REVIEW

Diagnosis of HIV-associated tuberculosis

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Purpose of review
The current review highlights recent advances in tuberculosis (TB) diagnostics that are relevant for clinicians engaged in the care of HIV-positive adults.

Recent findings
The first section focuses on newly available tools, highlighting recent progress. The second section focuses on new diagnostic approaches that are not yet widely available or implemented, but have considerable potential to improve the management of TB/HIV coinfected persons. The final section speculates about future directions that may be fruitful.

Summary
Advances in *Mycobacterium tuberculosis* nucleic acid amplification-based genotypic tests stand to improve case detection and drug susceptibility testing in the near term. Identification of human gene expression signatures that are associated with TB and/or TB risk, and the identification of novel *M. tuberculosis* targets coupled with exploitation of advances in biosensor technology may transform TB diagnosis in the future.

Keywords
diagnosis, diagnostics, HIV, tuberculosis

INTRODUCTION

Tuberculosis (TB) in people living with HIV (PLHIV) has several facets that warrant special consideration during the development and clinical application of TB diagnostic tests. These facets of TB/HIV are linked to the immunosuppression that is the hallmark of untreated HIV, and present challenges during the TB diagnostic process. There is the potential for low bacillary burden in respiratory specimens even when pulmonary TB is present, and the propensity for extrapulmonary TB. Persons with untreated HIV are at increased risk of TB and TB death compared with HIV-negative individuals. These facets of HIV-associated TB have laid bare the inadequacy of sputum smear microscopy as the principle diagnostic test, and underscore the need for highly sensitive, specific, rapid tests that provide comprehensive information that can be used to formulate an individualized, microbiologically appropriate treatment regimen. The first section of this review focuses on newly available tools, highlighting progress in the field over the last 2 years. The second section focuses on new diagnostic approaches that are not yet widely available or implemented, but have considerable potential. The final section speculates about future directions that may be fruitful.

NEW TOOLS THAT HAVE ENTERED CLINICAL PRACTICE

Nucleic acid amplification tests

The cardinal features of nucleic acid amplification tests (NAATs) are enzymatic amplification of target nucleic acid sequences to a concentration sufficiently high to be detected, followed by biochemical detection. Knowledge of mycobacterial genomes has allowed selection of targets and detection probes that are species-specific, and therefore NAATs can be used for *Mycobacterium tuberculosis* case detection and species identification. NAATs can also be used to detect alleles associated with drug resistance and susceptibility, thereby providing genotypic drug susceptibility testing. NAATs detect bacillary DNA – dependence on concentration of bacilli in a specimen is a consequent limitation.

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KEY POINTS

- Highly sensitive nucleic acid amplification based tests hold promise for improving the TB diagnostic yield in people living with HIV who have concomitant TB.
- Progress is being made in the development of rapid tests that provide comprehensive genotypic drug susceptibility results.
- Progress is being made toward identification of host gene expression signatures that are associated with TB and TB risk.
- Application of proteomics, lipidomics, and metabolomics methodologies, when combined with advances in biosensor technology, may catalyze a new wave of novel TB diagnostics.

GeneXpert tests

Xpert MTB/RIF (Cepheid Inc., Sunnyvale, California, USA) is an automated, integrated, cartridge-based NAAT recommended by the WHO as the initial test for TB to increase case detection and improve identification of rifampin resistance directly from sputum [1–3]. Yet, its sensitivity for TB detection is inadequate when few bacilli are present in a clinical specimen, as is often the case in individuals with HIV-associated pulmonary TB [2].

