of declining CD4 count on sputum-based Xpert®MTB/RIF still remains unclear.	The findings from both studies need to be considered preliminary and context-specific, and further work, especially focusing on the outcome-related impact of extrapulmonary or disseminated TB in patients who have paucibacillary SS, is required.
69	2012	South Africa	Xpert®MTB/RIF –generated cycle-threshold (CT) values have poor clinical utility as a rule-in test for SS positivity (cut-point ≤20.2; SENS 32.3%, SPEC 97.1%) but moderately good rule-out value (cut-point >31.8; NPV 80.0%). Thus, 20% of individuals with CT values >31.8 were erroneously ruled out as SS-. This group had a significantly lower SS bacillary load relative to correctly classified SS+ patients (CT ≤ 31.8; P < .001).	Xpert®MTB/RIF -generated average CT values >31.8 provide moderately good rule-out value for SS positivity. Whether individuals falling above this cut-point, compared with those below, will display reduced disease transmission requires prospective validation. These data have public health implications for the roll-out of Xpert®MTB/RIF and inform public health and contact tracing strategies.

70	2012	Primary care clinic, Johannesburg, South Africa,	An unexpected case of R resistance was investigated on Xpert®MTB/RIF using repeat Xpert®MTB/RIF , SS microscopy, MTBDRplus® assay, C, DST, spoligotyping and rpoB gene sequencing. A false-positive result was most likely, given the wild type rpoB gene sequence and exclusion of both mixed infection and mixture of drug-susceptible and drug-resistant populations.	This report highlights the need for health care workers' understanding of assay performance characteristics when decentralising the diagnosis of drug-resistant TB. These issues should not, however, diminish enthusiasm for the Xpert®MTB/RIF assay.
71	2012	Tanzania	28/164 children (17.1%) had confirmed TB. Xpert®MTB/RIF detected 100% (95% CI, 59.0%–100%) of SS+ cases and 66.6% (95% CI, 43.0%–85.4%) of C+ but SS- cases. In the per-sample analysis, Xpert®MTB/RIF displayed a similar SENS (54.7% [95% CI, 42.7%–66.2%]) compared with C methods. Xpert®MTB/RIF detected 3-fold more confirmed TB cases than SS microscopy but with equal rapidity. Four additional cases (8.5%) with clinical TB but negative C were diagnosed by Xpert®MTB/RIF . Testing second and third samples increased SENS by 20% and 16%, respectively. When TB was reliably excluded, Xpert®MTB/RIF specificity was 100%. HIV infection did not affect diagnostic accuracy of Xpert®MTB/RIF. .	Xpert®MTB/RIF was easy to perform and displayed similar diagnostic accuracy as C methods in children with suspected TB. Rapid turnaround times should reduce treatment delay and improve patient outcome, although SENS remains suboptimal and access was dependent on local laboratory infrastructure.
72	2012	Italy	1,476 consecutive extra-pulmonary clinical specimens including both paediatric (494) and adult samples were investigated with Xpert®MTB/RIF. In comparison with a reference standard consisting of combination of C and clinical diagnosis of TB, an overall SENS and SPEC of 81.3% and 99.8%were found for Xpert®MTB/RIF while the SENS of microscopy was 48%. For biopsies, urines, pus and cerebrospinal fluids SENS exceeded 85% while it was slightly under 80% for gastric aspirates. SENS was lower than 50% for cavitary fluids. High SENS and SPEC (86.9% and 99.7% respectively) were also obtained for paediatric specimens.	Although the role of C remains central in the microbiological diagnosis of EPTB, the SENS of Xpert®MTB/RIF in rapidly diagnosing the disease made it a much better choice compared to SS microscopy. The ability of ruling out the disease still remained suboptimal.
73	2012	South Africa	535 children [median age 19 months, 117 (21·9%) HIV-infected] underwent one induced sputum (IS) and nasopharyngeal aspirate (NPA); 396 had two paired specimens. A positive SS, Xpert®MTB/RIF or C occurred in 30 (5.6%), 81 (15.1%) and 87 (16.3%) respectively. C yield was higher from IS (84/87, 96.6%) vs NPA (61/87, 70.1%, p<0.001). Amongst children with two paired specimens, 63 C confirmed cases occurred [60 (95.2%) IS vs. 48 (76.2%) NPA, p=0.002]. SENS of two Xpert®MTB/RIF tests was similar on IS and NPAs (45/63, 71% vs 41/63, 65%, p=0.444); SENS of SS was lower on IS (21/63, 33%) and NPA (16/63, 25%). Incremental yield from a second IS was 9 cases (17.6%) by C and 9 (25%) by Xpert®MTB/RIF ; a second NPA increased C yield by 10 (26.3%) and Xpert®MTB/RIF 11 (36.7%). Xpert SPEC was 99.1% (98.1 - 100) on IS and 98.2 (96.8 - 99.6) on NPAs. Xpert®MTB/RIF provided faster results than culture (median 0 vs 15 days, p<0·001).	Xpert®MTB/RIF on 2 NPAs was useful in children with suspected PTB particularly in settings where IS and culture were not feasible.
74	2012	Zambia	A model proposed to integrate TB and HIV screening, diagnosis, and treatment into existing antenatal care using Xpert®MTB/RIF technology (as per WHO recommendations).	Pilot studies were urgently required to evaluate strategies for the integration of TB screening into antenatal clinics using new diagnostic technologies, in order to reduce morbidity and mortality for both the mother and child, particularly in women who are co-infected with HIV.
98	2012	South Africa	In this diagnostic sub-study of a TB prevalence survey conducted in gold mining companies in South Africa, 6,893 participants provided a SS specimen. 187/6893 (2.7%) were positive for MTB in C, 144/6893 (2.1%) were positive for MTB by Xpert®MTB/RIF , and 91/6893 (1.3%) were positive for AFB by microscopy. SENS, SPEC, PPV and NPV for detection of MTB by Xpert®MTB/RIF were 62.6% (95% CI 55.2, 69.5), 99.6% (99.4, 99.7), 81.3% (73.9, 87.3), and 98.9 (98.6, 98.8); agreement between Xpert®MTB/RIF and C was 98.5% (98.2, 98.8). SENS of microscopy was 17.6% (12.5, 23.9). When individuals with a history of TB treatment were excluded from the analysis, Xpert®MTB/RIF SPEC was 99.8 (99.7, 99.9) and PPV was 90.6 (83.3, 95.4) for detection of MTB. For the testing scenario of 7000 specimens with 2.7% of specimens C+ for MTB, costs were $165,690 for Xpert®MTB/RIF and $115,360 for the package of microscopy plus C.	In the context of a TB prevalence survey, the Xpert®MTB/RIF diagnostic yield was substantially higher than that of microscopy yet lower than that of liquid C. Xpert may be useful as a sole test for TB case detection in prevalence surveys, particularly in settings lacking capacity for liquid C.

