New Diagnostics for Latent and Active Tuberculosis: State of the Art and Future Prospects

Madhukar Pai, M.D., Ph.D.¹ and Richard O’Brien, M.D.²

ABSTRACT

Tuberculosis (TB) continues to be the world’s most important infectious cause of morbidity and mortality among adults. Nearly 9 million people develop TB disease each year, and an estimated 1.6 million die from the disease. Despite this enormous global burden, case detection rates are low, posing serious hurdles for TB control. Conventional TB diagnosis continues to rely on antiquated tests such as sputum smear microscopy, culture, tuberculin skin test, and chest radiography. These tests have several limitations and perform poorly in populations affected by the HIV epidemic. Conventional tests for detection of drug resistance are time consuming, tedious, and inaccessible in most settings. In this review, we describe recent advances in the diagnosis of latent and active TB, and detection of drug resistance. Although the perfect test will not be ready for large-scale rollout and integration into routine TB care services for some time, substantial progress has been made in expanding the TB diagnostic product pipeline. With the resurgence of interest in the development of new tools for TB control, and the recent influx of funding and political support, it is likely that the next few years will see the introduction of new diagnostic tools into routine TB control programs.

KEYWORDS: Tuberculosis, diagnosis, new tools, sensitivity, specificity

Despite the enormous global burden of tuberculosis (TB), case detection continues to be a problem.¹ It is estimated that approximately half of all patients with TB are still not diagnosed and appropriately treated. Each year, ~1.6 million deaths occur due to TB. The problem is significantly worsened by HIV infection and the increasing prevalence of multidrug resistant (MDR) and extensively drug resistant (XDR) TB.

Conventional TB diagnosis continues to rely on sputum smear microscopy, culture, tuberculin skin test, and chest radiography. These tests have been used for nearly a century and have several limitations. Existing tools may be insufficient to control TB in developing countries, especially in countries ravaged by the HIV epidemic.² Hence there is an urgent need for newer or improved methods for diagnosis to accelerate the fight against TB and to meet the target of elimination by 2050. A recent modeling study showed that a rapid and universally accessible test that is not affected by HIV status, with a sensitivity of 85% for smear-positive and smear-negative cases, and a specificity of 97% can save an estimated 392,000 adjusted lives annually, or 22% of the global TB deaths.³

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The involvement of agencies such as the Stop TB Partnership’s Working Group on New Diagnostics, the World Health Organization (WHO), the Special Program for Research and Training in Tropical Diseases (TDR), and the Foundation for Innovative New Diagnostics (FIND) has led to a resurgence of interest in the development of new TB diagnostics. Indeed, the development of new tools is a key component of the Global Plan to Stop TB, 2006–2015, the new global Stop TB Strategy. With the anticipated launch of new tools within the next few years, the Stop TB Partnership has recently established a Retooling Task Force to develop a framework for catalyzing policy makers and practitioners at global and national levels toward accelerated introduction of new tools into TB control programs.

In the past few years, the TB diagnostics pipeline has rapidly expanded. The current pipeline of products, together with their current stage of development, has been comprehensively described by Perkins and Cunningham (Table 1). Figure 1 illustrates the types of technology platforms that are currently in the pipeline. The technologies range from simple microscopic and growth detection systems to sophisticated molecular and immune-based systems. In this review, we describe recent advances in latent and active TB diagnosis, and progress made in the development of new tools for the diagnosis of MDR-TB.

### ADVANCES IN LATENT TB DIAGNOSIS

#### Interferon-Gamma Release Assays

Until recently, the diagnosis of latent TB infection (LTBI) depended solely on the tuberculin skin test (TST), an imperfect test with known limitations. The biggest advance in recent times has been the development of T cell–based interferon-gamma release assays (IGRAs). IGRAs are in vitro blood tests that are based on interferon-gamma release after stimulation by TB–specific antigens (e.g., ESAT-6 and CFP-10). Two IGRAs are now commercially available—the QuantiFERON-TB Gold In-Tube Assay (Cellestis Ltd, Carnegie, Victoria, Australia), and the T-SPOT.TB assay (Oxford Immunotec Limited, Abingdon, Oxon, UK).

