STATE-OF-THE-ART REVIEW

Molecular Diagnosis of TB in the HIV Positive Population

Amy Y. Vittor, MD, PhD, Joseph M. Garland, MD, and Robert H. Gilman, MD, DTMH

ABSTRACT

Background: Tuberculosis (TB) remains a major contributor to morbidity and mortality in HIV-positive individuals, causing 1.1 million incident cases and 0.32 million deaths in 2012. Diagnosis of TB is particularly challenging in HIV-coinfected individuals, due to a high frequency of smear-negative disease, atypical presentations, and extrapulmonary TB.

Objective: The aim of this article was to review the current literature on molecular diagnostics for TB with an emphasis on the performance of these diagnostic tests in the HIV-positive population.

Methods: We searched the PubMed database using at least one of the terms *TB, HIV, diagnostics, Xpert MTB/RIF, nucleic acid amplification tests, drug susceptibility testing, RNA transcription*, and drew on World Health Organization publications.

Findings: With increased focus on reducing TB prevalence worldwide, a new set of tools for diagnosing the disease have emerged. Molecular tools such as Xpert MTB/RIF and line-probe assays are now in use or are being rolled out in many regions. The diagnostic performance of these and other molecular assays are discussed here as they pertain to the HIV-positive population.

Conclusions: Molecular diagnostics offer a useful addition and at times, alternative, to traditional culture methods for the diagnosis of TB. However, most of these tests suffer from decreased accuracy in the HIV-positive population.

Key Words: drug susceptibility testing, HIV, molecular diagnostics, RNA transcription signatures, tuberculosis Xpert MTB/RIF

© 2014 The Authors. Published by Elsevier Inc. on behalf of Icahn School of Medicine at Mount Sinai. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). *Annals of Global Health 2014;80:476-485*

INTRODUCTION

Tuberculosis (TB) is a major cause of morbidity and mortality worldwide, particulary in the HIV-infected population. In 2012, 8.6 million new cases of TB occurred, 13% of which were diagnosed in HIV-positive individuals. Of the 1.3 million deaths attributed to TB in 2012, 25% were in HIV-positive individuals.[1] People living with HIV are 20 to 37 times more likely to develop TB than those without HIV, and TB accounted for 1 in 4 deaths among HIV-positive individuals, with the African region accounting for the highest percent (75%) of TB cases among this population.[1,2]

HIV infection and TB are co-occuring epidemics. Unfortunately, diagnosis of TB is challenging in this population because concurrent HIV infection is associated with sputum smear-negative disease and a higher proportion of extrapulmonary TB.[3] The conventional method of diagnosis in most high-burden countries, sputum smear microscopy, has especially poor sensitivity in HIV-coinfected individuals.[4] Traditional culture methods have an unacceptably long turnaround time, or are not readily available due to the need for relatively sophisticated laboratories and skilled technicians.

In response to these facts, the 2010 update of the World Health Organization's (WHO) Global Plan to Stop TB gave more weight to and increased the projected funding need for laboratory capacity compared with the prior iteration of this roadmap.[2] Furthermore, recognizing the lack of laboratory infrastructure, the Global Laboratory Initiative was created in 2007, with the goal of delivering rapid, quality-assured tests in areas of need. Many new diagnostics have emerged since the Global Plan was rolled out. The WHO has endorsed the use of 10 new

2214-9996/© 2014 The Authors. Published by Elsevier Inc. on behalf of Icahn School of Medicine at Mount Sinai. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

From the Department of Infectious Disease, University of Florida Emerging Pathogens Institute, Gainesville, FL (AYV); Department of Infectious Disease, University of Pennsylvania, Jonathan Lax Center/Philadelphia FIGHT, Philadelphia, PA (JMG); Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD (RHG). Address correspondence to R.H.G.; e-mail: rgilman@jhsph.edu

The authors do not have any conflicts of interest to declare.

http://dx.doi.org/10.1016/j.aogh.2015.01.001

diagnostics and approaches since 2007, including liquid culture, several noncommercial culture methods, line-probe assays for drug susceptibility testing (DST), a reduction in the number of smears from three to two, the light-emitting diode microscopy, and the Xpert MTB/RIF assay. Here we review molecular diagnostics, highlighting their role in HIV-infected individuals.

Diagnostic Challenges in the HIV—TB-Coinfected Population

Given the high mortality in the HIV—TB-coinfected population, the need to detect TB accurately and rapidly is great, yet the performance of most tests is diminished in this group of patients. HIV-positive individuals have a higher rate of smear-negative disease because they are less likely to have cavitary lesions due to the impairment of granuloma formation.[5,6] Approximately 24% to 61% of HIV—TB-coinfected patients are smear negative.[7] These patients have a higher mortality rate, probably due to profound immunosuppression as well as delayed diagnosis.[8] Furthermore, HIV-positive individuals often do not manifest typical symptoms of TB (prolonged cough, fever, night sweats, weight loss).[9,10] HIV-positive patients also are more likely to have extrapulmonary TB than HIV-negative patients.[11] The likelihood of extrapulmonary TB increases as the CD4 count decreases.[12] Although 40% of patients with extrapulmonary TB may have concurrent pulmonary TB, the most widely available method of diagnosis, sputum smear microscopy, is of little diagnostic value for the remaining 60%.[13]

Because of these challenges, symptom screening and sputum smear microscopy often lead to false-negative results and excess mortality from missed diagnosis. The extent of mortality caused by undiagnosed TB in HIV-positive patients is well illustrated in a series of studies examining cause of death in HIV-positive patients in TB-prevalent countries, with autopsy-confirmed rates of TB varying between 32% and 45%.[14-21] However, presumptive treatment for TB in non-TB cases can lead to excess mortality as well as a result of missed alternate diagnoses and medication toxicity.[22,23]

The emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) in the past decade has highlighted the urgent need for both accurate diagnosis and DST. Approximately 3.6% of all new TB cases are caused by MDR strains, of which 10% are XDR-TB.[24] The WHO estimates that by 2015, 20% of TB costs in low- and middle-income countries will be consumed by MDR-TB treatment.[24] The association between MDR-TB and HIV infection remains unclear, but data from Estonia, Latvia, and Moldova suggest that HIV-positive individuals there are at higher risk for acquiring MDR-TB,[25] and a study in Peru showed that 43% of HIV—TB-coinfected patients had MDR-TB, compared with 4% of HIV-negative TB patients.[26] In the African region, the burden of MDR-TB remains poorly characterized due to a lack of surveillance. What is clear is that HIV patients with MDR-TB face a very high mortality rate. If diagnosis and appropriate therapy are delayed, mortality is as high as 70% to 87%.[27,28] A cohort study in Peru demonstrated that 55% of HIV—MDR-TB-coinfected patients died within 2 months of TB diagnosis, well before conventional DST results usually become available.[28] Since the introduction of molecular and culture-based tools for DST, MDR-TB detection has risen dramatically with concomitant increases in patient enrollment in MDR-TB treatment programs.[1]

Thus, the ideal test for TB would provide results accurately and rapidly, not be infractructure or labor-intensive, and would remain cost-effective. Because of the difficulty in obtaining sputum samples, the ideal test would make use of bodily fluids more easily obtained than sputum. Although they do not supplant culture-based methods yet, which remain the gold standard but have severe limitations due to wait time for results and the need for technical skill and specialized laboratories, molecular diagnostics have advanced the field substantially in recent years.