106	2012	Central hospital laboratory, South Korea	Xpert®MTB/RIF detected MTB in 71 (100%) specimens (32 SS+, 39 SS-). 100% (62/62) concordance with drug resistance confirmed by phenotypic method and 98.4% (61/62) concordance with sequencing. One specimen containing approximately 50% of mutant p.His526Tyr was falsely interpreted as wild-type. The minimal detection ratio was 5:1 of mutant vs. wild-type cells. The median time saved was 18.5 days (range 9-30) for the diagnosis of TB and 81.5 days (65-136) for R susceptibility in SS-, C+ patients.	Xpert®MTB/RIF showed: high SENS in detecting MTB with information on R resistance; rapid time to diagnosis compared to conventional tests. Location and number of mutations may affect test sensitivity.
107	2012	Central hospital laboratory, South Africa	Xpert®MTB/RIF was compared vs.Genotype® MTBDRplus (version 2) on SS+ and SS- patient specimens. 282 consecutive specimens were tested by the two new molecular assays and routine diagnostics. Both assays showed similar diagnostic performance characteristics. SEN of the Genotype® MTBDRplus (v2.0) and Xpert®MTB/RIF assay for the detection of C- MTB was 73.1% and 71.2% respectively; SPEC for both assays was 100%. Both assays diagnosed MTB in 57–58% of SS-cases suggesting that the performances depend on bacillary load. Detection of MTB in C- specimens confirmed that molecular-based assays should not be used for treatment monitoring. SENS and SPEC for R resistance detection was 100% in both assays. Genotype® MTBDRplus (v2.0) assay provided additional information on H susceptibility.	The Genotype® MTBDRplus (v2.0) assay will complement the Xpert®MTB/RIF screening assay by validating R susceptibility, providing information on H susceptibility and providing pharmacogenetic information useful in guiding treatment.
108	2012	Reference centre, Lima, Peru	Detection of TB by Xpert®MTB/RIF was compared to a composite reference standard of Löwenstein-Jensen (LJ) and liquid culture. Detection of R resistance was compared to the LJ proportion method. 131 patients were included, the median CD4 cell count was 154.5 cells/mm3 and 45 (34.4%) had TB. For TB detection among HIV patients, SENS of Xpert®MTB/RIF was 97.8% (95% CI 88.4–99.6) (44/45); SPEC was 97.7% (95% CI 91.9–99.4) (84/86); PPV was 95.7% (95% CI 85.5–98.8) (44/46); NPVwas 98.8% (95% CI 93.6–99.8) (84/85). Xpert®MTB/RIF detected 13/14 SS- TB cases, outperforming smear microscopy [97.8% (44/45) vs. 68.9% (31/45); p = 0.0002]. For R resistance detection, SENS of Xpert®MTB/RIF was 100% (95% CI 61.0–100.0) (6/6); SPEC was 91.0% (95% CI 76.4–96.9) (30/33); PPV was 66.7% (95% CI 35.4–87.9) (6/9); NPV was 100% (95% CI 88.7 –100.0) (30/30).	In HIV patients with a high clinical suspicion of TB, Xpert®MTB/RIF performed well for TB diagnosis and outperformed smear microscopy.
109	2012	District hospital, India	Performances of LED auramine fluorescent microscopy and Xpert®MTB/RIF for diagnosis of TB in HIV-infected patients were compared. Although at higher cost, Xpert®MTB/RIF outperformed LED fluorescent microscopy in all type of specimens, especially in cerebrospinal fluid where the number of positive results was increased 11 times. Pleural fluid, ascitic fluid, pus, and stool specimens also yielded positive results with the Xpert®MTB/RIF assay. When collecting two additional early-morning sputum samples, the increase in the number of positive results with the Xpert®MTB/RIF assay was lower than previously reported for HIV-infected patients. R resistance was observed in 2.2% of the cases.	Xpert®MTB/RIF assay can improve the rapid diagnosis of TB meningitis and other types of extrapulmonary tuberculosis in HIV-infected patients.
110	2012	Central Hospital, Tanzania	Among 219 enrolled contacts, the yield of active TB was 2.3%. SENS of SS microscopy was 60% (95%CI 14.7-94.7), SENS of Xpert®MTB/RIF MTB/RIF was 100% (95%CI 47.81-100.0).	As all C+ cases tested positive by Xpert®MTB/RIF on the first submitted sample, the evaluation of one sample only could be sufficient for TB diagnosis in this context.
111	2012	Reference Hospital Santiago, Chile	166 subjects were enrolled; 50.6% provided two sputum samples, 33.1% only one sputum sample and 16.3% a mouth wash sample. The prevalence of TB was 8.1% (13/160). Diagnostic SENS increased from 66.7% (95%CI 39.1-86.2) for SS to 91.7% (95%CI 64.6-98.5) for Xpert®MTB/RIF , with comparable SPEC at 98.6% (146/148, 95%CI 95.2-99.6) and 99.3% (147/148, 95%CI 96.3-99.9). Xpert®MTB/RIF allowed early detection of R resistance in 16.6% of cases, with rapid adjustment to MDR-TB treatment.	Xpert®MTB/RIF provided earlier TB diagnosis in 25% more cases than SS alone. Its implementation should be considered for TB diagnosis in HIV-positive patients even outside TB-endemic areas.
112	2012	South Africa	Xpert®MTB/RIF testing of two induced sputum specimens detected approximately 75% of children with C-confirmed disease. Urine lipoarabinomannan has shown promise as a rapid diagnostic in a subgroup of HIV-infected severely immunocompromised adults, but there have been no data in children so far.	The availability of Xpert®MTB/RIF was an important advance that could improve case detection in children and enable rapid detection of mycobacterial drug resistance.

Annex 1: Synopsis of the available studies on Xpert®MTB/RIF presented in the order they appear in the text for the following areas: assay evaluation, assay development, detection of extrapulmonary TB, detection of paediatric TB, diagnostic algorithms, use in prevalence surveys and quality assurance.

Legend:

AFB: Alcohol-acid fast bacilli
ART: Antiretroviral therapy
C+/-: Culture positive/negative
CFU: Colony Forming Unit
CI: Confidence Intervals
CT: Cycle-threshold
EQA: External quality assurance
H: Isoniazid
IQR: Interquartile range
LED: Light-emitting diode
MDR-TB: Multidrug-resistant TB
MTB: *Mycobacterium tuberculosis*
NPV: Negative predictive value
NTM: Non-tuberculous mycobacteria
PPV: Positive predictive value
R: Rifampicin
SENS: Sensitivity
SPEC: Specificity
SS+/-: Sputum smear positive/negative
TB: Tuberculosis
XDR-TB: Extensively drug-resistant TB

Ref N.	Year	Setting	Main Findings	Main Conclusions
8	1998	USA	A set of overlapping molecular beacons was used to analyze an 81-bp region of the MTB rpoB gene for mutations that confer resistance to the antibiotic R. In a blinded study of 52 R-resistant and 23 R-susceptible clinical isolates, this method correctly detected mutations in all of the resistant strains and in none of the susceptible strains.	The assay was carried out entirely in sealed PCR tubes and was simple to perform and interpret. This approach can be used to analyze any DNA sequence of moderate length with single base pair accuracy
9	2000	Spain and USA	A rapid closed-tube PCR assay using fluorogenic reporter molecules called molecular beacons to detect reportedly common MTB mutations associated with resistance to H and R was developed. The overall SENS and SPEC of the assay for H resistance were 85 and 100%, respectively, and those for R resistance were 98 and 100%, respectively. R resistance mutations were detected equally well in isolates from both study populations; however, H resistance mutations were detected in 94% of the isolates from Madrid but in only 76% of the isolates from New York (P = 0.02). In New York, H resistance mutations were significantly more common in the MDR isolates (94%) than in single-drug-resistant isolates (44%; P < 0.001). No association between previously described mutations in the kasA gene and H resistance was found. The first mutations that cause H resistance may often occur in sequences that have not been commonly associated with H resistance, possibly in other as yet uncharacterized genes	The molecular beacon assay was simple, rapid, and highly sensitive for the detection of R-resistant MTB isolates and for the detection of H resistance in MDR isolates.
10	2001	USA	The study reports on a highly SENS PCR assay that takes less than 3 h and reliably identifies R-resistant MTB in DNA extracted directly from SS. When 148 MTB clinical isolates of known susceptibility to R were tested, mutations associated with R resistance were detected in 63 of the 65 R-resistant isolates, and no mutations were found in any of the 83 R-susceptible isolates. When DNA extracted directly from the SS of 11 patients infected with R-resistant TB was tested, mutations were detected in all of the samples.	The use of this rapid assay should enable early detection and treatment of drug-resistant TB in clinical settings.
11	2010	Vietnam and Uganda	Analytic tests of MTB DNA demonstrated a limit of detection (LOD) of 4.5 genomes per reaction. Studies using sputum spiked with known numbers of MTB CFU predicted a clinical LOD of 131 CFU/ml. Killing studies showed that the assay's buffer decreased MTB viability by at least 8 logs, substantially reducing biohazards. Tests of 23 different commonly occurring R resistance mutations demonstrated that all 23 (100%) would be identified as R-resistant. An analysis of 20 NTM species confirmed high assay specificity. A small clinical validation study of 107 clinical sputum samples from suspected TB cases in Vietnam detected 29/29 (100%) SS+ C+ cases and 33/39 (84.6%) or 38/53 (71.7%) SS-,C+ cases, as determined by growth on solid medium or on both solid and liquid media, respectively. MTB was not detected in 25/25 (100%) of the C- samples. A study of 64 SS- smear-C+ sputa from retreatment TB cases in Uganda detected 63/64 (98.4%) C+ - cases and 9/9 (100%) cases of R resistance. R resistance was excluded in 54/55 (98.2%) susceptible cases. SPEC rose to 100% after correcting for a conventional susceptibility test error.	This highly sensitive and simple-to-use system can detect MTB directly from SS in less than 2 h.
12	2010	USA	79 MTB isolates and 89 NTM isolates were studied .The Xpert®MTB/RIF assay correctly identified all 79 MTB isolates and correctly excluded all 89 NTM isolates. R resistance was correctly identified in all 37 resistant isolates and in none of the 42 susceptible isolates. Dynamic range was assessed by adding 102 to 107 Colony Forming Units (CFU) of MTB into MTB-negative sputum samples. The assay showed a log-linear relationship between cycle threshold and input CFU over the entire concentration range. Resistance detection in the presence of different mixtures of R-resistant and R-susceptible DNA was assessed. Resistance detection was dependent on the particular mutation and required between 65% and 100% mutant DNA to be present in the sample for 95% certainty of resistance detection	The Xpert®MTB/RIF assay was relatively resistant to contamination by MTBDRplus amplicons and could safely be used in the same laboratory environment. This was the result of inefficient amplification of the MTBDRplus amplicon, which has only limited overlap with the outer priming regions of the Xpert®MTB/RIF assay. The Xpert®MTB/RIF itself contains amplicons within the sealed cartridge, reducing or eliminating the need for the precautions typically associated with other nucleic acid amplification tests.
13	2010	USA	The bioaerosols generated by the Xpert®MTB/RIF assay was compared to AFB microscope slide SS preparation. The Xpert®MTB/RIF assay sample treatment reagent (SR) was also studied for its sterilizing capacity, stability, and effect on assay SENS after prolonged treatment. Neither the sample preparation steps for the Xpert®MTB/RIF assay nor its automated processing produced any culturable bioaerosols. In testing of SR sterilizing capacity, clinical SS samples from strongly SS+ TB patients treated with SR at a 2:1 ratio eliminated MTB growth in all but 1/39 or 3/45 samples cultured on solid or liquid medium, respectively. These few unsterilized samples had a mean 13.1-day delay in the time to C+. SR treatment at a 3:1 ratio eliminated growth in all samples. SR retained a greater than 6-log-unit	These results suggest that benchtop use of the Xpert®MTB/RIF assay limits infection risk to the user.