The Xpert MTB/RIF Ultra ('Ultra', Cepheid Inc.) was developed to improve the sensitivity for detection of *M. tuberculosis* (through incorporation of two different multicopy amplification targets and improvements in assay chemistry and cartridge design) and to improve the accuracy of rifampin resistance detection [4▪]. Analytical laboratory data showed an approximately 1-log improvement in the lower limit of detection compared with Xpert MTB/RIF, as well as improved detection of rifampin resistance in mixed infections, avoidance of false-positive rifampin resistance results in paucibacillary specimens, and improved differentiation of certain silent mutations [4▪]. In a multicenter study of the clinical diagnostic accuracy of Ultra vs. conventional Xpert MTB/RIF, Ultra sensitivity was superior to that of Xpert MTB/RIF for case detection among participants with sputum smear-negative pulmonary TB [Ultra 63% vs. Xpert MTB/RIF 46% (difference 17%, 95% confidence interval (CI) 10, 24)] and among HIV-positive participants [Ultra sensitivity 90% vs. Xpert MTB/RIF 77% (difference 13%, 95% CI 6.4, 21)] [5▪]. For case detection, Ultra specificity was lower than that of Xpert MTB/RIF [Ultra 96% vs. Xpert MTB/RIF 98% (difference −2.7%, 95% CI −3.9, −1.7)], with the difference most marked in participants with previous TB. Ultra and Xpert performed similarly in detecting rifampin resistance [5▪]. For Ultra, the sensitivity gains are expected to increase diagnostic yield in PLHIV, but the impact of reduced specificity may vary across different populations. Mathematical modeling to estimate the potential impact of Ultra (as a replacement for Xpert MTB/RIF) showed that Ultra was likely to have mortality benefit in settings with high TB and HIV prevalence [6▪]. Ongoing diagnostic accuracy studies that include long-term follow-up may help to clarify the biologic and clinical basis of Ultra-positive/culture-negative result. Results from an ongoing initial deployment in South Africa will help to clarify the settings and circumstances in which Ultra may have the greatest benefit. The finding of positive Ultra tests in some culture-negative individuals with previously treated TB is not unique to Ultra, and has been observed for Xpert MTB/RIF as well as for early generation manual NAATs [7▪,8]. Based on a technical expert consultation, the WHO has stated that Ultra can replace Xpert in all settings [9]. Roll-out beyond South Africa is expected to commence in 2018.

Ultra's increased analytical sensitivity is likely to increase test yield from nonrespiratory specimens. Bahr *et al.* [10▪▪] prospectively evaluated 129 HIV-positive adults with suspected meningitis using Xpert MTB/RIF, Ultra, and culture of centrifuged cerebrospinal fluid. Among 23 participants classified as having probable or definite TB meningitis according to a uniform case definition, Ultra sensitivity was 70% (95% CI 47, 87); sensitivities of Xpert MTB/RIF and culture were 43% (23, 66) and 43% (23, 66).

Molecular line-probe assays for the detection of resistance to second-line antituberculosis drugs

Early detection of *M. tuberculosis* drug resistance is a major barrier to successful TB treatment and control. Line-probe assays are NAATs in which labeled amplification products are hybridized to oligonucleotide probes immobilized on a strip. In 2008, WHO recommended line probe assays for detection of multidrug-resistant (MDR)-TB [11]. Early versions of line probe tests for second-line drug resistance assessed a limited number of genetic loci and therefore had suboptimal performance, especially with respect to sensitivity [12]. However, the GenoType MTBDRsl VER 2.0 (Hain Lifescience, Nehren, Germany) accommodates a more complete set of mutations in *gyrA* and *gyrB* (fluoroquinolone resistance), as well as *rrs* and the *eis* promoter region (second-line injectable resistance). The GenoType MTBDRsl VER 2.0 performed well when used to test 268 rifampin-resistant or MDR isolates from South Africa – sensitivity and specificity for fluoroquinolone resistance were 100% (95.8, 100) and 98.9%

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(96.1, 99.9), respectively, and for second-line injectable drugs were 89.2% (79.1, 95.6) and 98.5% (95.7, 99.7), respectively [13▪]. In 2016, WHO recommended the use of such next-generation line probe assay as the initial test to detect resistance to fluoroquinolones and the second-line injectable drugs, instead of phenotypic culture-based assays [14]. Existing line-probe assays are technically demanding and require that different steps in the testing process be conducted in separate rooms to minimize amplicon carry-over. Suitable laboratory infrastructure, specially trained technical personnel, and appropriate quality assurance testing are necessary.