As reviewed recently, IGRAs have very high specificity and are unaffected by prior bacille Calmette-Guérin (BCG) vaccination or sensitization to nontuberculous mycobacteria. The sensitivity of IGRAs in active TB is ~75% to 90%, and there are data that suggest that IGRAs may be more sensitive than the TST in immunocompromised populations. Table 2 is a comparison of the performance and operational characteristics of the TST and IGRAs.

Overall, IGRAs are useful tests for the detection of LTBI, and their use is rapidly expanding in low-incidence countries. However, IGRAs cannot distinguish between latent and active TB. Furthermore, data are lacking in children and HIV-infected and immunocompromised populations, and there are limited data on the ability of IGRAs to predict future development of TB disease. Although IGRAs cannot replace conventional tests for active TB, they may have some potential to assist in the diagnosis of active TB in some settings, among selected populations such as young children, immunocompromised persons, and individuals with smear-negative and extrapulmonary disease. In these populations, microbiological diagnosis is often hard to establish, and IGRAs may offer supporting evidence to help establish a diagnosis of active TB.

The scope and role of IGRAs in developing countries remain unclear. Several field evaluation projects are ongoing, and these studies should provide strong evidence for making policies for high-burden countries.

#### A Specific Tuberculin Skin Test

A known limitation of the conventional TST is the lack of specificity of the purified protein derivative (PPD), a crude antigen mixture. An obvious solution to this problem is to replace PPD with antigens that are specific to *Mycobacterium tuberculosis*. This is the principle underlying an improved, specific skin test developed by the Statens Serum Institut, Copenhagen, Denmark. A recent randomized phase I clinical trial evaluated the safety of intradermal recombinant dimer ESAT-6 (rdESAT-6) compared with PPD and to determine the optimum dose range.

No serious adverse events were observed but the study was not sufficiently powered to demonstrate complete safety. Further work is ongoing to improve this novel skin test and to include other specific TB antigens such as CFP-10.