			killing capacity despite storage at temperatures spanning 4 to 45°C for at least 3 months.	
17	2010	Peru, Azerbaijan, South Africa, and India	Among C+ patients, a single, direct Xpert®MTB/RIF test identified 551/561 patients with SS+ TB (98.2%) and 124 of 171 with SS- TB (72.5%). The test was specific in 604 of 609 patients without TB (99.2%). Among patients with SS- C+ TB, the addition of a second Xpert®MTB/RIF test increased SENS by 12.6% and a third by 5.1%, to a total of 90.2%. As compared with phenotypic DST, Xpert®MTB/RIF MTB/RIF testing correctly identified 200/205 patients (97.6%) with R-resistant bacteria and 504/514 (98.1%) with R-susceptible bacteria. Sequencing resolved all but two cases in favour of the Xpert®MTB/RIF assay.	The Xpert®MTB/RIF test provided sensitive detection of TB and R resistance directly from untreated SS in less than 2 hours with minimal hands-on time.
18	2011	Multicentre study in South Africa, Peru, India, Azerbaijan, Philippines, and Uganda	6648 participants were enrolled. Once-off Xpert®MTB/RIF testing detected 933 (90·3%) of 1033 C-confirmed cases of TB, compared with 699 (67·1%) of 1041 for microscopy. Xpert®MTB/RIF test SENS was 76·9% in SS-, C+ patients (296 of 385 samples), and 99·0% specific (2846 of 2876 non-tuberculosis samples). Xpert®MTB/RIF test SENS for R resistance was 94·4% (236 of 250) and SPEC was 98·3% (796 of 810). Unlike microscopy, Xpert®MTB/RIF test SENS was not significantly lower in patients with HIV co-infection. Median time to detection of TB for the Xpert®MTB/RIF was 0 days (IQR 0—1), compared with 1 day (0—1) for microscopy, 30 days (23—43) for solid C, and 16 days (13—21) for liquid C. Median time to detection of resistance was 20 days (10—26) for line-probe assay and 106 days (30—124) for conventional DST. Use of the Xpert®MTB/RIF test reduced median time to treatment for SS- TB from 56 days (39—81) to 5 days (2—8). The indeterminate rate of Xpert®MTB/RIF testing was 2·4% (126 of 5321 samples) compared with 4·6% (441 of 9690) for C.	The Xpert®MTB/RIF can effectively be used in low-resource settings to simplify patients' access to early and accurate diagnosis, thereby potentially decreasing morbidity associated with diagnostic delay, dropout and mistreatment.
27	2011	Turkey	253 pulmonary and 176 extrapulmonary specimens obtained from 429 patients were included in the study. 110 (89 C+ and 21 C- for MTB) of the 429 patients were considered to have TB. In pulmonary specimens, SENS of Xpert®MTB/RIF were 100% (27/27) and 68.6% (24/35) for SS+ and SS- specimens respectively. It had lower SENS in extrapulmonary specimens; for SS+ 100% (4/4) and SS- 47.7% (21/44). The test accurately detected the absence of TB in all 319 patients studied. The Xpert®MTB/RIF assay also detected one R-resistant and 88 R-susceptible specimens which were confirmed by phenotypic DST.	The Xpert®MTB/RIF test was a simple method and routine staff with minimal training could use the system. The test appeared to be as SENS as C with SS+ specimens but less SENS with SS- pulmonary and extrapulmonary specimens that included low number of bacilli.
28	2011	Kuwait	Clinical specimens included 206 pulmonary and 29 extra-pulmonary samples .72 (60 pulmonary and 12 extra-pulmonary) samples yielded MTB by C, while 56 (78%) C+ samples (46 pulmonary and 10 extra-pulmonary) were also SS+. Xpert®MTB/RIF showed98% agreement for SS+, C+ samples and 69% agreement for SS-, C+ samples for detection of MTB. Overall concordance with C was 92%.There was 98% and 64% agreement for SS+ and SS- pulmonary specimens, respectively. Xpert®MTB/RIF showed 100% agreement with C for both SS+, C+ and SS-, C+ extra-pulmonary specimens.	The rapidity and simplicity of the closed cartridge Xpert®MTB/RIF test made it a good TB diagnostic test for routine use in reference laboratories of countries with low to intermediate incidence of TB, and may also help in reducing further transmission of infection in such settings
29	2011	Athens, Greece.	The Xpert®MTB/RIF assay was evaluated with microscopically negative and -positive pulmonary- and extrapulmonary specimens. For the pulmonary samples, SENS, SPEC, PPV and NPV were 90.6%, 94.3%, 93.5%, and 91.7%, and for the extrapulmonary samples, these were 100%, 91.6%, 50%, and 100%, respectively. For microscopically negative specimens, the respective values were 86.3%, 93%, 79%, and 95.6%. The assay correctly detected R resistance in all but one specimen, which harboured a mixed population.	The Xpert®MTB/RIF assay was highly effective for TB diagnosis and identification of R resistant strains in smear-negative samples.
30	2011	Singapore	162 respiratory and non-respiratory specimens were compared for the Xpert®MTB/RIF and the Amplified Mycobacterium Tuberculosis Direct (MTD) assay. Based on C as the gold standard, the overall SENS and SPEC for all sample types for the Xpert®MTB/RIF was 90.9 and 89%, respectively, whilst for the MTD assay, the overall SENS and SPEC was 97.3 and 87.1 %, respectively. A higher proportion of total equivocal results were obtained for the MTD assay at 10.5% (17/162) whilst the Xpert®MTB/RIF assay generated 5.5% (9/162) of invalid reads. The limited number of R-resistant isolates (n=4 present in this study hindered a proper assessment of the efficacy of Xpert®MTB/RIF in detecting R resistance.	In the specific laboratory context, the MTD test has a similar performance to the Xpert assay. The MTD test is however a fully manual test. In contrast, the Xpert MTB/RIF is a self-contained, integrated test that offers minimal hands-on time with low potential for PCR contamination. The concurrent detection for R associated mutations is also an added advantage