Molecular Tests to Detect TB

Manual nucleic acid amplification tests. Manual nucleic acid amplification tests (NAATs) include in-house and commercial tests, and usually employ polymerase chain reaction (PCR) or ligase chain reaction methods to amplify target TB genes such as *IS6110*, *MBP64*, *MTP40*, *IS986*, and *hsp65*. These are performed directly on sputum samples or on culture colonies, and results can be available on the same day. False negatives occur as a result of low bacillary load or enzymes in sputum that inhibit the amplification reaction, whereas false positives tend to arise from contamination.[29] Many commercial NAATs are available, and hundreds of studies on their efficacy have been published in recent years. Most tests perform well in sputum smear-positive specimens, but have suboptimal sensitivity in sputum smear-negative samples. The cost of commercial tests ranges between US $25 and $50, whereas in-house tests are approximately US $15.[30]

Meta-analyses of commercial NAATs (AMTD, E-MTD, Roche Amplicor, Cobas Amplicor, BDProbe-Tec, BDProbeTec-ET, Lcx test [discontinued]) on sputum samples generally have reported sensitivities of 90% to 100% and specificities of 71% to 96% in sputum smear-positive samples, but significantly lower sensitivities of 22% to 89% and specificities of 97% to 99% in sputum smear-negative samples[29,31-40] (Table 1). Some studies used either liquid or solid culture media, whereas others use both. The use of an inferior reference, such as solid cultures, may negatively bias the performance of the assay being examined. The performance of in-house NAATs on respiratory samples is difficult to interpret due to great heterogeneity with sensitivities ranging from 9% to 100% and specificities from 6% to 100%.[41] The use of

Table 1. Diagnostic Accuracy in Sputum Smear-positive and Smear-negative Samples with Liquid and/or Solid Culture as Gold Standard

Assay	HIV-negative		HIV-positive		Smear positive	Smear negative	Overall	References
	Sensitivity (%)	Specificty (%)	Sensitivity (%)	Specificty (%)	Sensitivity (%)	Sensitivity (%)	Specificty (%)	
Commercial NAATs	13-95	100	42-89	93-9%	90-100	22-89	66-100	29,31-40
In-house NAATs	13-98	94-100	25-100	86-93	9-100	8%-100	6-100	31, 33, 35, 85
Xpert MTB/RIF	83-98	96-100	70-94	92-96	95-99	47-78	94-100	34, 43, 45, 46, 86
TB LAMP	N/A	N/A	N/A	N/A	97-98	30-56	100	59, 60, 87

MTB RIF, *Mycobacterium tuberculosis* rifampin; N/A, not applicable; NAAT, nucleic acid amplification test; TB LAMP, tuberculosis loop-mediated isothermal amplification.

IS6110 as a target and nested PCR methods has been associated with better accuracy.[41] For diagnosis of extrapulmonary TB, NAATs generally do not perform as well, demonstrating pooled sensitivity and specificity of 53% and 95%, respectively for serum, and 30% to 76% and 73% to 99%, respectively for pleural TB. For TB meningitis, pooled sensitivity was 60% (range: 25%-74%) and specificity was 93% (range: 88%-99%).[31]

A few studies have examined the performance of NAATs in HIV- and TB-coinfected patients. One such study used the AMPLICOR PCR NAAT for diagnosis of pulmonary TB.[32] The authors reported a sensitivity >99% in smear-positive samples, and 82% in smear-negative samples (with solid culture as the reference), which did not differ significantly for HIV-positive and HIV-negative individuals. Overall specificity was 84%. Table 2 demonstrates the positive and negative predictive values (PPV, NPV) for this test given high or low TB prevalence. The PPV is quite low in the latter scenario due to the relatively poor specificity. Davis et al.[33] studied the utility of the Amplified *Mycobacterium tuberculosis*. Direct (MTD) test (GenProbe Inc, San Diego, CA) and a novel in-house NAAT (targeting the *secA1* gene) using solid cultures as a reference in smear-negative patients only, the majority of whom were HIV positive. The sensitivity of the two NAATs was poor in relation to the reference (culture; 25% for the in-house assay, and 42% for AMTD; specificity 93% for both). The high rate of false positives seen with NAATs may be due to the use of solid culture as a reference, which fails to detect 10% to 15% of true positives. As such, the specificities reported in these studies may be misleading. However, laboratory contamination can lead to a true source of false positives for NAATs. This problem is overcome with the cartridge-based Xpert MTB/RIF described here, adding to its utility.

In summary, commercial and in-house manual NAATs have many limitations, including low sensitivity in sputum smear-negative TB, and the need for PCR laboratory capacity, skilled personnel, and biosafety requirements. Furthermore, they do not perform well in the diagnosis of extrapulmonary TB. Because sensitivity of the leading commercial NAATs in sputum smear-positive cases tends to be good, the main role for

Table 2. PPVs and NPVs for Select Tests, Calculated for Low and High Prevalence*

Assay	Assay Characteristics (%)	TB Prevalence (%)	PPV (%)	NPV (%)	Refs
AMPLICOR	Sensitivity: 99 sm+	5	23	99	32
	82 sm−				
	Specificity: 84	50	85	91	
Xpert MTB/RIF	Sensitivity: 98 sm+	5	82	99	46
	67 sm−				
	Specificity: 99	50	99	88	
TB LAMP	Sensitivity: 98 sm+	5	42	99	60
	56 sm-				
	Specificity: 94	50	93	83	

MTB RIF, *Mycobacterium tuberculosis* rifampin; NPV, negative predictive value; PPV, positive predictive value; sm+, sputum smear positive; sm-, sputum smear negative; TB, tuberculosis; TB LAMP, tuberculosis loop-mediated isothermal amplification.
*Assuming smear-negative rate of 40%; prevalence rates noted are those expected in HIV-positive patient populations with clinical presentation compatible with TB.

Table 3. PPVs and NPVs for RIF Resistance in Select Drug Resistance Tests, Calculated for High and Low MDR TB Prevalence Among Patients with TB

Assay	Assay Characteristics (%)	MDR TB Prevalence (%)	PPV (%)	NPV (%)	References
Genotype MTBDR*plus*	Sensitivity: 100	30	91	100	88
	Specificity: 96	5	57	100	
INNO-LiPA Rif.TB	Sensitivity: 96	30	91	98	74
	Specificity: 96	5	56	100	
Xpert MTB/RIF	Sensitivity: 95	30	95	98	46
	Specificity: 98	5	71	100	

MDR TB, multidrug-resistant tuberculosis; MTB RIF, *Mycobacterium tuberculosis* rifampin; NPV, negative predictive value; PPV, positive predictive value.