### ADVANCES IN ACTIVE TB AND MDR/XDR-TB DIAGNOSIS

#### Improved (Optimized) Smear Microscopy

Although much work is being done to develop new diagnostics, in most resource-limited countries, sputum smear microscopy remains the primary means for bacteriologic diagnosis of TB. Given the known limitations of smear microscopy, considerable research has been done to identify methods that can increase the sensitivity and optimize the yield of smear microscopy. A series of recent systematic reviews has shown that microscopy can be optimized using at least three different approaches: chemical and physical processing...
<table>
<thead>
<tr>
<th>Technology, Test</th>
<th>Stage of Development</th>
<th>Developer(s)/Supplier(s)</th>
<th>Level of the Health System</th>
<th>DST Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASE DETECTION</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Growth-based detection</td>
<td>Commercialized reagents and prepared media</td>
<td>Multiple</td>
<td>Referral</td>
<td>Y</td>
</tr>
<tr>
<td>Conventional solid media</td>
<td>Commercialized, under study for feasibility and impact of use in resource-limited settings</td>
<td>BD, bioMérieux, Trek</td>
<td>Referral</td>
<td>Y</td>
</tr>
<tr>
<td>Automated liquid culture systems</td>
<td>In evaluation</td>
<td>Salubris</td>
<td>Referral</td>
<td>Y</td>
</tr>
<tr>
<td>TK colorimetric media</td>
<td>Academic evaluations published</td>
<td>Noncommercial testing methods</td>
<td>Referral</td>
<td>Y</td>
</tr>
<tr>
<td>MODS assay, thin-layer culture, others</td>
<td>Commercialized, improved test in development</td>
<td>Biotec</td>
<td>Referral</td>
<td>Y</td>
</tr>
<tr>
<td>Phage-based detection</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DIRECT VISUALIZATION</td>
<td></td>
<td></td>
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<tr>
<td>Conventional microscopy with acid-fast staining</td>
<td>In routine use</td>
<td>Multiple</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>Fluorescent microscopy with nonspecific cell-wall staining</td>
<td>In routine use</td>
<td>Multiple</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>Fluorescent microscopy with LED light source</td>
<td>In development</td>
<td>Various</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>Fluorescent microscopy with molecular probes (FISH)</td>
<td>In development</td>
<td>ID-FISH Technology</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>Automated microscopy</td>
<td>In development</td>
<td>Various</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>Computer-assisted microscopy</td>
<td>In development</td>
<td>Various</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>VOC DETECTION</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electronic nose analysis of headspace gas</td>
<td>In development</td>
<td>Scensitive</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>GC/MS analysis of exhaled air</td>
<td>In development</td>
<td>Menssana Research</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>Handheld surface acoustic wave-GC</td>
<td>In development</td>
<td>Electronic Sensor Technology</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>Giant African pouch rats</td>
<td>In evaluation</td>
<td>Apopo</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>Honeybees</td>
<td>In development</td>
<td>Inscentinel</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>Antigen detection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB-derived antigen detection in urine or other clinical material</td>
<td>In development</td>
<td>Chemogen, Proteome Systems, TB DiaDirect, others</td>
<td>Health center</td>
<td>N</td>
</tr>
<tr>
<td>TB-derived antigen detection in exhaled air vapor</td>
<td>In evaluation</td>
<td>Rapid Biosensor Systems</td>
<td>Health center</td>
<td>N</td>
</tr>
<tr>
<td>ANTIBODY DETECTION</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detection of diagnostic antibody responses to TB</td>
<td>Many commercially available, improved tests in development</td>
<td>Various</td>
<td>Health center</td>
<td>N</td>
</tr>
<tr>
<td>MOLECULAR DETECTION</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Automated, nonintegrated NAAT</td>
<td>Commercialized</td>
<td>GenProbe, Roche, BD, others</td>
<td>Reference</td>
<td>N</td>
</tr>
<tr>
<td>Automated, integrated NAAT</td>
<td>In development</td>
<td>Cepheid</td>
<td>Referral</td>
<td>Y</td>
</tr>
<tr>
<td>Simplified manual NAAT (LAMP)</td>
<td>In development</td>
<td>Eiken</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>Nonamplified probe detection</td>
<td>In development</td>
<td>Investigen, others</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>Transrenal DNA detection</td>
<td>In development</td>
<td>Xenomics, others</td>
<td>Referral or microscopy</td>
<td>N</td>
</tr>
<tr>
<td>Manual amplification and hybridization</td>
<td>In evaluation</td>
<td>Innogenetics, Hain</td>
<td>Reference</td>
<td>N</td>
</tr>
</tbody>
</table>
(e.g., bleach) and concentration of sputum (e.g., centrifugation), fluorescence microscopy, and the examination of two (not three) sputum specimens. The World Health Organization (WHO) recently revised its policies on smear microscopy. It now recommends the number of specimens to be examined for screening of TB cases can be reduced from three to two, in places where a well-functioning external quality assurance

### Table 1 (Continued)

<table>
<thead>
<tr>
<th>Technology, Test</th>
<th>Stage of Development</th>
<th>Developer(s)/Supplier(s)</th>
<th>Level of the Health Systema</th>
<th>DST Utilityb</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPECIES IDENTIFICATIONd</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminous probe of culture isolate</td>
<td>Commercially available</td>
<td>GenProbe</td>
<td>Reference</td>
<td>N</td>
</tr>
<tr>
<td>Fluorescent probe of smear-positive sputum</td>
<td>In development</td>
<td>ID-FISH</td>
<td>Referral</td>
<td>N</td>
</tr>
<tr>
<td>Reverse hybridization line probe from culture isolates</td>
<td>Commercially available</td>
<td>Innogenetics, Hain</td>
<td>Reference</td>
<td>N</td>
</tr>
<tr>
<td>Dipstick detection of TB antigens in positive cultures</td>
<td>In demonstratione</td>
<td>Tauns</td>
<td>Referral</td>
<td>N</td>
</tr>
<tr>
<td>Species-specific amplification or sequencing</td>
<td>Research use</td>
<td>Various</td>
<td>Reference</td>
<td>N</td>
</tr>
<tr>
<td>LTBI DETECTION</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculin skin test with PPD</td>
<td>Commercialized</td>
<td>Multiple</td>
<td>Microscopy</td>
<td>N</td>
</tr>
<tr>
<td>MPT-64 skin patch</td>
<td>In evaluation</td>
<td>Sequella</td>
<td>Health center</td>
<td>N</td>
</tr>
<tr>
<td>Whole-blood IFN-γ release assay</td>
<td>Commercialized; in evaluation for disease-endemic countries</td>
<td>Cellestis</td>
<td>Referral</td>
<td>N</td>
</tr>
<tr>
<td>ELISPOT IFN-γ release assay</td>
<td>Commercialized; in evaluation for disease-endemic countries</td>
<td>Oxford Immunotech</td>
<td>Referral</td>
<td>N</td>
</tr>
<tr>
<td>Skin testing with TB-specific antigens</td>
<td>In early evaluation</td>
<td>Statens Serum Institut</td>
<td>Microscopy</td>
<td>N</td>
</tr>
</tbody>
</table>