31	2011	North Carolina, USA	The Cepheid Xpert MTB/RIF research-use-only (RUO) assay and a laboratory-developed test (LDT) targeting IS6110 were evaluated and compared to mycobacterial C as the gold standard in112 specimens from 90 patients, including 89 pulmonary specimens and 23 extrapulmonary specimens. Of the specimens tested, 37 (33%) were C+ for MTB complex; 29 were pulmonary, and 8 were extrapulmonary. Of the C+ specimens, 83% of the pulmonary specimens and 50% of the extrapulmonary specimens were SS+. There was complete concordance between the SS+ C+ specimens, independent of the anatomical site (100% SENS). The SENS of the MTB/RIF RUO assay for SS- specimens was 60% for pulmonary and 75% for extrapulmonary specimens, while the IS6110 LDT SENS were 40% and 0%, respectively. There was also complete concordance among the C- specimens tested. Both assays showed 95% SPEC, with four C- specimens testing as positive. A review of patient records indicated that there was a high likelihood of the presence of MTB complex DNA in the false-positive specimens. Biosafety analysis was performed and showed an acceptable reduction in organism viability using the processing methods described above	Both molecular assays are suitable for the detection of MTB isolates in SS+ pulmonary and extrapulmonary specimens, while SENS of the detection of MTB isolates in SS- specimens was variable.
32	2011	Multisite clinical trial, USA	2,008 samples were tested. Decreasing MTB Ct was associated with increasing SS microscopy grade for smears of concentrated sputum pellets (rs = −0.77) and directly from SS (rs = −0.71). A Ct cut off of approximately 27.7 best predicted SS+ status. The association between MTB Ct and time-to-detection in liquid C (rs = 0.68) and semi-quantitative colony counts (rs = −0.56) was weaker than SS. Tests of paired same-patient SS showed that high-viscosity SS samples contained ×32 more MTB than non-viscous samples. Comparisons between the grade of the acid-fast bacilli SS and Xpert®MTB/RIF quantitative data across study sites enabled the identification a site outlier in microscopy.	Xpert®MTB/RIF quantitation offers a new, standardized approach to measuring bacterial burden in the sputum of TB patients.
33	2011	Primary health care clinic, Johannesburg, South Africa	Consecutive adults with suspected TB attending a primary health care clinic were prospectively enrolled and evaluated for TB according to national guidelines, including assessment for SS- TB by chest X-ray, clinical evaluation, and HIV testing. A single SS sample underwent routine decontamination, AFB SS microscopy, liquid C, and phenotypic DST. Residual sample was batched for molecular testing. 311 participants, HIV prevalence 70% (n = 215), with 120 (38.5%) C+ TB cases. Compared to liquid C, SENS of all the test methodologies, determined with a limited and potentially underpowered sample size (n = 177), were 59% (47%–71%) for SS microscopy, 76% (64%–85%) for MTBDRplus, 76% (64%–85%) for LCTB, and 86% (76%–93%) for , Xpert®MTB/RIF with specificities all >97%. Among HIV+ individuals, SENS of the Xpert®MTB/RIF assay was 84% (69%–93%), while the other molecular tests had SENS reduced by 6%. TB detection among SS-, C+ samples was 28% (5/18) for MTBDRplus, 22% (4/18) for LCTB, and 61% (11/18) for Xpert®MTB/RIF. A few (n = 5) R-resistant cases were detected using phenotypic DST. Xpert®MTB/RIF detected four of these five cases (fifth case not tested) and two additional phenotypically susceptible cases.	The Xpert®MTB/RIF test has superior performance for rapid diagnosis of MTB over existing AFB SS microscopy and other molecular methodologies in an HIV- and TB-endemic region. Its place in the clinical diagnostic algorithm in national health programs needs exploration.
34	2011	South Africa	Xpert®MTB/RIF was evaluated using single archived spot-SS samples from 496 South African patients with suspected TB. Overall, Xpert®MTB/RIF detected 95% (95% CI, 88–98%; 89 of 94) of SS+ C+ cases and SPEC was 94% (91–96%; 320 of 339). SENS in SS- cases was 55% (35–73%; 12 of 22) when the analysis was restricted to 1 ml of unprocessed SS and C time-to-positivity of less than or equal to 28 days. Compared with SS microscopy (n = 94), Xpert®MTB/RIF detected an additional 17 cases (n = 111) representing an 18% (11–27%; 111 vs. 94) relative increase in TB case detection. Compared with SS microscopy, the inclusion of Xpert®MTB/RIF -positive C- TB cases (ruled-in by an alternative diagnostic method) resulted in the detection of a further 16 cases (n = 127), thus significantly increasing the TB case detection rate to 35% (95% CI, 26–45%; 94 to 111 vs. 94 to 127; P < 0.01), the overall SPEC to 99.1% (97–100%; 320 of 323; P < 0.001), and SENS in SS- TB to 60% (P = 0.12). Performance was strongly correlated with SS status and C time-to-positivity. In patients infected with HIV compared with patients uninfected with HIV, Xpert®MTB/RIF showed a trend to reduced SENS (P = 0.09) and significantly reduced NPV (P = 0.01). The NPV for RMP resistance was 99.4%.	Xpert®MTB/RIF outperformed SS microscopy, established a diagnosis in a significant proportion of patients with SS- TB, detected many highly likely TB cases missed by C, and accurately ruled out R-resistant TB. Sample-specific factors had limited impact on performance. Performance in patients infected with HIV, especially those with advanced immunosuppression, warrants further study.

35	2011	HIV clinic, South Africa	The accuracy of the X Xpert®MTB/RIF assay for diagnosing TB and drug resistance was assessed in comparison with other tests, including fluorescence SS microscopy and automated liquid C (gold standard) and DST. Of 515 patients enrolled, 468 patients (median CD4 cell count, 171 cells/µl; IQR 102–236) produced at least one SS sample, yielding complete sets of results from 839 samples. MTB was cultured from 81 patients (TB prevalence, 17.3%). The overall SENS of the Xpert®MTB/RIF assay for C+ TB was 73.3% (SPE, 99.2%) compared to 28.0% (SPE, 100%) using SS microscopy. All SS+, C+- disease was detected by Xpert®MTB/RIF from a single sample (SENS, 100%), whereas SENS for SS-, C+ TB was 43.4% from one SS sample and 62.3% from two samples. Xpert®MTB/RIF correctly identified R resistance in all four cases of MDR-TB but incorrectly identified resistance in three other patients whose disease was confirmed to be drug sensitive by gene sequencing (SPEC, 94.1%; PPV, 57%).	In this population of individuals at high risk of TB, intensive screening using the Xpert®MTB/RIF assay increased case detection by 45% compared with SS microscopy, strongly supporting replacement of microscopy for this indication. However, despite the ability of the assay to rapidly detect R-resistant disease, SPEC for drug-resistant TB was sub-optimal.
36	2011	University Center for Chronic Diseases, Groesbeek, The Netherlands	The Authors analysed 89 unprocessed clinical samples (86 SS samples, 1 pleural fluid,1 gastric fluid, 1 bronchial washing). 26 samples were obtained fresh, in clinical routine, 63 samples previously stored at −70°C. SENS for detecting MTB in C+ samples was 93.8% (60/64) and exceeded SS microscopy (40/64, 62.5%). SPEC for detecting MTB was 92.0% (23/25) and for R resistance 100% (8/8). In the 40 SS+ samples that grew MTB, SENS was 100%. One SS+,C- gastric fluid sample was positive with Xpert®MTB/RIF;In 48 SS- samples, SENS was 83.3% (20/24), and SPEC was 95.8% (23/24).The combined SENS for detecting MTB in SS- and SS+ sputum samples was 93.8% (60/64), and SPEC was 92.0% (23/25). The sample set included 8 samples from R-resistant TB patients (4 fresh, 4 frozen; 6 MDR-TB,2 R-monoresistant); all were recognised as R-resistant by the Xpert®MTB/RIF assay. No false positives or -negatives were noted; SENS and SPEC for detecting R-resistance was 100%. No significant differences in SENS and SPEC in fresh (SENS 100%, SPEC 90.1%, n = 26) vs. frozen samples (SENS 91.8%, SPEC 92.9%,n = 63) were detected.	The test was technically simple to conduct and required neither PCR facilities nor biosafety precautions other than those of routine SS handling. These characteristics render it a promising close-to-patient test for TB in various settings. The Xpert®MTB/RIF thus combines all the characteristics required of a close-to-patient test. The analysis was rapid, simple, safe and required hardly any training. This platform is highly promising for close to-patient TB diagnostics.
37	2011	Tanzania	In 292 samples, the diagnostic performance of Xpert®MTB/RIF was compared to standard SS microscopy and C. Xpert®MTB/RIF achieved 88.4% (95%CI = 78.4% to 94.9%) SENS among patients with C+ and 99% (95%CI = 94.7% to 100.0%) SPEC in patients who had no TB. HIV status did not affect test performance in 172 HIV-infected patients (58.9% of all participants). Seven additional cases (9.1% of 77) were detected by Xpert®MTB/RIF among the group of patients with clinical TB who were C- . In 45 sputum specimens that grew NTM the assay's SPEC was 97.8% (95%CI = 88.2% to 99.9%).	The Xpert®MTB/RIF assay was a highly sensitive, specific and rapid method for diagnosing TB which has potential to complement the current reference standard of TB diagnostics and increase its overall sensitivity. Its usefulness in detecting SS and C- patients needs further study. Further evaluation in high burden TB and HIV areas under programmatic health care settings to ascertain applicability, cost-effectiveness, robustness and local acceptance are required.
38	2011	France	The Authors evaluated 117 clinical specimens (97 C+ and 20 C- for MTBC) frozen in sediment. The 97 clinical specimens included 60 respiratory and 37 non respiratory specimens, of which 36 were SS+ and 61 were SS-.Among the 97 C+ specimens, 4 had R-resistant isolates. Both methods were highly SPEC and exhibited excellent SENS (100%) with SS+ specimens. SENS of the Xpert®MTB/RIF test with the whole SS- specimens was more reduced than that of the IS6110-TaqMan assay (48 versus 69%, P = 0.005). Both methods exhibited similar SENS with SS- respiratory specimens, but the Xpert®MTB/RIF test had lower SENS with SS- non respiratory specimens than the IS6110-TaqMan assay (37 versus 71%, P = 0.013). Finally, SENS of the Xpert®MTB/RIF test and the IS6110-TaqMan assay were 79% and 84%, respectively with respiratory specimens, and 53% and 78% respectively (P = 0.013), with non-respiratory specimens. The Xpert®MTB/RIF test correctly detected R resistance in SS+ specimens but not in the one SS- specimen.	The Xpert®MTB/RIF test was a simple rapid method well adapted to a routine laboratory that appeared to be as SENS as the IS6110-TaqMan assay with respiratory specimens but less SENS with paucibacillary specimens, such as SS- non respiratory specimens.
39	2011	University hospital Caen, France	91 respiratory and 89 non-respiratory samples were evaluated. Overall, 31 (17.2%) of the 180 samples, including 17 respiratory and 14 non-respiratory (respectively 17 and 12 PCR-positive), yielded MTB on C. SENS and SPEC of PCR were respectively 100% and 100%, and 85.7% and 97.3% for respiratory and non-respiratory samples.	Although the Xpert®MTB/RIF testis validated only for respiratory samples, findings suggested that it could be useful for the diagnosis of extra-pulmonary TB.