NAATs may be to rule out TB in sputum smear-positive cases (eg, due to non-TB mycobacteria) in settings with low TB prevalence.

Xpert MTB/RIF. An automated, cartridge-based real-time PCR system was developed collaboratively by FIND, Cepheid (Sunnyvale, CA), and the University of Medicine and Dentistry of New Jersey, which detects *Mycobacterium tuberculosis* (MTB) in sputum samples with minimal need for technical expertise. The Xpert MTB/RIF assay detects the presence of MTB as well as rifampin (RIF) resistance within 2 hours by PCR amplification of the RIF resistance-determining region of the MTB *rpo*B gene. The sputum sample is treated, reducing MTB viability by at least 6 logs, thereby nearly eliminating biohazard risk.[42] It is then transferred into a preloaded cartridge. Extraction, amplification, and detection are automated. According to Boehme et al.,[43] the minimum number of bacilli that can be detected is 131 cfu/mL, but as few as 10 cfu/mL were detected in 35% of samples. In their multisite study of 1730 individuals, sensitivity of smear-negative TB samples was 72.5% for the first sputum sample, 85.1% for 2 samples, and 90.2% for 3 samples. Specificity was 98%.

With these results, the Xpert MTB/RIF platform was endorsed by the WHO in December 2010, and by June 2014, nearly 16,000 modules had been purchased by 108 of the 145 countries eligible for concessional pricing.[44] Since this initial study of Xpert MTB/RIF, many others have examined its ability to perform in various settings. Scott et al.[34] compared the diagnostic accuracy of Xpert MTB/RIF with 2 NAATs (LightCycler Mycobacterium Detection [LCTB] assay and MTBDR*plus* assay), smear microscopy and liquid culture (gold standard) on a single sputum specimen in South Africa. Among smear-negative and culture-positive patients, Xpert MTB/RIF outperformed other NAATs (sensitivity 61% vs 28% [MTBDR*plus*] and 22% [LCTB]) on a single sputum sample. Sensitivities were similar between the HIV-positive and the HIV-negative groups for Xpert MTB/RIF (83% vs 84%), but lower for LCTB and MTBDR*plus* (70% vs 75% in both cases).

In a separate study using archived single sputum samples from South African patients with suspected TB, Theron et al.[45] found that the sensitivity of Xpert MTB/RIF was good in smear-positive cases (95%), but as seen in the prior study, was lower in smear-negative cases (55%) using liquid culture as the reference.

A 2014 Cochrane review of Xpert MTB/RIF summarized the diagnostic performance of this test in respiratory samples (where culture was the reference) by extracting data from randomized clinical trials, cross-sectional studies, and cohort studies.[46] This review included 27 studies (9557 participants) that took place in a wide spectrum of settings (60% were in low- or middle-income countries; TB incidence rates per 100,000 population ranged from 4 to 993). The pooled sensitivity and specificity of Xpert MTB/RIF for the detection of MTB was 89% (range: 85%-92%) and 99% (range: 98%-99%), respectively. In smear-negative, culture-positive patients, Xpert MBT/RIF had a pooled sensitivity and specificity of 67% (range: 60%-75%) and 99% (range: 98%-99%), respectively. In a subanalysis of the performance of Xpert MTB/RIF in smear-negative, culture-positive HIV-positive patients, the authors reported pooled sensitivity of 61% (range: 40%-81%). The PPV and NPV based on these estimates of test performance are shown in Table 2. If the Xpert MTB/RIF is used for diagnosis in a population with high TB prevalence with a 40% rate of smear-negative TB, it is worth bearing in mind that for every 100 people who test negative, 12 will be falsely negative.

Extrapulmonary TB can be difficult to diagnose due to a wide variety of symptoms and low bacillary load.[47] In a low TB-prevalent setting, Xpert MTB/RIF had a sensitivity of 27% to 44% for pleural TB (using liquid and solid culture as reference),[47,48] 71% to 88% for TB lymphadenitis,[47,48] and 85% for TB meningitis.[48] In India, which has a high prevalence of TB, the sensitivity was better for pleural TB, reaching 63%, similar for TB lymphadenitis at 73% to 86%, and poorer for TB meningitis at 29%.[49] For suspected TB meningitis, an expert review panel on the use of Xpert MTB/RIF for diagnosing extrapulmonary TB strongly recommends

this test in preference to conventional microscopy and culture as the initial diagnostic test for cerebrospinal fluid specimens due to the need for rapid results in this serious condition.[50]

Although the Xpert MTB/RIF cartridges and modules are available to countries with a high TB burden at concessional prices ($9.98 per cartridge; $17,000 per 4-module[51]), there has been concern about the ability to roll-out Xpert MTB/RIF widely and sustain the effort.[52] In an analysis of cost per disability-adjusted life-year averted in TB suspects in three different settings with high or low HIV, TB, and MDR-TB rates, it was estimated that the replacement or addition of Xpert MTB/RIF for smear microscopy and clinical diagnosis was cost-effective in each prevalence setting.[53] However, cost-effectiveness was greatly reduced when TB prevalence (smear-positive and negative) fell below 7% to 9%.

Andrews et al.[54] modeled clinical outcomes and the cost-effectiveness of screening all HIV-positive patients referred for antiretroviral therapy (ART); data were compared between smear alone, smear and culture, and 1 or 2 samples tested with Xpert MTB/RIF in a peri-urban setting in South Africa. This was based on the recognition that symptom screening fails to identify 10% to 20% of positive sputum cultures in HIV-positive patients.[55-57] They modeled outcomes using these diagnostic methods in both symptomatic patients and all-comers, and found that sputum smear and symptom screening resulted in a 2-month increase in life expectancy, whereas screening all-comers using 2 Xpert MTB/RIF samples led to a 7- to 9-month increase in life expectancy. They concluded that the 2-sample Xpert MTB/RIF screening of all-comers referred for ART is cost-effective in areas with a TB prevalence >7.5%.