aThe health care system is divided here for conversion into four levels: reference laboratory, a national or regional laboratory performing specialty mycobacterial tests, not focused on patient care; referral laboratory, a laboratory with TB-specific expertise performing such tests as TB culture; microscopy laboratory, a laboratory performing only microscopy for TB detection; and health center, a clinical facility not routinely providing any mycobacteriology testing. Listed in the table is the level of intended or appropriate use.

bIndicates that methodology may also be used to detect drug resistance.

cLo¨ wenstein-Jensen, Ogawa, 7H10, and other media.

dBeyond NAATs with species-specific primer sequences.

eDemonstration is a phase in FIND’s development pathway, coming after evaluation, in which the feasibility and impact of programmatic use are measured.

DST, drug susceptibility testing; ELISPOT, enzyme-linked immunospot; FISH, fluorescence in situ hybridization; GC, gas chromatography; IFN, interferon; LAMP, loop-mediated, isothermal amplification; LED, light-emitting diode; LTBI, latent TB infection; MODS, microscopic-observation drug susceptibility assay; MS, mass spectrometry; NAAT, nucleic acid amplification testing; PPD, purified protein derivative; VOC, volatile organic compound.

From Perkins and Cunningham, reproduced with permission.

![Figure 1](https://via.placeholder.com/150)

**Figure 1** Types of technology platforms that are currently in the tuberculosis diagnostics pipeline. ESAT-6, early secreted antigen target 6; IFN-γ, interferon-gamma; LAMP, loop-mediated, isothermal amplification; LED, light-emitting diode; MODS, microscopic-observation drug susceptibility assay; VOC, volatile organic compounds.
system exists, where the workload is very high and human resources are limited.\textsuperscript{19}

Given the enhanced sensitivity of fluorescence microscopy (FM), work is also ongoing to develop simpler, field-friendly FM technology. Recently, it has been shown that low-cost ultrabright light-emitting diodes (LEDs) with a long lifespan could be a good replacement of expensive lamps used in conventional FM. Recent, emerging data suggest that LEDs show good excitation of auramine and other fluorescent dyes.\textsuperscript{20}

Table 2 A Comparison of Tuberculin Skin Test with Interferon-Gamma Release Assays

<table>
<thead>
<tr>
<th>Performance and Operational Characteristics</th>
<th>TST</th>
<th>QFT-Gold</th>
<th>T-SPOT.TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated sensitivity (in patients with active TB)</td>
<td>70–90%</td>
<td>75–80%</td>
<td>90–95%</td>
</tr>
<tr>
<td>Sensitivity in immunocompromised individuals</td>
<td>Low (due to anergy)</td>
<td>Limited data in immunocompromised populations; higher chance of indeterminate result in those with low CD4 + counts</td>
<td>Sensitivity retained in immunocompromised individuals</td>
</tr>
<tr>
<td>Estimated specificity (in healthy persons with no known TB disease/exposure)</td>
<td>50–95% (variable; affected by BCG: lower when BCG given after infancy)</td>
<td>95–100% (not affected by BCG vaccination)</td>
<td>90–100% (not affected by BCG vaccination)</td>
</tr>
<tr>
<td>Cross-reactivity with BCG</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cross-reactivity with nontuberculous mycobacteria</td>
<td>Yes</td>
<td>Less likely</td>
<td>Less likely</td>
</tr>
<tr>
<td>Association between test-positivity and subsequent risk of active TB during follow-up</td>
<td>Moderate to strong positive association</td>
<td>Insufficient evidence</td>
<td>Insufficient evidence</td>
</tr>
<tr>
<td>Correlation with Mycobacterium tuberculosis exposure</td>
<td>Yes</td>
<td>Yes (correlated better with exposure than TST in some head-to-head comparisons)</td>
<td>Yes (correlated better with exposure than TST in some head-to-head comparisons)</td>
</tr>
<tr>
<td>Benefits of treating test-positives (based on randomized, controlled trials)</td>
<td>Yes</td>
<td>No evidence</td>
<td>No evidence</td>
</tr>
<tr>
<td>Reliability (reproducibility)</td>
<td>Moderate</td>
<td>Limited evidence but may be high</td>
<td>Limited evidence but may be high</td>
</tr>
<tr>
<td>Interreader variability</td>
<td>Yes</td>
<td>No</td>
<td>Variation in counting spots (if done manually)</td>
</tr>
<tr>
<td>Boosting phenomenon</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Potential for conversions and reversions</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Patient visits to complete testing protocol</td>
<td>Two</td>
<td>One</td>
<td>One</td>
</tr>
<tr>
<td>Material costs</td>
<td>Low</td>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>Sample processing and assay complexity</td>
<td>NA</td>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>Laboratory infrastructure required</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Time to obtain a result</td>
<td>48–72 hours</td>
<td>24–48 hours longer if assays batched</td>
<td>24–48 hours longer if assays batched</td>
</tr>
<tr>
<td>Trained personnel required</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