40	2011	Western United States	A total of 217 specimens were submitted to evaluate the GeneXpert MTB/RIF assay (for research use only).Overall agreement compared to C was 89% (98% for SS+ and 72% for SS-) for detection of MTB.	Overall, the GeneXpert assay was simple, fast, accurate, and cost-comparative to other commercially available PCR assays for the direct detection of MTB.
41	2011	Spain	64 of 85 (75.3%) SS- respiratory (n = 78) and non respiratory (n = 7) samples with C+ MTB complex (MTC) were detected by the GeneXpert (GX) system using the Xpert®MTB/RIF assay. In addition, GX found rpoB mutations in all six of the R-resistant strains detected. The test was negative in 20 C- and 20 NTM C+ samples (100% SPEC).	The global data obtained in the present study indicate that GX has a high SENS, since all samples analyzed had a low mycobacterial load. The relatively high cost of GX was an important issue that TB control programs should consider prior to implementation of this assay. Its clinical and epidemiological advantages should be weighed against the resources available in each setting. In summary, the GX technique demonstrated a high capacity for detecting MTC and for predicting MDR in SS- clinical samples. Moreover, its rapidity, simplicity, and low laboriousness make the technique a good candidate for routine use in many clinical laboratories whenever the clinical criteria for its application are met.
50	2011	South Africa	452 children (median age 19·4 months, IQR 11·1—46·2) had at least one induced sputum specimen; 108 children (24%) had HIV infection. 27 children (6%) had a positive SS result, 70 (16%) had a positive C result, and 58 (13%) had a positive Xpert®MTB/RIF test result. With mycobacterial C as the reference standard, Xpert®MTB/RIF tests when done on two induced sputum samples detected twice as many cases (75.9%, 95% CI 64.5—87.2) as did SS microscopy (37.9%, 25·1—50·8), detecting all of 22 SS+ cases and 22 of 36 (61·1%, 44.4—77.8) SS- cases. For SS- cases, the incremental increase in SENS from testing a second specimen was 27.8% for Xpert®MTB/RIF , compared with 13·8% for C. SPEC of Xpert®MTB/RIF was 98.8% (97.6—99.9). Xpert®MTB/RIF results were available in median 1 day (IQR 0—4) compared with median 12 days (9—17) for C (p<0·0001).	Xpert®MTB/RIF testing of two induced sputum specimens was warranted as the first-line diagnostic test for children with suspected pulmonary TB.
51	2011	South Africa	The Authors compared the turn-around-time, detection-threshold, dynamic range, reproducibility, relative discriminative ability, of 4 mycobacterial load determination techniques: automated liquid culture (BACTEC-MGIT-960), [3H]-uracil incorporation assays, luciferase-reporter construct bioluminescence, and quantitative PCR(Xpert -MTB/RIF) using serial dilutions of Mycobacterium bovis and Mycobacterium tuberculosis H37RV. Mycobacterial colony-forming-units(CFU) using 7H10-Middlebrook solid media served as the reference standard. All 4 assays correlated well with the reference standard, however, bioluminescence and uracil assays had a detection threshold ≥1×103 organisms. By contrast, BACTEC-MGIT-960 liquid C, although only providing results in days, was user-friendly, had the lowest detection threshold (<10 organisms), the greatest discriminative ability (1 vs. 10 organisms; p = 0.02), and the best reproducibility (coefficient of variance of 2% vs. 38% compared to uracil incorporation; p = 0.02). Xpert®MTB/RIF correlated well with mycobacterial load, had a rapid turn-around-time (<2 hours), was user friendly, but had a detection limit of ~100 organisms.	Choosing a technique to quantify mycobacterial burden for laboratory or clinical research depends on availability of resources and the question being addressed. Automated liquid culture had good discriminative ability and low detection threshold but results were only obtained in days. Xpert®MTB/RIF provided rapid quantification of mycobacterial burden, but had a poorer discrimination and detection threshold.
52	2011	South Africa	20 cases with confirmed TB pleural effusion. Xpert®MTB/RIF SENS and SPEC in pleural fluid was 25% and 100%, respectively. All positive Xpert®MTB/RIF results were also pleural fluid C+.	Xpert®MTB/RIF testing in pleural fluid samples is feasible, but of low SENS and linked to a positive pleural fluid C. There is an indication for high SPEC, which must be verified with larger studies including more patients with a pleural effusion due to other causes than TB. Before this is attempted the methods for collection, storage and preparation of pleural fluid samples need to be optimized in order to increase Xpert®MTB/RIF SENS on pleural fluid.

53	2011	Spain	340 non-respiratory samples were processed using two real-time PCR assay kits: Xpert®MTB/RIF and Cobas TaqMan MTB. SENS and SPEC of the Xpert assay were 95% and 100%, respectively, compared to 78% and 98% for the Cobas assay.	Both molecular techniques represent an important contribution to the detection of MTB, since they can provide results in a matter of hours, whereas the reference C method takes days. Real-time PCR techniques afford greater SENS and SPEC and a much-reduced response time,as well as enabling visualization of amplification curves. One limitation of these techniques is that, in detecting MTB DNA, they cannot distinguish between viable and nonviable microorganisms. For that reason, although these assays are semi-quantitative, they should not be used for monitoring patient progress or treatment efficacy
54	2011	South Africa	To determine the diagnostic utility of the Xpert® 52 MTB/RIF, FNAB (fine needle aspiration biopsy) were collected from 50 consenting patients by aspirating TB lymphadenitis..Aspirates underwent C (MGIT 960), genotypic DST (Genotype MTBDR*plus* assay) and Xpert MTB/RIF. Compared to the reference standard, Xpert MTB/RIF correctly identified 29 out of 30 TB cases (sensitivity 96.7%, 95%CI, 86.6-100). The possible "false negative" result had a prolonged transit interval of 9 days before Xpert MTB/RIF testing, which may have affected the result. Xpert MTB/RIF was positive in two cases with negative cytomorphology and culture (specificity 88.9%, 95%CI, 69.6-100). The Xpert MTB/RIF test was positive in all 6 smear negative culture positive cases and correctly identified the 1 of the 2 R resistant cases. The average time to result for microbiological C was 18.5 days (range 9-55 days), while the Xpert MTB/RIF test result was available within 2 hours of commencing the test.	This study demonstrated the excellent diagnostic accuracy of the Xpert® MTB/RIF test in patients with TB lymphadenitis.
55	2011	India	547 extrapulmonary specimens were split and processed simultaneously for both C (solid and liquid) and Xpert®MTB/RIF testing. For culture, SENS was low, 53% (150/283 specimens). Xpert®MTB/RIF SENS and SPEC results were assessed in comparison to a composite reference standard made up of SS and C results and clinical, radiological, and histological findings. SENS of the Xpert®MTB/RIF assay was 81% (228/283 specimens) (64% [89/138] for SS- cases and 96% [139/145] for SS+ cases), with 99.6% SPEC. SENS was found to be high for the majority of specimen types (63 to 100%) except for cerebrospinal fluid, with SENS of 29% (2/7 specimens). The Xpert®MTB/RIF correctly identified 98% of phenotypic R--resistant cases and 94% of phenotypic R-susceptible cases. Sequencing of the 6 discrepant samples resolved 3 of them, resulting in an increased specificity of 98%.	The results of this study suggested that the Xpert®MTB/RIF test also showed good potential for the diagnosis of extrapulmonary TB and that its ease of use made it applicable for countries where TB is endemic.
56	2011	Germany	521 non-respiratory specimens were comparatively investigated with the Xpert®MTB/RIF assay and conventional liquid and solid C methods. 20 (3.8%) of the 521 specimens gave no interpretable result. Whereas SENS of the X Xpert®MTB/RIF with tissue specimens was 69.0% (20 out of 29 C+ cases detected), 100% SENS was found with urine and stool specimens. The combined SENS and SPEC of the Xpert®MTB/RIF assay were calculated to be 77.3% and 98.2%, respectively.	The Xpert®MTB/RIF assay could be applied to extrapulmonary specimens with a high SENS and SPEC, which, coupled with its speed and simplicity, made this technique a very useful tool for the diagnosis of extrapulmonary TB.
57	2011	CapeTown, South Africa	In this cost-analysis study the Authors assessed the accuracy and/or laboratory-associated cost-of-diagnosis of SS microscopy,chest-radiography, and interferon-γ-release assays (IGRAs; T-SPOT-TB and QFT-GIT), combined with a single Xpert-MTB/RIF, for the diagnosis of TB in 480 suspects. When conducted prior to Xpert®MTB/RIF testing: (i) SS-microscopy followed by Xpert®MTB/RIF (if SS-) had the lowest cost-of-diagnosis of any strategy investigated; (ii) a combination of SS-microscopy, chest-radiography (if SS-) and Xpert®MTB/RIF (if imaging compatible with active-TB) did not further reduce the cost per TB case diagnosed; (iii) a normal chest radiograph ruled-out TB in 18% of suspects [57/324; NPV 100%(57/57)]. When downstream adjunct tests were applied to Xpert®MTB/RIF -negative individuals: (i) radiology ruled-out TB in 24% [56/234; NPV 100%(56/56)]; (ii) SS-microscopy ruled-in TB in 21%(7/24) of C+ individuals; (iii) IGRAs were not useful in either context.	In resource-poor settings, SS-microscopy combined with Xpert®MTB/RIF had the highest accuracy and lowest cost-of-diagnosis compared to either technique alone. In Xpert®MTB/RIF-negative individuals, chest radiography had poor rule-in value but couldreliably rule-out TB in ~1 in 4 of such cases. These data informed the programmatic utility of Xpert®MTB/RIF in high burden settings. Detailed cost-effectiveness analyses are required.
58	2011	South Africa	A pilot program using dried culture spots (DCS) of inactivated MTB is described. Of 274 DCS results received, 2.19% generated errors; the remaining yielded 100% correct MTB detection. Probe A cycle threshold (Ct) variability of three DCS batches ≤3.47Ct. Longer-term DCS stability is on-going.	This study provided preliminary demonstration through the use of inactivated MTB coupled with easier transportation of DCS material that an EQA program can be safely provided. Future design of an