Although the Xpert MTB/RIF represents a significant step forward toward developing a rapid, accurate test that can be used at the peripheral health center level, it still misses one-fourth to one-third of patients in the critical smear-negative group (when 1 sample is obtained). This assay is also not able to assess resistance to drugs other than rifampin (see discussion on drug resistance). As the studies just described concluded, unless the burden of TB and HIV is high, Xpert MTB/RIF may not be cost-effective. In South Africa, the prevalence of TB in people starting ART was 16% to 35%, whereas in Southeast Asia, it was 6% to 15%.[58] In these populations, TB rates are high enough to ensure that Xpert MTB/RIF screening of people about to start ART is cost-effective. According to an expert panel convened in 2013 for the use of Xpert MTB/RIF, this platform should be used instead of conventional microscopy, culture, and DST as the initial diagnostic test in patients with HIV and those suspected of having MDR-TB.[50]

Loop-mediated isothermal amplification. Loop-mediated isothermal amplification (LAMP) is a rapid molecular diagnostic tool that also can be implemented without the need for skilled personnel. LAMP allows for the rapid amplification of genetic material (<1 hour) in a closed-tube system by heating the sample in an isothermal bath (62°C), eliminating the need for a thermocycler. The amplified product can be visualized by eye with good accuracy and reproducibility. Boehme et al.[59] evaluated the diagnostic accuracy of LAMP for TB in Peru, Bangladesh, and Tanzania. Sensitivity was 98% for smear-positive sputum samples, but only 49% for smear-negative samples. Specificity was 99%. Six tests and 2 controls were run in an average of 54 minutes. Despite running the tests in a single room without biosafety cabinets for each step, DNA contamination was not observed.

In 2008, Eiken Chemical modified the original TB LAMP assay and developed a kit that was evaluated by Mitarai et al.[60] in Japan. Although the original kit required centrifugation and washes with a buffer, the new one does not. Two sputum samples were obtained from 170 TB suspects and 170 controls. Using solid and liquid cultures as the reference, they found that LAMP (untreated or digested and concentrated sputum) performed well for smear-positive samples (sensitivity 98%), but was suboptimal for smear-negative samples (sensitivity 56% for untreated samples, 30% for treated samples). Specificity was 94% for untreated samples and 100% for treated samples. Similar results were found in subsequent reference and peripheral health center evaluation studies.[6] In these field studies, sensitivity in smear-positive samples was 97%, and for smear-negative samples, 57% to 62%. Specificity was 95% to 96%. False positives were noted to arise from nonspecific amplification in high heat and humidity. Due to the short turnaround time (<1 hour), simplicity, and low cost, TB LAMP may have a place in peripheral health care settings despite its low sensitivity in smear-negative samples and the need for extensive training and quality assurance. This matter is currently under consideration by the WHO, which has agreed that there is currently insufficient evidence to recommend in favor or, or against, this test in lieu of smear microscopy.[61] Data specific to HIV patients is not yet available, but TB LAMP's low sensitivity in smear-negative samples may limit its utility.

Using nonsputum samples to detect pulmonary TB. Obtaining sputum samples can be very challenging, especially in children under the age of 10 years.[62] Therefore, there is a great interest in using bodily fluids and solids that can easily be obtained, such as urine and stool, for the diagnosis of pulmonary and/or disseminated TB. Urine and stool MTB DNA detection methods offer some promise on this front.

Transrenal MTB DNA. Transrenal DNA, or small fragments of microbial DNA (<200 base pairs) filtered through the kidneys, can be detected using nucleic acid amplification for diagnosis of both pulmonary and extrapulmonary TB. Green et al.[63] published a review on

the topic, and discussed reasons for variable sensitivities (7%-100%) reported in the literature. Special storage, specimen collection frequencies, and nucleic acid extraction methods are required to deal with the small size of the DNA fragment, its dilute nature in urine, and its tendency to degrade when stored. Of 7 studies evaluating the diagnostic accuracy of transrenal DNA detection for nonrenal or urethral TB, 4 compared test performance in HIV-positive and HIV-negative patients. Although specimen processing varied from study to study, all 4 studies found higher sensitivity among HIV-positive subjects (16%-100%) compared with HIV-negative individuals (6%-38%). If consistency in test performance can be achieved by optimizing and standardizing specimen processing, this may be a valuable tool for diagnosing TB in the HIV-positive population.

Urine-based Xpert MTB/RIF. A few studies have examined the use of urine in the Xpert MTB/RIF platform in patients with HIV in South Africa clinically suspected of having TB.[64,65] Similar trends were seen in these 2 studies; sensitivity was markely higher in those with lower CD4 counts, likely due to increased bacillary burden and renal involvement in this population. In Peter et al.,[64] the sensitivity of urine Xpert MTB/RIF (48%) was comparable to the sensitivity of smear microscopy in this study population (52%). Sensitivity was improved (54%) in those with CD4 counts <200 compared with those with CD4 counts >200 (31%). In smear-negative patients, the sensitivity was 39% and specificity was 98%. The overall sensitivity was improved to 70% with a decrease in specificity (89%) when Xpert MTB/RIF followed the urine lipoarabinomannan antigen-based assay.

In a similar study by Lawn SD et al.,[65] the ability of Xpert MTB/RIF to detect TB in urine was lower, but the same trend was observed as in the prior study (CD4 <50: sensitivity 44%, CD4 50-150: sensitivity 25%, and CD4 >150: sensitivity 3%). Xpert MTB/RIF on sputum was positive in 58% from the first sample, and in 70% for those with 2 samples. The smear-negative rate was high in this study population (71%). The urine test was able to accurately diagnose patients who were sputum culture negative (specificity 100%). This study used less urine (2 mL) compared with the first study (2-10 mL), which may have contributed to the lower sensitivies observed. Although the ability to detect MTB in urine using the Xpert MTB/RIF platform is suboptimal, it may be of value in HIV patients with advanced immunosuppression who are unable to produce sputum.

Stool PCR. Because much of the sputum produced is swallowed and enters the digestive tract, Cordova et al.[66] hypothesized that MTB DNA could be detected in stool from adults with pulmonary TB. Paired sputum and stool samples were obtained from patients, 39% of whom were HIV positive. The stool was analyzed by hemi-nested PCR of the IS6110 sequence, followed by RIF susceptibility testing by heteroduplex PCR assay. Stool PCR was found to have 86% sensitivity and 100% specificity when compared with sputum culture, and was similar for HIV-negative and HIV-positive patients. The stool heteroduplex PCR assay for RIF susceptibility had 98% agreement with sputum culture DST. Subsequently, a study of 1693 HIV-positive patients in southeast Asia revealed that 44% of those with culture-confirmed TB also had a positive stool culture,[67] and small pediatric studies detected TB in 47% to 75% of TB culture-positive children using Xpert MTB/RIF on stool samples.[68,69] Although the technical aspects of these nonsputum methods for detecting pulmonary and/or disseminated TB are still being developed, these methods hold the potential promise of easier specimen collection should they yield good results.