BCG, bacille Calmette-Guérin; IGRA, interferon-gamma release assay; TST, tuberculin skin test. Adapted from references 8,10,11,46.
conventional FM equipment, opening the door for wide-
spread use of LED-based FM in developing countries.

In view of these potential advantages, FIND has
partnered with Carl Zeiss MicroImaging GmbH
(Oberkochen, Germany) to develop an inexpensive,
robust LED microscope for routine use in high-burden
countries. The microscope is configured for both
white light and fluorescence applications and will allow
switching between the two. Low power consumption
will permit operation with a rechargeable battery built
into the system. White light applications, using a
mirror in settings of no electrical power, will also be
possible. Preliminary data suggest that LED micro-
scopy is feasible and slightly more sensitive than stan-
dard FM. Field evaluation projects are currently
ongoing in several countries.

Automated Liquid Cultures
Although mycobacterial culture is highly sensitive, it is
not widely available in most developing countries. Also,
solid culture [e.g., Löwenstein–Jensen (LJ)] is slow and
results are often not available for weeks. Expanding
culture capacity is urgently needed to address challenges
posed by the epidemics of HIV-associated TB and drug
resistant TB, especially in resource-limited settings.
Liquid culture systems such as BACTEC and MGIT
(Becton Dickinson, Sparks, MD) reduce the delays in
obtaining results to days rather than weeks. For drug
susceptibility testing (DST), the delay may be reduced to
as little as 10 days, compared with 28 to 42 days with
conventional solid media. Liquid systems are more
sensitive for detection of mycobacteria and may increase
the case yield by 10% over solid media. With increased
sensitivity and reduced delays, liquid systems may con-
tribute significantly to improved patient management.
Liquid systems are, however, more prone to contami-
nation. In addition, they are expensive and require
expertise and infrastructure.

Recently, the WHO released a policy statement
on the use of liquid culture systems. The WHO policy
recommends phased implementation of these systems as
part of a country-specific comprehensive plan for lab-
oratory capacity strengthening, and addresses key issues,
including biosafety, customer support, staff training,
maintenance of infrastructure and equipment, specimen
transport, and reporting of results.

MODS
The microscopic-observation drug-susceptibility
(MODS) assay for the detection of TB and MDR-TB,
directly from sputum, relies on 3 principles: 1) M.
tuberculosis grows faster in liquid medium than in solid
medium; 2) characteristic cord formation can be visual-
ized microscopically (“strings and tangles” appearance
in liquid medium at an early stage; and 3) the incorpo-
ration of drugs permits rapid and direct DST concom-
itantly with the detection of bacterial growth. MODS
uses an inverted light microscope and Middlebrook 7H9
broth culture to rapidly detect early growth of M. tuber-
culosis of bacterial cells in the broth medium with or
without antimicrobial drugs (for DST). In a recent large
study in Peru, sensitivity of detection was 97.8% for
MODS culture, 89.0% for automated mycobacterial
culture, and 84.0% for LJ culture ($p < 0.001$); the median
time to culture positivity was 7 days, 13 days, and
26 days, respectively ($p < 0.001$), and the median time
to the results of susceptibility tests was 7 days, 22 days,
and 68 days, respectively. Thus MODS has excellent
accuracy in field conditions and requires only an inverted
light microscope. The cost of MODS culture has
been estimated to be substantially lower than the cost
of LJ culture and DST and that of automated myco-
bacterial culture. However, labor costs may be higher for
MODS culture.