				Xpert®MTB/RIF EQA program could be similarly based on line probe assay programs using one pan-susceptible strain, one R-monoresistant strain with a common rpoB mutation, one MDR strain, one NTM strain and a negative control each placed on a DCS card and distributed 3/6 monthly.
59	2012	Italy	Review of the findings by Tortoli et al (Ref 72) done by a per-sample analysis of 268 diagnoses of extrapulmonary TB (EPTB) at a range of anatomic sites (SENS: 81.3%; 95% CI: 76.2–85.8) and data for 1206 samples in which EPTB was excluded (SPEC: 99.8%; 95% CI: 99.4–100).	The AA conclude that this study (Ref 72) was an important addition to the growing body of literature demonstrating the utility of Xpert®MTB/RIF for EPTB diagnosis when applied to diverse types of clinical samples.
60	2012	USA	The Authors determined the lower limit of detection (LOD) of the GeneXpert MTB/RIF assay with non-respiratory specimens and investigated the utility of flotation procedures for concentrating the bacilli. Clinical specimens (9 CSF, 13 gastric aspirate, 8 tissue, and 17 stool) were spiked with single-celled MTB and the LOD of the GeneXpert was determined. Flotation studies were conducted with sucrose and NaCl and the cycle thresholds of the MTB/RIF assay were compared between treated and untreated samples.There was no significant difference between the LOD of the Xpert®MTB/RIF with saline (median 33 CFU/ml) and CSF (median 25 CFU/ml) ($P > 0.05$) or gastric aspirate samples (median 58 CFU/ml) ($P > 0.05$). The LOD with spiked tissue (median 1,525 CFU/ml) and stool samples (median 6,800 CFU/ml) was significantly elevated compared to saline ($P \leq 0.05$ and ≤ 0.0005, respectively). Flotation studies with sucrose or NaCl did not consistently result in lowered cycle thresholds in stool or gastric aspirates but >10 cycle reduction was achieved in two of the three pooled CSF samples.	Unlike with tissue and stool samples, there was no significant PCR inhibition in the Xpert®MTB/RIF assay with CSF and gastric aspirates. Although pre-concentration of CSF samples with sucrose and NaCl may enhance detection of MTB by PCR, further advances are needed to concentrate the bacilli and eliminate PCR inhibitors in paucibacillary non-respiratory samples.
61	2012	South Africa	The Authors determined the diagnostic yield of the Xpert MTB/ RIF assay for TB when testing small volumes of urine from ambulatory HIV-infected patients before starting ART therapy in South Africa. Among 602 patients recruited, 535 produced at least 1 sputum sample and a specimen of urine. Sputum C results were available from 516 patients and these yielded 85 diagnoses of C+ TB. The remainder (n = 431) were sputum C negative.Compared with a gold standard of sputum culture, SENS of urine Xpert®MTB/RIF among those with CD4 cell counts of <50, 50–100, and >100 cells per microliter were 44.4%, 25.0%, and 2.7% ($P = 0.001$), respectively.	Urine Xpert®MTB/RIF testing provided a means of rapid TB diagnosis in patients with advanced immunodeficiency and poor prognosis. These data were indicative of high rates of TB dissemination and renal involvement in this clinical population.
62	2012	Spain	Among 108 SS- extrapulmonary samples showing a C+ for MTB complex (43 body fluids and 65 non liquid specimens), 63 (58.3%) were positive with the Xpert®MTB/RIF assay . SENS was quite low for samples from sterile locations (especially for pleural fluids: 26.9%) but high for some non-liquid samples, like abscess aspirates (76.5%).	Xpert®MTB/RIF may be a useful tool to be considered for extrapulmonary TB diagnosis.
63	2012	University Teaching Hospital Lusaka, Zambia	C+ TB was found in 201/881 patients (22.8%). Xpert®MTB/RIF SPEC was 95.0% (95% CI, 92.4%–96.8%); SENS was 86.1% (95% CI, 80.3%–90.4%). In SS-, C+ cases, the assay had 74.7% SENS (95% CI, 64.6%–82.8%), identifying 71 additional TB cases that were not detected by SS. 18/111 patients with TB who were tested (16.2%) had MDR- TB. SENS and SPEC of Xpert®MTB/RIF for detecting C+, Resistant TB was 81.3% (95% CI, 53.7%–95.0%) and 97.5% (95% CI, 90.4%–99.6%), respectively.	Xpert®MTB/RIF performed better than SS in an inpatient setting in a country where TB and HIV infection are highly endemic. Assessment of its usefulness and cost-effectiveness for increased detection of TB cases missed by SS and for concomitant screening for MDR-TB among adult inpatients attending tertiary care referral centres in other countries with a high burden of TB and HIV infection is warranted.
64	2012	Different Hospitals, sub-Saharan Africa	42% (116/242) of patients had C+ TB. 18% (20/54) were SS scarce. In SS-scarce patients, SENS of urine Xpert®MTB/RIF MTB/RIF and LAM ELISA test was 40% (95%CI: 22–61) and 60% (95%CI: 39–78), respectively. Urine Xpert®MTB/RIF SPEC was 98% (95%CI: 95–100). Combined SENS of urine LAM ELISA and Xpert®MTB/RIF was better than Xpert®MTB/RIF alone [Xpert®MTB/RIF and LAM: 70% (95%CI: 48–85) vs. Xpert®MTB/RIF: 40% (95%CI: 22–61), $p = 0.03$]. Significant predictors of urine Xpert®MTB/RIF positivity were CD4<50 cells/ml ($p = 0.001$), elevated protein-to-creatinine ratio ($p<0.001$) and LAM ELISA positivity ($p<0.001$). Urine centrifugation and pelleting significantly increased the SENS of Xpert®MTB/RIF over unprocessed urine in paired samples [42% (95%CI: 26–58) vs. 8% (95%CI: 0–16), $p<0.001$]. Urine Xpert®MTB/RIF -generated CT (cycle-threshold)values correlated poorly with markers of bacillary burden (SS grade and time-to-positivity).	This preliminary study indicates that urine-based Xpert®MTB/RIF , alone or in combination with LAM antigen detection, maypotentially aid the diagnosis of TB in HIV-infected patients with advanced immunosuppression when SS-based diagnosis is not possible. Concentration of urine prior to Xpert®MTB/RIF testing significantly improves SENS.