RNA expression in host blood. Several novel studies have examined the ability to discern unique patterns of RNA expression in the host when a patient is actively infected with TB.[70,71] One such study derived a disease risk score using RNA transcripts for HIV-positive and HIV-negative patients with culture-confirmed TB, with diseases other than TB, and for healthy individuals with latent TB infection (LTBI).[70] The authors calculated disease risk scores based on a training group in Malawi, and validated these scores in a test group in South Africa. In HIV-positive patients, the disease risk score achieved a sensitivity and specificity of 94% and 90%, respectively, for distinguishing TB from LTBI, and 95% and 84%, respectively, for TB and other diseases. The classification was slightly better at differentiating TB and LTBI in HIV-negative patients (sensitivity 95% and specificity 94%). For TB and other diseases, the risk score performed considerably better in HIV-negative patients (sensitivity 100% and specificity 96%). The decreased specificity in the HIV-positive group (TB vs other diseases) may be attributable to the use of culture as a gold standard, which may fail to detect TB in paucibacillary and extrapulmonary disease. Therefore, a portion of the false positives in this study may be true positives. The results from this study have good generalizability due to the inclusion of HIV-positive patients with a spectrum of diseases that may be considered in the differential along with TB.

A recent study by Anderson et al. (2014)[71] examined the transcriptional signature of TB in host blood in a pediatric cohort with and without HIV. TB diagnosis in the pediatric population is particularly challenging. Children often are not able to expectorate sputum spontaneously, and often require hospitalization to obtain gastric aspirates and induced sputum samples. Moreover, presentations of childhood TB tend to be paucibacillary and extrapulmonary, rendering most cases microbiologically unconfirmed.[72,73] In this study,

Table 4. Diagnostic accuracy of commercially available molecular tests for multidrug resistant TB*

	Rifampin		Isoniazid				
Assay	Sensitivity (%)	Specificty (%)	Sensitivity (%)	Specificty (%)	Sample Type	DST Reference	References
Genotype MTBDR*plus*	99	99	94	100	Smear-positive sputum	Agar proportion method	80
	100	100	93	100	Smear-positive sputum	MGIT AST†	81
	100	100	100	100	Culture isolates	MGIT AST	81
	100	96	81	100	Smear-positive sputum	MGIT DST‡	88
INNO-LiPA Rif.TB	96	96	N/A	N/A	Culture isolates	Sequencing, agar proportion method	74-76
	87-96	87-96	N/A	N/A	Smear-positive sputum	Liquid culture	
Xpert MTB/RIF	95	98	N/A	N/A	Sputum (smear-positive and smear-negative)	MGIT DST, MTBDR*plus*, sequencing	46

AST, antimicrobial susceptibility testing; INH, isoniazid; MGIT, Mycobacterium Growth Indicator tube; DST, drug susceptibility testing; MTB RIF, *Mycobacterium tuberculosis* rifampin; N/A, not applicable.
*Data are not reported separately for HIV-positive and HIV-negative individuals.
†MGIT AST for INH, RIF, streptomycin, ethambutol.
‡MGIT DST for INH, RIF.

a training cohort consisting of HIV-positive and HIV-negative children was established in Malawi and South Africa, and the transcription signatures were then validated in a Kenyan cohort. With culture as the reference, the overall sensitivity of the risk score derived from the transcription signature was 83% (compared with 54% for the Xpert MTB/RIF) and specificity was 84% (compared with 100% for Xpert MTB/RIF). Specific results for the HIV-positive population were not reported. Prospective studies that allow for improved accuracy in disease classification will be instrumental in evaluating the performance of these biomarkers.The disease risk scores developed in these studies are computationally simple and use a minimal number of RNA transcripts. These elements make it possible to envision a cost-effective platform in the future.

Molecular Drug Susceptibility Tests

Globally, only 2% of new and 6% of previously treated TB cases underwent testing for MDR-TB in 2010; in 2012, these numbers increased to 5% and 9%, respectively.[1] As concern over MDR- and XDR-TB grows, new susceptibility testing methods take on an increasing importance.

Xpert MTB/RIF. As mentioned previously, Xpert MTB/RIF detects RIF resistance by targeting the MTB *rpo*B gene. The presence of RIF resistance is thought to be a good marker for MDR-TB, which is defined as resistance to at least RIF and isoniazid (INH).[43] A 2014 Cochrane review calculated pooled sensitivity of 93% (95% CI, 87%-97%) and specificity of 97% (95% CI, 91%-99%).[46] Trebucq et al.[52] point out that where MDR-TB prevalence is low (<15% of TB cases), the PPV of the RIF-resistance testing of Xpert MTB/RIF is low and requires further confirmatory testing. Furthermore, the Cochrane review reports a lower pooled sensitivity of 91% (95% CI, 79%-97%) when the proportion of RIF resistance is ≤15%. As shown in Table 3, when MDR-TB prevalence is 5%, for every 100 people testing positive for RIF resistance, 29 will test falsely positive.

Line-probe assays. Currently, two line-probe assays (LPAs) are available for detecting MDR-TB: INNO-LiPA Rif.TB assay (Innogenetics, Gent, Belgium) and GenoType *plus* assay (Hain Lifescience, Nehren, Germany). The GenoType MTBDR*plus* assay simultaneously detects MTB directly from sputum or from liquid or solid culture, as well as mutations in the *rpo*B gene conferring RIF resistance, *kat*G gene conferring high-level INH resistance, and the *inh*A gene conferring low-level INH resistance, by means of PCR and reverse hybridization. The INNO-LiPA Rif.TB assay detects the presence of MTB on solid culture but is not labeled for use on sputum samples. It detects RIF resistance, but does not test for INH resistance. The performance of this test on culture isolates was good when evaluated in a high MDR-TB setting, with a sensitivity of 96% and specificity of 96%,[74] but was more variable in sputum samples (Table 4).[75,76] FIND has negotiated lower prices for the use of the GenoType MTBDR*plus* assay in high

countries with MDR burden.[77,78] Both LPAs have been in use in many developed countries for more than a decade.[79]

The MTBDR*plus* assay has been endorsed by the WHO and has been rolled out in 27 countries with high MDR-TB burden in response to the emergence of MDR-TB and XDR-TB.[79] Barnard et al.[80] studied the accuracy of the LPA for detection of MDR in South Africa, using indirect DST on agar slants as a reference. Results for the LPA were obtained within 1 to 2 days. The sensitivities for RIF and INH resistance were both 99%, and specificities were 99% and 100%, respectively, for smear-positive sputum. Smear-negative sputum was also examined, 80% of which gave an interpretable result (sample size was too small for sensitivity/specficity calculations). Anek-Vorapong et al.[81] evaluated the MTBDR*plus* assay in culture isolates and in smear-positive sputum, and found the sensitivity for INH resistance was 100% for the former but 93% for the latter. Detection of RIF resistance was excellent for both specimen types (100% sensitivity for both).

An additional LPA, MDRTB*sl* (Hain Lifescience, Nehren, Germany), has been developed to detect resistance to second-line TB therapy (amikacin, kanamycin, capreomycin, ethambutol, and fluoroquinolones) and is currently being evaluated by FIND and partners.[79] Thus far, it appears that sensitivities are moderate for detecting resistance in culture isolates to ethambutol (57%-77%), kanamycin (41%-77%), and better for capreomycin (80%-97%), amikacin (80%-100%), and fluoroquinolone (87%-94%).[82-84] These sensitivities were lower yet when sputum samples were tested directly.