Nucleic Acid Amplification Tests
Nucleic acid amplification tests (NAATs) have been
evaluated for TB diagnosis and detection of drug resist-
ance for nearly 2 decades. Several meta-analyses have
shown that NAATs have high specificity for TB diag-
nosis, but modest and variable sensitivity, particularly in
smear-negative TB and extrapulmonary disease. Also,
they are expensive and require special infrastruc-
ture and expertise. Considerable effort has been made to
develop advanced versions of NAATs with better per-
formance and operating characteristics. These include
assays such as loop-mediated isothermal amplification
(LAMP), Xpert MTB (Cepheid, Sunnyvale, California,
USA), and the GenoType MTBDRplus assay (Hain
Lifescience GmbH, Nehren, Germany).

Recently, LAMP was developed by scientists at
Eiken Chemical Co. in Japan, which has characteristics
that could enable the development of a simple, rapid, and
sensitive detection test for TB and potentially other
diseases. FIND and Eiken have developed a TB
detection assay that pairs simple manual specimen proc-
essing steps with LAMP, an isothermal amplification
system that generates large amounts of target DNA in
real time and results in visible amplification readout,
with no need for instrumentation, probing, or opening
the reaction tube to determine the result. Preliminary
data on a multicenter evaluation of a first version of this
assay were published in 2007 and showed the feasibility
of performing the assay without sophisticated training or
equipment. In that study, LAMP significantly outper-
formed microscopy. Sensitivity of LAMP in smear-
and culture-positive sputum specimens was 97.7%; specificity
in culture-negative samples was 99%. The current
version of the assay requires no equipment other than
a heat block and utilizes reagents that are stable without a cold chain. Further refinements in the LAMP assay have been made, and further evaluation and field demonstration projects are under way.

Existing NAATs for TB are complicated because of the need for three separate steps for (1) specimen processing and nucleic acid extraction, (2) nucleic acid amplification, and (3) detection of amplified products. By combining these three steps into a single, automated process, the GeneXpert microfluidic and molecular testing platform developed by Cepheid, Inc. (Sunnyvale, CA) attempts to overcome these known limitations. A specially designed cartridge allows all specimen processing steps to be performed in an automated manner by a single device that also performs real-time polymerase chain reaction (PCR) and sequence-specific detection of target amplicons. The Xpert MTB test being developed uses molecular beacons and six-color fluorescence detection for real-time identification of both \textit{M. tuberculosis} and rifampin resistance in less than 120 minutes. Feasibility studies performed in Peru and Latvia have confirmed the assay’s ease of use and its utility for desktop detection of MDR-TB (Catharina Boehme, FIND, pers. comm.). Larger field trials are under way.

With the emergence of XDR-TB, considerable attention has been focused on the rapid diagnosis of drug resistance. Molecular assays detect gene mutations that identify drug resistance. Among the molecular assays, line probe assays have shown great promise. Line-probe assays are a family of novel DNA strip–based tests that use PCR and reverse hybridization methods for the rapid detection of mutations associated with drug resistance. Commercially available line-probe assays include the INNO-LiPA Rif. TB kit (Innogenetics NV, Gent, Belgium) and the GenoType MTBDRplus assay (Hain Lifescience GmbH, Nehren, Germany). A recent laboratory evaluation study from South Africa estimated the accuracy of the GenoType MTBDRplus assay performed directly on AFB smear-positive sputum specimens. Compared with conventional DST, the sensitivity, specificity, positive, and negative predictive values were 98.9%, 99.4%, 97.9%, and 99.7% for detection of rifampin resistance; 94.2%, 99.7%, 99.1%, and 97.9% for detection of isoniazid resistance; and 98.8%, 100%, 100%, and 99.7% for detection of multidrug resistance compared with conventional results.

With overall performance characteristics that are superior to conventional culture and DST and the possibility for high throughput with substantial cost savings, these molecular tests may revolutionize MDR-TB diagnosis.