High-throughput nucleic acid amplification tests

High-throughput NAAT platforms can accommodate dozens of specimens simultaneously and therefore are intended mainly for reference laboratories. Some of the platforms are polyvalent, that is, they can test for multiple analytes using the same assay principle. The assays furthest along in evaluation are the Abbott RealTi*m*e MTB and companion RealTi*m*e MTB RIF/INH assays. These are qualitative real-time PCR assays intended for direct testing of respiratory specimens and use with the Abbott *m*2000 platform. To optimize sensitivity for case detection, the RealTi*m*e MTB assay targets two conserved multicopy insertion elements of *M. tuberculosis*. The RealTi*m*e MTB RIF/INH assay targets regions within *rpoB*, *katG*, and the *inhA* promoter and therefore provides genotypic drug susceptibility testing results for rifampin and isoniazid. The claimed analytical sensitivities of the RealTi*m*e MTB assay and the RealTi*m*e MTB RIF/INH assay are 17 and 60 colony forming unit/ml, respectively, which are similar to the analytical lower limits of detection for Ultra (15.6 cfu/ml for *M. tuberculosis* detection and 105 cfu/ml for detection of rifampin susceptibility) [4▪,15]. Studies of clinical diagnostic accuracy for detection of *M. tuberculosis* in respiratory specimens have shown promising results, and of particular interest are studies in which the Abbott assays were compared with Xpert MTB/RIF [16▪,17,18]. In a prospective study of 206 adults (73% HIV-positive) in South Africa, the sensitivity and specificity of the Abbott RealTi*m*e MTB assay were 85.5% (74.2–93.1) and 92.4% (86.4–96.3), whereas for Xpert MTB/RIF were 91.9% (82.2–97.3) and 97.7% (93.5–99.5) when performed on the same processed sputum pellets [16▪]. Among 35 participants with smear-negative/culture-positive pulmonary TB, the Abbott RealTi*m*e MTB assay correctly identified 26/35 (74.3%) cases, whereas Xpert MTB/RIF correctly identified only 9/35 (25.7%), with the increased yield of the Abbott test potentially reflecting use of multicopy targets [16▪].

Loop-mediated isothermal amplification

Loop-mediated isothermal amplification (LAMP) is a NAAT method that is mostly temperature-independent and does not require sophisticated thermal cycling instrumentation. Loopamp *M. tuberculosis* complex detection kit (Eiken Chemical Company, Tokyo, Japan) is a commercially available LAMP assay that is mostly manual, requires less than 1 h to perform, and can be read with the unaided eye using ultraviolet light [19]. Using a reference standard of two cultures to define a TB-negative participant, pooled sensitivity of Loopamp across 10 studies was 76.0% (69.9, 81.2), and Loopamp was consistently more sensitive than smear microscopy; pooled specificity was 98.0% (96.0, 99.0) [19]. Accuracy in PLHIV was evaluated in four studies – sensitivity was 63.8% (49.0, 76.4) and was lower among HIV-positive adults than among all adults; specificity was 98.8 (85.1, 99.9) [20]. In 2016, the WHO issued policy guidance that, in adults with signs/symptoms consistent with TB, Loopamp may be used as a replacement for sputum smear microscopy to diagnose pulmonary TB, and as a follow-on test to smear microscopy (conditional recommendations based on very low-quality evidence) [20]. Due to limited evidence, it is unclear whether Loopamp has additional diagnostic value over sputum smear microscopy for testing PLHIV who have TB signs/symptoms.

Tests that detect targets other than nucleic acids

Urine lipoarabinomannan

Lipoarabinomannan (LAM) is a glycolipid component of the *M. tuberculosis* cell wall. As a bacterial product, it has the potential to discriminate active TB from latent TB infection, independent of host immune response. Its suitability for testing urine makes it attractive for use in populations in whom sputum is uninformative or not obtainable. The Alere Determine TB LAM Ag test (Abbott, Abbott Park, Illinois, USA) test is a commercially available lateral flow assay that detects urinary LAM in about 30 min without special equipment. The sensitivity of the Determine TB LAM Ag test in HIV-negative individuals is too low to be clinically useful [21]. However, its sensitivity is appreciably higher in PLHIV and is inversely related to CD4 count, with sensitivity of over 60% in patients with CD4 less than 50 cells/μl – the group with the highest mortality and in which sputum-based tests perform least

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well [22–25]. The WHO endorsed the determine TB LAM Ag test in 2015 to assist in the diagnosis of TB in PLHIV with low CD4 counts (<100 cells/μl) or severe illness [26]. Since then, detection of urinary LAM using the Determine TB LAM Ag test has been shown to be an independent predictor of mortality in patients with HIV-associated TB in sub-Saharan Africa [27]. A multicenter, randomized, controlled clinical trial showed that LAM testing and TB treatment can reduce mortality in hospitalized HIV-positive patients with advanced immunosuppression and severe illness [28].