The inability to use the LPA on negative sputum smears reduces its utility in the HIV—TB-coinfected population. Skilled personnel, laboratory equipment, and biosafety requirements further limit the use of LPAs to reference laboratories in most developing countries. Xpert MTB/RIF is able to circumvent these issues, but is only able to test for RIF resistance. Despite these limitations, molecular methods have the distinct advantage of being rapid and their use is therefore warranted in high-burden settings.

CONCLUSION

The Global Plan to Stop TB partnership goals are to reduce TB prevalence by 50% compared with 1990 levels and to eliminate TB as a public health problem by 2050 (defined as <1 case per million globally).[2] A crucial step toward accomplishing these goals is improving diagnostic accuracy. Progress has been made in recent years due to concerted international efforts to reduce the burden of disease, particularly in the face of high mortality from TB-HIV coinfection, MDR-TB, and XDR-TB. The diagnosis of TB in HIV-positive patients remains particularly challenging due to the high rate of sputum smear-negative and extrapulmonary TB disease. The dissemination of new molecular tools such as Xpert MTB/RIF in high-burden countries offers the potential for rapid diagnosis with fair sensitivity for sputum-smear negative TB, as well as RIF resistance testing. This is limited by the relatively high cost of the cartridges and equipment, but analyses have shown that this technology is cost-effective when used in select settings with high TB prevalence. In such settings, rapid diagnosis may not only benefit the patient, but also curtail transmission.

The goals for new diagnostics in the Global Plan to Stop TB 2011-2015 are to have 1) a simple, rapid, and affordable test for use at peripheral health centers; 2) a test for MDR-TB at peripheral health centers; and 3) a test for LTBI that can identify people at high risk for disease progression.[2] The Xpert MTB/RIF, LPAs, and possibly TB-LAMP mark a significant step toward accomplishing the first 2 goals. However, molecular methods have not been able to match the sensitivity of liquid culture systems, which remain the gold standard for diagnosing TB in HIV-positive patients (as well as in HIV-negative individuals). Current molecular methods are also not yet able to supplant culture for DST. Thus, effective integration of molecular tests, biomarkers, antigen assays, microscopy, and novel culture methods is necessary, tailored to the specific conditions of each country according to the prevalence of TB, HIV, and MDR-TB. Further studies are needed on the cost-effectiveness and diagnostic performance of different algorithms incorporating a variety of methods for TB detection and DST (eg, using the MTBDR*sl* vs culture after Xpert MTB/RIF if the sample tests positive for RIF) to make the best use of existing and burgeoning methods.

References

1. Global Tuberculosis Control: Report 2013. Geneva, World Health Organization, 2011 (WHO/HTM/TB/2013.11).
2. Global Plan to Stop TB 2011-2015: transforming the fight towards elimination of tuberculosis. Geneva: World Health Organization; 2010.
3. Aaron L, Saadoun D, Calatroni I, et al. Tuberculosis in HIV-infected patients: a comprehensive review. Clin Microbiol Infect 2004;10: 388—98.
4. Monkongdee P, McCarthy KD, Cain KP, et al. Yield of acid-fast smear and mycobacterial culture for tuberculosis diagnosis in people with human immunodeficiency virus. Am J Respir Crit Care Med 2009;180:903—8.
5. Elliot AM, Namaambo K, Allen BW, et al. Negative sputum smear results in HIV-positive patients with pulmonary tuberculosis in Lusaka, Zambia. Tub Lung Dis 1993;74:191—4.
6. Palmieri A, Girardi E, Pellicelli AM, et al. Pulmonary tuberculosis in HIV-infected patients presenting with normal chest radiograph and negative sputum smear. Infection 2002;30:68—74.
7. Getahun H, Harrington M, O'Brien R, et al. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection of AIDS in resource-constrained settings: informing urgent policy changes. Lancet 2007;369:2042—9.
8. Kang'ombe CT, Harries AD, Ito K, et al. Long-term outcome in patients registered with tuberculosis in Zomba, Malawi: mortality at 7 years according to initial HIV status and type of TB. Int J Tuberc Lung Dis 2004;8:829—36.
9. Wood R, Middelkoop K, Myer L, et al. Undiagnosed tuberculosis in a community with high HIV prevalence: implications for tuberculosis control. Am J Respir Crit Care Med 2007;175:87—93.