65	2012	City Hospital Auckland, New Zealand	All AFB SS+ respiratory specimens, were processed and analysed by Xpert® MTB/RIF assay using C (MGIT 960 system) as the gold standard, A total of 169 specimens (89 SS+ respiratory specimens; 9 smear-positive extra-pulmonary specimens and 71 positive MGIT liquid C vials) from 169 patients were analysed. With the use of culture as the "gold standard", the overall SENS and SPE of the MTB/RIF assay for the detection of MTB were 100% (141/141) and 100% (28/28), respectively. The MTB/RIF assay detected R resistance in 13/169 (7.7%) specimens. However, using standard phenotypic methods, R resistance was detected in only 7/13 (54%) isolates. In 2 of the remaining 6 isolates, amplification and sequencing of the rpoB gene revealed mutations associated with increased but low-level R resistance.	Similar to previous studies, the Authors found the MTB/RIF assay highly SENS and SPE for the detection of MTB, when used for both smear-positive pulmonary and extrapulmonary specimens as well as for isolates in liquid C media. However, the Authors found that the assay was less reliable for the detection of R resistance, producing false-positive results in 4/13 (31%) specimens. Further work is therefore required to evaluate the performance of the MTB/RIF assay for the detection of R resistance in a range of clinical settings and on a range of specimen types.
66	2012	Supra-national Reference Laboratory, Milan, Italy	An innovative approach allowed selective amplification of DNA derived from viable MTB in clinical specimens, which was useful for monitoring mycobacterial load in pulmonary TB patients during anti-TB treatment. The protocol was based on pre-treatment of samples with propidium monoazide (PMA).PMA did not significantly affect PCR yield of specimens collected at time zero, confirming that the SS positivity of these samples was mostly due to highly damaged bacteria. Moreover, Delta Ct (difference in amplification yield between samples with and without PMA pre-treatment) calculated between t0 and t1 in PMA-untreated samples was found to be too low to represent a real decrease in bacterial load due to therapy. All patients were successfully treated and cured at the end of therapy, consistent with the reduction of live bacteria detected by the PMA assay.	Quantitative molecular techniques combined with the PMA method could be an alternative to SS and C for monitoring early treatment response and for preliminary evaluation of personalised regimens. The use of this assay can allow earlier evaluation of treatment efficacy, showing a clear decrease in the vital mycobacterial load. Absence of the response to therapy might also be promptly identified by the test allowing a regimen change and limiting the spread of infection and further resistance development
67	2012	HIV service, South Africa	523/602 patients screened had ≥1 Xpert®MTB/RIF and C result, yielding 89 C+ TB diagnoses. Of these, 37 (42%) of TB patients were Xpert®MTB/RIF -negative when a single SS sample was tested, compared with 25 (28%) when 2 samples were tested. Compared to patients with Xpert®MTB/RIF -positive TB, those with Xpert®MTB/RIF -negative TB (using either definition) had substantially higher CD4 cell counts, lower plasma viral loads, higher hemoglobin concentrations, and higher body mass index. Their TB was also less advanced, with a lower frequency of prolonged cough (≥2 weeks), less extensive radiographic abnormalities, and a lower frequency of detectable lipoarabinomannan antigenuria and mycobacteriuria. Xpert®MTB/RIF -negative cases were all SS- with prolonged time to C positivity (median, 21 days). Despite greater delays in starting TB treatment, Xpert®MTB/RIF -negative patients were less likely to die during follow-up.	Compared to patients with Xpert positive TB diagnosed during pre-ART screening, Xpert negative cases had less advanced immunosuppression and less advanced TB and did not have adverse outcomes despite substantial delays in starting TB treatment.
68	2012	Primary care clinic, Cape Town, South Africa	Lawn and colleagues described the clinical characteristics of patients with pulmonary TB who tested negative by Xpert®MTB/RIF but had radiographically less advanced TB and fewer adverse outcomes compared with those who tested positive, prior to commencing antiretroviral therapy in Cape Town, South Africa (ref 67). In a primary care clinic based in the same city, and using archived samples from a prospectively recruited cohort, Theron et al. demonstrated Xpert®MTB/RIF –negative TB patients to have a significantly diminished SS bacillary load compared with those who tested positive. They also described the use of different tests, including chest radiography, when further investigating individuals. Theron's et al data imply that, contrary to what was suggested by Lawn and colleagues, Xpert®MTB/RIF was more likely to miss HIV-infected individuals with advanced immunosuppression. Given the small sample sizes of both studies, and the conflicting findings, the impact of declining CD4 count on sputum-based Xpert®MTB/RIF still remains unclear.	The findings from both studies need to be considered preliminary and context-specific, and further work, especially focusing on the outcome-related impact of extrapulmonary or disseminated TB in patients who have paucibacillary SS, is required.
69	2012	South Africa	Xpert®MTB/RIF –generated cycle-threshold (CT) values have poor clinical utility as a rule-in test for SS positivity (cut-point ≤20.2; SENS 32.3%, SPEC 97.1%) but moderately good rule-out value (cut-point >31.8; NPV 80.0%). Thus, 20% of individuals with CT values >31.8 were erroneously ruled out as SS-. This group had a significantly lower SS bacillary load relative to correctly classified SS+ patients (CT ≤ 31.8; P < .001).	Xpert®MTB/RIF -generated average CT values >31.8 provide moderately good rule-out value for SS positivity. Whether individuals falling above this cut-point, compared with those below, will display reduced disease transmission requires prospective validation. These data have public health implications for the roll-out of Xpert®MTB/RIF and inform public health and contact tracing strategies.

70	2012	Primary care clinic, Johannesburg, South Africa,	An unexpected case of R resistance was investigated on Xpert®MTB/RIF using repeat Xpert®MTB/RIF , SS microscopy, MTBDRplus® assay, C, DST, spoligotyping and rpoB gene sequencing. A false-positive result was most likely, given the wild type rpoB gene sequence and exclusion of both mixed infection and mixture of drug-susceptible and drug-resistant populations.	This report highlights the need for health care workers' understanding of assay performance characteristics when decentralising the diagnosis of drug-resistant TB. These issues should not, however, diminish enthusiasm for the Xpert®MTB/RIF assay.
71	2012	Tanzania	28/164 children (17.1%) had confirmed TB. Xpert®MTB/RIF detected 100% (95% CI, 59.0%–100%) of SS+ cases and 66.6% (95% CI, 43.0%–85.4%) of C+ but SS- cases. In the per-sample analysis, Xpert®MTB/RIF displayed a similar SENS (54.7% [95% CI, 42.7%–66.2%]) compared with C methods. Xpert®MTB/RIF detected 3-fold more confirmed TB cases than SS microscopy but with equal rapidity. Four additional cases (8.5%) with clinical TB but negative C were diagnosed by Xpert®MTB/RIF . Testing second and third samples increased SENS by 20% and 16%, respectively. When TB was reliably excluded, Xpert®MTB/RIF specificity was 100%. HIV infection did not affect diagnostic accuracy of Xpert®MTB/RIF. .	Xpert®MTB/RIF was easy to perform and displayed similar diagnostic accuracy as C methods in children with suspected TB. Rapid turnaround times should reduce treatment delay and improve patient outcome, although SENS remains suboptimal and access was dependent on local laboratory infrastructure.
72	2012	Italy	1,476 consecutive extra-pulmonary clinical specimens including both paediatric (494) and adult samples were investigated with Xpert®MTB/RIF. In comparison with a reference standard consisting of combination of C and clinical diagnosis of TB, an overall SENS and SPEC of 81.3% and 99.8%were found for Xpert®MTB/RIF while the SENS of microscopy was 48%. For biopsies, urines, pus and cerebrospinal fluids SENS exceeded 85% while it was slightly under 80% for gastric aspirates. SENS was lower than 50% for cavitary fluids. High SENS and SPEC (86.9% and 99.7% respectively) were also obtained for paediatric specimens.	Although the role of C remains central in the microbiological diagnosis of EPTB, the SENS of Xpert®MTB/RIF in rapidly diagnosing the disease made it a much better choice compared to SS microscopy. The ability of ruling out the disease still remained suboptimal.
73	2012	South Africa	535 children [median age 19 months, 117 (21·9%) HIV-infected] underwent one induced sputum (IS) and nasopharyngeal aspirate (NPA); 396 had two paired specimens. A positive SS, Xpert®MTB/RIF or C occurred in 30 (5.6%), 81 (15.1%) and 87 (16.3%) respectively. C yield was higher from IS (84/87, 96.6%) vs NPA (61/87, 70.1%, p<0.001). Amongst children with two paired specimens, 63 C confirmed cases occurred [60 (95.2%) IS vs. 48 (76.2%) NPA, p=0.002]. SENS of two Xpert®MTB/RIF tests was similar on IS and NPAs (45/63, 71% vs 41/63, 65%, p=0.444); SENS of SS was lower on IS (21/63, 33%) and NPA (16/63, 25%). Incremental yield from a second IS was 9 cases (17.6%) by C and 9 (25%) by Xpert®MTB/RIF ; a second NPA increased C yield by 10 (26.3%) and Xpert®MTB/RIF 11 (36.7%). Xpert SPEC was 99.1% (98.1 - 100) on IS and 98.2 (96.8 - 99.6) on NPAs. Xpert®MTB/RIF provided faster results than culture (median 0 vs 15 days, p<0·001).	Xpert®MTB/RIF on 2 NPAs was useful in children with suspected PTB particularly in settings where IS and culture were not feasible.
74	2012	Zambia	A model proposed to integrate TB and HIV screening, diagnosis, and treatment into existing antenatal care using Xpert®MTB/RIF technology (as per WHO recommendations).	Pilot studies were urgently required to evaluate strategies for the integration of TB screening into antenatal clinics using new diagnostic technologies, in order to reduce morbidity and mortality for both the mother and child, particularly in women who are co-infected with HIV.
98	2012	South Africa	In this diagnostic sub-study of a TB prevalence survey conducted in gold mining companies in South Africa, 6,893 participants provided a SS specimen. 187/6893 (2.7%) were positive for MTB in C, 144/6893 (2.1%) were positive for MTB by Xpert®MTB/RIF , and 91/6893 (1.3%) were positive for AFB by microscopy. SENS, SPEC, PPV and NPV for detection of MTB by Xpert®MTB/RIF were 62.6% (95% CI 55.2, 69.5), 99.6% (99.4, 99.7), 81.3% (73.9, 87.3), and 98.9 (98.6, 98.8); agreement between Xpert®MTB/RIF and C was 98.5% (98.2, 98.8). SENS of microscopy was 17.6% (12.5, 23.9). When individuals with a history of TB treatment were excluded from the analysis, Xpert®MTB/RIF SPEC was 99.8 (99.7, 99.9) and PPV was 90.6 (83.3, 95.4) for detection of MTB. For the testing scenario of 7000 specimens with 2.7% of specimens C+ for MTB, costs were $165,690 for Xpert®MTB/RIF and $115,360 for the package of microscopy plus C.	In the context of a TB prevalence survey, the Xpert®MTB/RIF diagnostic yield was substantially higher than that of microscopy yet lower than that of liquid C. Xpert may be useful as a sole test for TB case detection in prevalence surveys, particularly in settings lacking capacity for liquid C.