On the horizon

Novel GeneXpert test for detection of resistance to isoniazid, fluoroquinolones, and aminoglycosides

Recent publications describe the development, analytical performance, and clinical diagnostic accuracy testing of a new GeneXpert cartridge (Cepheid Inc.) for genotypic detection of susceptibility/resistance to isoniazid, fluoroquinolones, and aminoglycosides directly from sputum [29,30▪]. Using DNA sequencing as the reference standard, sensitivities of the new assay for detecting mutations associated with resistance were 98.1% for isoniazid, 95.8% for fluoroquinolones, 92.7% for kanamycin, and 96.8% for amikacin, with specificity for all drugs 99.6% or greater [30▪]. With a run time of just over 2 h, suitability for testing of sputum, and compatibility with existing GeneXpert instruments (after a software upgrade and 10-color calibration), this assay holds promise as a rapid point-of-care test to guide therapeutic decisions for almost all patients with TB.

Mycobacterium tuberculosis DNA sequencing

M. tuberculosis DNA sequencing approaches increasingly are being used to guide clinical management, especially with respect to detection of genetic changes associated with drug resistance. Targeted approaches, in which only prespecified genetic regions are sequenced, are relatively simple but with the caveat that potentially important sequence information outside of the targeted region will be missed. Whole genome sequencing approaches can provide comprehensive genetic information about drug susceptibility/resistance and strain background, but several challenges must be overcome before whole genome sequencing approaches are rolled out more broadly for clinical care. First, the relevance of many genetic changes and combinations of genetic changes is unclear – several initiatives such as ReSeqTB and the TB Drug Resistance Mutation Database should facilitate a better understanding [31,32]. Second, whole genome sequencing engenders the need for robust data handling systems. A third technical challenge is the low bacillary burden present in many primary clinical specimens. It seems likely that these challenges will be met successfully and that comprehensive, rapid, direct sequencing will be used routinely in the future to guide management of patients with TB.

Point-of-care C-reactive protein for tuberculosis screening

WHO recommends systematic screening for TB in all PLHIV. A major implementation barrier is the absence of an inexpensive, rapid, point-of-care test that has sufficiently high sensitivity that people who screen negative have a low probability of active TB, and sufficiently high specificity that confirmatory testing is needed only for a small subgroup of high-risk persons. The WHO target product profile for such a screening test is sensitivity at least 90% and specificity at least 70% [33]. Symptom-based screening performs well below these targets in many relevant settings [34].

C-reactive protein (CRP) is an acute-phase reactant whose concentration in serum increases in response to inflammation and can be measured using cheap point-of-care assays on whole blood obtained by fingerprick. Yoon *et al.* [35▪] recently reported results from a prospective study conducted in Uganda to determine whether the iCHROMA (Boditech, Chuncheon, South Korea) point-of-care CRP assay met the minimum WHO target product profile for a TB screening test. Adults with HIV and CD4 of 350 cells/μl or less who were initiating combined antiretroviral therapy (ART) were enrolled, had CRP testing performed, and had two spot sputum specimens tested for *M. tuberculosis* using culture and Xpert MTB/RIF. Among 1177 participants (median CD4 165 cells/μl), CRP sensitivity was 89% (95% CI 83, 93) and specificity was 72% (95% CI 69, 75) [35▪]. Whether CRP performs similarly in other settings, and whether these target accuracy thresholds are useful in routine practice are open questions that are worthy of investigation.

Blood cell transcription profiling to estimate tuberculosis risk

Recent work by several groups has focused on identification of sets of human genes whose relative expression levels are associated with TB. One potential application of a blood gene expression signature is TB case detection. In 2010, Berry *et al.* [36] described a 393 transcript signature for active TB that, after TB treatment, reverted to that of healthy controls. They

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also described an 86-transcript signature that discriminated TB from other diseases and was dominated by neutrophil-driven interferon-inducible genes [36]. Another important potential application is prediction of risk of future progression to active TB, thereby allowing limited prevention resources to be targeted to individuals with the greatest risk for personal TB morbidity and transmission to others. Walzl and team have been systematically chipping away at the challenge of identifying transcriptional signatures that predict risk of progression. In a landmark 2016 article they described an unbiased screen of blood RNA profiles among a cohort of South African adolescents with latent TB infection who were followed for 2 years [37]. After identifying a signature through unbiased screens, they adapted the signature to targeted assays and reconfirmed the findings in the original cohort and two independent cohorts. They concluded that a 16-gene signature predicted risk of progression to TB in HIV-negative South African adolescents and in healthy household contacts from the South Africa and the Gambia. More recently, they refined this to a PCR-based 4-gene signature and demonstrated that there was little population-associated variability among healthy household contacts from South Africa, the Gambia, and Ethiopia [38▪]. An important caveat is that HIV-positive persons were excluded from most of these studies. Currently it is not clear whether the identified signatures are predictive of TB progression in PLHIV and to what extent predictive ability is influenced by HIV-associated immunosuppression and inflammatory dysregulation.