10. Ramirez-Cardich ME, Kawai V, Oberhelman RA, et al. Clinical correlates of tuberculosis co-infection in HIV-infected children hospitalized in Peru. Int J Infect Dis 2006;10:278—81.
11. Gilks CF, Brindle RJ, Otieno LS, et al. Extrapulmonary and disseminated tuberculosis in HIV-1-seropositive patients presenting to the acute medical services in Nairobi. AIDS 1990;4:981—5.
12. Jones BE, Young SM, Antoniskis D, et al. Relationship of the manifestations of tuberculosis to CD4 cell counts in patients with human immunodeficiency virus infection. Am Rev Respir Dis 1993;148:292—7.
13. Leeds IL, Magee MJ, Kurbatova EV, et al. Site of extrapulmonary tuberculosis is associated with HIV infection. Clin Infect Dis 2012;55:75—81.
14. Cox JA, Lukande RL, Lucas S, et al. Autopsy causes of death in HIV-positive individuals in sub-Saharan Africa and correlation with clinical diagnoses. AIDS Rev 2010;12:183—94.
15. Cox J, Lukande RL, Nelson AM, et al. An autopsy study describing causes of death and comparing clinico-pathological findings among hospitalized patients in Kampala, Uganda. PLoS One 2012;7:e33685.
16. Pronyk PM, Kahn K, Hargreaves JR, et al. Undiagnosed pulmonary tuberculosis deaths in rural South Africa. Int J Tuberc Lung Dis 2004;8:796—9.
17. Lucas SB, Hounnou A, Peacock C, et al. The mortality and pathology of HIV infection in a west African city. AIDS 1993;7:1569—79.
18. Ansari NA, Kombe AH, Kenyon TA, et al. Pathology and causes of death in a group of 128 predominantly HIV-positive patients in Botswana, 1997-1998. Int J Tuberc Lung Dis 2002;6:55—63.
19. Nelson AM, Perriens JH, Kapita B, et al. A clinical and pathological comparison of the WHO and CDC case definitions for AIDS in Kinshasa, Zaire: is passive surveillance valid? AIDS 1993;7:1241—5.
20. Rana FS, Hawken MP, Mwachari C, et al. Autopsy study of HIV-1-positive and HIV-1-negative adult medical patients in Nairobi, Kenya. J Acquir Immune Defic Syndr 2000;24:23—9.
21. Lanjewar DN, Duggal R. Pulmonary pathology in patients with AIDS: an autopsy study from Mumbai. HIV Med 2001;2:266—71.
22. Hargreaves NJ, Kadzakumanja O, Whitty CJ, et al. "Smear-negative" pulmonary tuberculosis in a DOTS programme: poor outcomes in an area of high HIV seroprevalence. Int J Tuerc Lung Dis 2001;5:847—54.
23. Pukenyte E, Lescure FX, Rey D, et al. Incidence of and risk factors for severe liver toxicity in HIV-infected patients on anti-tuberculosis treatment. Int J Tuberc Lung Dis 2007;11:78—84.
24. Multidrug-resistant tuberculosis (MDR-TB): October 2013 update. Geneva: WHO; 2013.
25. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. Geneva: WHO; 2010.
26. Campos PE, Suarez PG, Sanchez J, et al. Multidrug-resistant Mycobacterium tuberculosis in HIV-infected persons. Peru. Emerg Infect Dis 2003;9:1571—8.
27. Wells CD. Global impact of multidrug-resistant pulmonary tuberculosis among HIV-infected and other immunocompromised hosts: epidemiology, diagnosis, and strategies for management. Curr Infect Dis Rep 2010;12:192—7.
28. Kawai V, Soto G, Gilman RH, et al. Tuberculosis mortality, drug resistance, and infectiousness in patients with and without HIV infection in Peru. Am J Trop Med Hyg 2006;75:1027—33.
29. Greco S, Girardi E, Navarra A, et al. Current evidence on diagnostic accuracy of commercially based nucleic acid amplification tests for the diagnosis of pulmonary tuberculosis. Thorax 2006;61:783—90.
30. Ling DI, Flores LL, Riley LW, et al. Commercial nucleic-acid amplification tests for diagnosis of pulmonary tuberculosis in respiratory specimens: meta-analysis and meta-regression. PLoS One 2008;3:e1536.
31. Dinnes J, Deeks J, Kunst H, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technol Assess 2007;11:1—196.
32. Kivihya-Ndugga L, van Cleeff M, Juma E, et al. Comparison of PCR with the routine procedure for diagnosis of tuberculosis in a population with high prevalences of tuberculosis and human immunodeficiency virus. J Clin Microbiol 2004;42:1012—5.
33. Davis JL, Huang L, Worodria W, et al. Nucleic acid amplification tests for diagnosis of smear-negative TB in a high HIV-prevalence setting: a prospective cohort study. PLoS One 2011;6:e16321.
34. Scott LE, McCarthy K, Gous N, et al. Comparison of Xpert MTB/RIF with other nucleic acid technologies for diagnosing pulmonary tuberculosis in a high HIV prevalence setting: a prospective study. PLoS Med 2011;8:e1001061.
35. Davis JL, Huang L, Kovacs JA, et al. Polymerase chain reaction of secA1 on sputum or oral wash samples for the diagnosis of pulmonary tuberculosis. Clin Infect Dis 2009;48:725—32.
36. Chen X, Yang Q, Kong H, Chen Y. Real-time PCR and Amplified MTD for rapid detection of Mycobacterium tuberculosis in pulmonary specimens. Int J Tuberc Lung Dis 2012;16:235—9.
37. Hofmann-Thiel S, Turaev L, Hoffmann H. Evaluation of the hyplex TBC PCR test for detection of Mycobacterium tuberculosis complex in clinical samples. BMC Microbiol 2010;10:95.
38. Tanaka H, Hirose H, Kato Y, et al. Clinical evaluation of TRCRapid M.TB for detection of Mycobacterium tuberculosis complex in respiratory and nonrespiratory specimens. J Clin Microbiol 2010;48:1536—41.
39. Laraque F, Griggs A, Slopen M, et al. Performance of nucleic acid amplification tests for diagnosis of tuberculosis in a large urban setting. Clin Infect Dis 2009;49:46—54.
40. Guerra RL, Hooper NM, Baker JF, et al. Use of the amplified mycobacterium tuberculosis direct test in a public health laboratory: test performance and impact on clinical care. Chest 2007;132:946—51.
41. Flores LL, Pai M, Colford JM Jr, et al. In-house nucleic acid amplification tests for the detection of Mycobacterium tuberculosis in sputum specimens: meta-analysis and meta-regression. BMC Microbiol 2005;5:55.
42. Banada PP, Sivasubramani SK, Blakemore R, et al. Containment of bioaerosol infection risk by the Xpert MTB/RIF assay and its applicability to point-of-care settings. J Clin Microbiol 2010;48:3551—7.
43. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 2011;363:1005—15.
44. TB diagnostics and laboratory strengthening. Available at: http://who.int/tb/laboratory/mtbrifrollout/en/. Accessed October 1, 2014.
45. Theron G, Peter J, van Zyl-Smit R, et al. Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. Am J Respir Crit Care Med 2011;184:132—40.
46. Steingart KR, Schiller I, Horne DJ, et al. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev 2014;1:CD009593.
47. Moure R, Martin R, Alcaide F. Effectiveness of an integrated real-time PCR method for detection of the Mycobacterium tuberculosis complex in smear-negative extrapulmonary samples in an area of low tuberculosis prevalence. J Clin Microbiol 2012;50:513—5.
48. Tortoli E, Russo C, Piersimoni C, et al. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. Eur Resp J 2012;40:442—7.
49. Vadwai V, Boehme C, Nabeta P, et al. Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? J Clin Microbiol 2011;49:2540—5.
50. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Policy update. Geneva: WHO; 2013.
51. FIND. FIND-negotiated prices for Xpert MTB/RIF and country list. Available at: http://www.finddiagnostics.org/about/what_we_do/successes/find-negotiated-prices/xpert_mtb_rif.html. Accessed October 1, 2014.
52. Trebucq A, Enarson DA, Chiang CY, et al. Xpert MTB/RIF for national tuberculosis programmes in low-income countries: when, where and how? Int J Tuberc Lung Dis 2011;15:1567—72.
53. Vassal A, van Kampen S, Sohn H, et al. Rapid diagnosis of tuberculosis with the Xpert MTB/RIF assay in high burden countries: a cost-effectiveness analysis. PLoS Med 2011;8:e1001120.
54. Andrews JR, Lawn SD, Rusu C, et al. The cost-effectiveness of routine tuberculosis screening with Xpert MTB/RIF prior to initiation of antiretroviral therapy: a model-based analysis. AIDS 2012;26:987—95.
55. Bassett IV, Wang B, Chetty S, et al. Intensive tuberculosis screening for HIV-infected patients starting antiretroviral therapy in Durban, South Africa. Clin Infect Dis 2010;51:823—9.
56. Lawn SD, Kranzer K, Edwards DJ, et al. Tuberculosis during the first year of antiretroviral therapy in a South African cohort using an intensive pretreatment screening strategy. AIDS 2010;24:1323—8.
57. Getahun H, Kittikraisak W, Heilig CM, et al. Development of a standardized screening rule for tuberculosis in people living with HIV in resource-constrained settings: individual participant data meta-analysis of observational studies. PLoS Med 2011;8:e1000391.