In June 2008, WHO announced a new policy statement endorsing the use of line probe assays for rapid screening of patients at risk of MDR-TB. The recommended use of line probe assays is currently limited to culture isolates and direct testing of smear-positive sputum specimens. Line probe assays are not recommended as a complete replacement for conventional culture and drug susceptibility testing.

However, the diagnosis of XDR-TB continues to be a challenge, especially because it requires additional data on resistance to several second-line drugs. Second-line drug resistance testing is poorly standardized, and gene mutations for second-line drugs are not well recognized.

### Antibody Detection (Serological) Tests

Although no guideline recommends the use of serological (i.e., antibody-detection) tests, dozens of commercial kits are sold, mostly in developing countries. Two recent systematic reviews have synthesized the available evidence on serological tests for TB. The evidence suggests that, at this point in time, published data on commercial serological tests for both pulmonary and extrapulmonary TB produce inconsistent estimates of sensitivity and specificity. For pulmonary TB, there was insufficient evidence to determine the accuracy of most commercial tests in smear microscopy—negative patients and none of the assays performed well enough to replace smear microscopy. For extrapulmonary TB, there were no studies of commercial tests of sufficient quality to enable their evaluation in patients with HIV infection or in children because the tests could be potentially helpful in these groups. Thus, at the present time, serological tests have little or no role to play in the diagnosis of TB.

Work is under way to develop improved versions of serological tests. Based on all the previous research, it seems unlikely that a single antigen will accurately detect TB. Rather, it is expected that several antigens, when used in combination, might generate an antibody profile or pattern that could be used to detect TB. This has been attempted with nonhuman primates with some success. If successful, this approach could pave the way for a point-of-care (POC) product in a strip test format for detection of specific anti-TB antibodies in whole blood (or other clinical specimens) with performance adequate for use at the primary care level. At this stage, it is unclear if such POC tests can be successfully developed for clinical use, especially in populations where TB/HIV coinfection is a concern. However, a better understanding of TB-specific biomarkers, and the application of new approaches such as metabolomics, genomics, and proteomics may facilitate the development of the much needed rapid POC test.

### Antigen Detection Tests

The detection of TB antigens is an exciting new development. Unlike antibody detection, antigen detection offers the possibility of high specificity and correlation with bacterial burden. Although antibody
Detection assays are significantly impaired by immunosuppression, antigen detection assays should not be adversely impacted. Promising early attempts to detect *M. tuberculosis* antigens in sputum, serum, and urine have been reported. For example, lipoarabinomannan (LAM), a heat-stable glycolipid, has been found in the urine of 75 to 80% of culture-confirmed TB cases in studies from Tanzania and Ethiopia.\(^41,42\) Based on these early data, a prototype urinary LAM detection test was produced by Chemogen, Inc. (Portland, ME). A commercial version of this test is now marketed as the Clearview TB ELISA (Inverness Medical Innovations, Inc., Scarborough, ME). Large-scale field demonstration projects are currently ongoing.

**Other Technologies under Development or Evaluation**

In addition to the tests already described, there are several other technologies being developed or evaluated for TB, including detection of volatile organic compounds in the breath, electronic nose analysis of exhaled air, bacteriophage-based systems, skin patch test, colorimetric rapid culture systems, transrenal DNA detection, thin-layer cultures, and molecular beacons. These technologies have been reviewed elsewhere.\(^2,8,9,43–46\)

**CONCLUSIONS**

After decades of little or no growth, the TB diagnostics pipeline has rapidly grown, with several technologies showing great promise. Clearly, this is evidence that good progress has been made in the development of new tests for TB. With the resurgence of interest in the development of new tools for TB control, and the recent influx of funding and political support, it is likely that the next few years will see the introduction of new tools into routine TB control programs. It is, however, important to ensure that policies on TB diagnostics are evidence-based.\(^45\) Improvement and dissemination of new tools should have a profound effect on TB control throughout the world.

**CONFLICTS OF INTEREST**

The authors have no financial conflicts of interest. ROB works for Foundation for Innovative New Diagnostics (FIND), a nonprofit agency that collaborates with several industry partners for the development of new diagnostics for neglected infectious diseases. No industry partner was involved in the preparation of this manuscript.

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