FUTURE DIRECTIONS

Exploiting advances in biosensor technology to detect novel and less novel *Mycobacterium tuberculosis* targets

Much progress in TB diagnostics over the past 2 decades has stemmed from advances in molecular genetics and genomics. Application of other methodologies including proteomics, lipidomics, and metabolomics hopefully will catalyze a next wave of novel TB diagnostics that move beyond nucleic acid targets. In considering the core components of any diagnostic test – target recognition, signal transduction, and analysis – it is clear that identification of appropriate *M. tuberculosis* targets has lagged behind the extraordinary progress in biosensor technology. However, some progress is being made in coupling *M. tuberculosis* targets with novel biosensing approaches.

Promising results from work with the Determine TB LAM Ag test have stimulated research to develop new urinary LAM detection strategies with improved sensitivity and specificity. Paris and colleagues recently reported on a promising approach that detects urinary LAM using hydrogel nanocages with incorporated copper-based dye that binds LAM with high affinity [39▪]. Importantly, this team addressed the existing barrier of scarce/poor quality antibodies against the complex carbohydrate target by using a chemical affinity bait. Among a small group of HIV-negative TB patients and controls, sensitivity was 96% and specificity was 81% and detection of urinary LAM was associated with mycobacterial burden. The assay requires further development along several lines before it could be applied in practice, but the published work demonstrates proof-of-principal and gives reason for optimism.

Building on progress in the field of biosensors, Bakhori *et al.* [40▪] recently described a plasmonic ELISA assay that detects antibody–antigen interactions. This assay exploits the enzymatic biocatalysis of catalase in the presence of hydrogen peroxide that is linked to the growth of gold nanoparticles, which produce red-colored or blue-colored solutions in the absence or presence of target, respectively. The tonality of the colored solutions can be detected by the unaided eye. The described work used as the target *M. tuberculosis* culture filtrate protein-10 (CFP-10), a secreted antigen against which mouse mAbs have been developed and are commercially available. The minimum concentration of CFP-10 that could be detected by the naked eye was 0.01 μg/ml. The assay was applied to 12 clinical sputum samples and results correlated with known TB status of those patients.

In a sophisticated riff off the century-old observation that the mycobacterial outer membrane binds and retains hydrophobic dyes (the basis of color – and fluorescent auramine – smear microscopy), Kamariza *et al.* [41▪] developed a trehalose dye conjugate that is metabolically incorporated into the mycobacterial outer membrane through the action of the mycobacterial acyl transferase Ag85 protein complex. Metabolic mycolylation of the dye and its subsequent integration into the outer membrane activate the dye's fluorescence and enable detection. This process is inhibited by anti-TB drugs and may have application for drug susceptibility testing. Proof of concept was demonstrated by applying the test to a small number of patient specimens.

CONCLUSION

Advances in technology and basic *M. tuberculosis* biology have catalyzed development of new diagnostic tests that are improving the TB diagnostic process and yield. That said, reliance of most of these new

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tests on respiratory specimens can limit their usefulness in PLHIV, in whom bacillary burden in sputum may be very low. Recent developments – including use of human gene expression signatures to predict risk, identification of novel *M. tuberculosis* targets and coupling them with novel ultrasensitive biosensor platforms, and progress in DNA sequencing – hold promise.

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Conflicts of interest

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The article describes the use of plasmonic ELISA for sensitive detection of *M. tuberculosis* with the naked eye.

41. ■ Kamariza M, Shieh P, Ealand CS, *et al.* Rapid detection of *Mycobacterium tuberculosis* in sputum with a solvatochromic trehalose probe. Sci Transl Med 2018; 10.

The article demonstrates proof-of-principle for an *M. tuberculosis* detection approach based on a trehalose probe whose fluorescence signal is specifically activated by metabolic incorporation into the mycobacterial outer membrane.

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