58. Lawn SD, Wood R. Tuberculosis in antiretroviral treatment services in resource-limited settings: addressing the challenges of screening and diagnosis. J Infect Dis 2011;204:S1159–67.
59. Boehme CC, Nabeta P, Henostroza G, et al. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. J Clin Microbiol 2007;45:1936–40.
60. Mitarai S, Okumura M, Toyota E, et al. Evaluation of a simple loop-mediated isothermal amplification test kit for the diagnosis of tuberculosis. Int J Tuberc Lung Dis 2011;15:1211–7.
61. The use of a commercial loop-mediated isothermal amplification assay (TB-LAMP) for the detection of tuberculosis. Expert Group Meeting Report. Geneva: WHO; 2013.
62. Zar HJ, Hanslo D, Apolles P, et al. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study. Lancet 2005;365: 130–4.
63. Green C, Huggett JF, Talbot E, et al. Rapid diagnosis of tuberculosis through the detection of mycobacterial DNA in urine by nucleic acid amplification methods. Lancet Infect Dis 2009;9:505–11.
64. Peter JG, Theron G, Muchinga TE, et al. The diagnostic accuracy of urine-based Xpert MTB/RIF in HIV-infected hospitalized patients who are smear-negative or sputum scarce. PLoS One 2012;7:e39966.
65. Lawn SD, Kerkhoff AD, Vogt M, et al. High diagnostic yield of tuberculosis from screening urine samples from HIV-infected patients with advanced immunodeficiency using the Xpert MTB/RIF assay. J Acquir Immune Defic Syndr 2012;60:289–94.
66. Cordova J, Shiloh R, Gilman RH, et al. Evaluation of molecular tools for detection and drug susceptibility testing of Mycobacterium tuberculosis in stool specimens from patients with pulmonary tuberculosis. J Clin Microbiol 2009;48:1820–6.
67. Oramasionwu GE, Heilig CM, Udomsantisuk N, et al. The utility of stool cultures for diagnosing tuberculosis in people living with the human immunodeficiency virus. Int J Tuberc Lung Dis 2013;17: 1023–8.
68. Nicol MP, Spiers K, Workman L, et al. Xpert MTB/RIF testing of stool samples for the diagnosis of pulmonary tuberculosis in children. Clin Infect Dis 2013;57:e18–21.
69. Walters E, Gie RP, Hesseling AC, et al. Rapid diagnosis of pediatric intrathoracic tuberculosis from stool samples using the Xpert MTB/RIF assay: a pilot study. Ped Infect Dis J 2012;31:1316.
70. Kaforou M, Wright VJ, Oni T, et al. Detection of tuberculosis in HIV-infected and –uninfected African adults using whole blood RNA expression signatures: a case-control study. PLoS Med 2013;10: e1001538.
71. Anderson ST, Kaforou M, Brent AJ, et al. Diagnosis of childhood tuberculosis and host RNA expression in Africa. N Eng J Med 2014;370:1712–23.
72. Perez-Velez CM, Marais BJ. Tuberculosis in children. N Eng J Med 2012;367:348–61.
73. Zar HJ, Connell TG, Nicol M. Diagnosis of pulmonary tuberculosis in children: new advances. Expert Rev Anti Infect Ther 2010;8: 277–88.
74. Makinen J, Marttila HR, Marjamaki M, et al. Comparison of two commercially available DNA line probe assays for detection of multidrug-resistant *Mycobacterium tuberculosis*. J Clin Microbiol 2006;44:350–2.
75. Skenders G, Holtz TH, Riekstina V, et al. Implementation of the INNO-LiPA Rif. TB line-probe assay in rapid detection of multidrug-resistant tuberculosis in Latvia. Int J Tuberc Lung Dis 2011;15:1546–52.
76. Ogwang S, Asiimwe BB, Traore H, et al. Comparison of rapid tests for detection of rifampin-resistant *Mycobacterium tuberculosis* in Kampala, Uganda. BMC Infect Dis 2009;9:139.
77. Molecular line probe assays for rapid screening of patients at risk of multi-drug resistant tuberculosis (MDR-TB). Geneva: WHO. Available at: http://www.who.int/tb/features_archive/expert_group_report_june08.pdf; 2008. Accessed October 1, 2014.
78. FIND. FIND negotiated prices and country list for Line Probe Assay and associated instrumentation. Available at: http://www.finddiagnostics.org/about/what_we_do/successes/find-negotiated-prices/mtbdrplus.html. Accessed October 1, 2014.
79. FIND. Line probe assay (1st line drugs). Available at: http://www.finddiagnostics.org/programs/tb/find_activities/line_probe_assay_1.html. Accessed October 1, 2014.
80. Barnard M, Albert H, Coetzee G, et al. Rapid molecular screening for multidrug-resistant tuberculosis in a high-volume public health laboratory in South Africa. Am J Respir Crit Care Med 2008;177:787–92.
81. Anek-Vorapong R, Sinthuwattanawibool C, Podewils LJ, et al. Validation of the Genotype MTBDRplus assay for detection of MDR-TB in a public health laboratory in Thailand. BMC Infect Dis 2010;10:123.
82. Brossier F, Veziris N, Aubry A, et al. Detection by Genotype MTBDRsl test of complex mechanisms of resistance to second-line drugs and ethambutol in multidrug-resistant Mycobacterium tuberculosis complex isolates. J Clin Microbiol 2010;48:1683–9.
83. Hillemann D, Rusch-Gerdes S, Richter E. Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of Mycobacterium tuberculosis strains and clinical specimens. J Clin Microbiol 2009;47:1767–72.
84. Ignatyeva O, Kontsevaya I, Kovalyov A, et al. Detection of resistance to second-line antituberculosis drugs by use of the Genotype MTBDRsl assay: a multicenter evaluation and feasibility study. J Clin Microbiol 2012;50:1593.
85. Araj GF, Talhouk RS, Itani LY, et al. Comparative performance of PCR-based assay versus microscopy and culture for the direct detection of Mycobacterium tuberculosis in clinical respiratory specimens in Lebanon. Int J Tuberc Lung Dis 2000;4:877–81.
86. Boehme CC, Nikol MP, Nabeta P, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 2011;377:1495–505.
87. George G, Mony P, Kenneth J. Comparison of the efficacies of loop-mediated isothermal amplification, fluorescence smear microscopy and culture for the diagnosis of tuberculosis. PLoS One 2011;6:e21007.
88. Albert H, Bwanga F, Mukkada S, et al. Rapid screening of MDR-TB using molecular line probe assay is feasible in Uganda. BMC Infect Dis 2010;10:41.