106	2012	Central hospital laboratory, South Korea	Xpert®MTB/RIF detected MTB in 71 (100%) specimens (32 SS+, 39 SS-). 100% (62/62) concordance with drug resistance confirmed by phenotypic method and 98.4% (61/62) concordance with sequencing. One specimen containing approximately 50% of mutant p.His526Tyr was falsely interpreted as wild-type. The minimal detection ratio was 5:1 of mutant vs. wild-type cells. The median time saved was 18.5 days (range 9-30) for the diagnosis of TB and 81.5 days (65-136) for R susceptibility in SS-, C+ patients.	Xpert®MTB/RIF showed: high SENS in detecting MTB with information on R resistance; rapid time to diagnosis compared to conventional tests. Location and number of mutations may affect test sensitivity.
107	2012	Central hospital laboratory, South Africa	Xpert®MTB/RIF was compared vs.Genotype® MTBDRplus (version 2) on SS+ and SS- patient specimens. 282 consecutive specimens were tested by the two new molecular assays and routine diagnostics. Both assays showed similar diagnostic performance characteristics. SEN of the Genotype® MTBDRplus (v2.0) and Xpert®MTB/RIF assay for the detection of C- MTB was 73.1% and 71.2% respectively; SPEC for both assays was 100%. Both assays diagnosed MTB in 57–58% of SS-cases suggesting that the performances depend on bacillary load. Detection of MTB in C- specimens confirmed that molecular-based assays should not be used for treatment monitoring. SENS and SPEC for R resistance detection was 100% in both assays. Genotype® MTBDRplus (v2.0) assay provided additional information on H susceptibility.	The Genotype® MTBDRplus (v2.0) assay will complement the Xpert®MTB/RIF screening assay by validating R susceptibility, providing information on H susceptibility and providing pharmacogenetic information useful in guiding treatment.
108	2012	Reference centre, Lima, Peru	Detection of TB by Xpert®MTB/RIF was compared to a composite reference standard of Löwenstein-Jensen (LJ) and liquid culture. Detection of R resistance was compared to the LJ proportion method. 131 patients were included, the median CD4 cell count was 154.5 cells/mm3 and 45 (34.4%) had TB. For TB detection among HIV patients, SENS of Xpert®MTB/RIF was 97.8% (95% CI 88.4–99.6) (44/45); SPEC was 97.7% (95% CI 91.9–99.4) (84/86); PPV was 95.7% (95% CI 85.5–98.8) (44/46); NPVwas 98.8% (95% CI 93.6–99.8) (84/85). Xpert®MTB/RIF detected 13/14 SS- TB cases, outperforming smear microscopy [97.8% (44/45) vs. 68.9% (31/45); p = 0.0002]. For R resistance detection, SENS of Xpert®MTB/RIF was 100% (95% CI 61.0–100.0) (6/6); SPEC was 91.0% (95% CI 76.4–96.9) (30/33); PPV was 66.7% (95% CI 35.4–87.9) (6/9); NPV was 100% (95% CI 88.7 –100.0) (30/30).	In HIV patients with a high clinical suspicion of TB, Xpert®MTB/RIF performed well for TB diagnosis and outperformed smear microscopy.
109	2012	District hospital, India	Performances of LED auramine fluorescent microscopy and Xpert®MTB/RIF for diagnosis of TB in HIV-infected patients were compared. Although at higher cost, Xpert®MTB/RIF outperformed LED fluorescent microscopy in all type of specimens, especially in cerebrospinal fluid where the number of positive results was increased 11 times. Pleural fluid, ascitic fluid, pus, and stool specimens also yielded positive results with the Xpert®MTB/RIF assay. When collecting two additional early-morning sputum samples, the increase in the number of positive results with the Xpert®MTB/RIF assay was lower than previously reported for HIV-infected patients. R resistance was observed in 2.2% of the cases.	Xpert®MTB/RIF assay can improve the rapid diagnosis of TB meningitis and other types of extrapulmonary tuberculosis in HIV-infected patients.
110	2012	Central Hospital, Tanzania	Among 219 enrolled contacts, the yield of active TB was 2.3%. SENS of SS microscopy was 60% (95%CI 14.7-94.7), SENS of Xpert®MTB/RIF MTB/RIF was 100% (95%CI 47.81-100.0).	As all C+ cases tested positive by Xpert®MTB/RIF on the first submitted sample, the evaluation of one sample only could be sufficient for TB diagnosis in this context.
111	2012	Reference Hospital Santiago, Chile	166 subjects were enrolled; 50.6% provided two sputum samples, 33.1% only one sputum sample and 16.3% a mouth wash sample. The prevalence of TB was 8.1% (13/160). Diagnostic SENS increased from 66.7% (95%CI 39.1-86.2) for SS to 91.7% (95%CI 64.6-98.5) for Xpert®MTB/RIF , with comparable SPEC at 98.6% (146/148, 95%CI 95.2-99.6) and 99.3% (147/148, 95%CI 96.3-99.9). Xpert®MTB/RIF allowed early detection of R resistance in 16.6% of cases, with rapid adjustment to MDR-TB treatment.	Xpert®MTB/RIF provided earlier TB diagnosis in 25% more cases than SS alone. Its implementation should be considered for TB diagnosis in HIV-positive patients even outside TB-endemic areas.
112	2012	South Africa	Xpert®MTB/RIF testing of two induced sputum specimens detected approximately 75% of children with C-confirmed disease. Urine lipoarabinomannan has shown promise as a rapid diagnostic in a subgroup of HIV-infected severely immunocompromised adults, but there have been no data in children so far.	The availability of Xpert®MTB/RIF was an important advance that could improve case detection in children and enable rapid detection of mycobacterial drug resistance.

Annex 1: Synopsis of the available studies on Xpert®MTB/RIF presented in the order they appear in the text for the following areas: assay evaluation, assay development, detection of extrapulmonary TB, detection of paediatric TB, diagnostic algorithms, use in prevalence surveys and quality assurance.

Legend:

AFB: Alcohol-acid fast bacilli
ART: Antiretroviral therapy
C+/-: Culture positive/negative
CFU: Colony Forming Unit
CI: Confidence Intervals
CT: Cycle-threshold
EQA: External quality assurance
H: Isoniazid
IQR: Interquartile range
LED: Light-emitting diode
MDR-TB: Multidrug-resistant TB
MTB: *Mycobacterium tuberculosis*
NPV: Negative predictive value
NTM: Non-tuberculous mycobacteria
PPV: Positive predictive value
R: Rifampicin
SENS: Sensitivity
SPEC: Specificity
SS+/-: Sputum smear positive/negative
TB: Tuberculosis
XDR-TB: Extensively drug-resistant TB