Rapid Diagnosis of *Mycobacterium tuberculosis* Infection and Drug Susceptibility Testing

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• ***Context.***—**The global control of tuberculosis remains a challenge from the standpoint of diagnosis, detection of drug resistance, and treatment. This is an area of special concern to the health of women and children, particularly in regions of the world with high infant mortality rates and where women have limited access to health care.**

Objective.—**Because treatment can only be initiated when infection is detected, and is guided by the results of antimicrobial susceptibility testing, there recently has been a marked increase in the development and testing of novel assays designed to detect *Mycobacterium tuberculosis* complex, with or without simultaneous detection of resistance to isoniazid and/or rifampin. Both nonmolecular and molecular assays have been developed. This review will summarize the current knowledge about the use of rapid tests to detect *M tuberculosis* and drug resistance.**

Data Sources.—**Review of the most recent World Health Organization Global Tuberculosis Report, as well as selected publications in the primary research literature, meta-analyses, and review articles.**

Conclusions.—**To a large extent, nonmolecular methods are refinements or modifications of conventional methods, with the primary goal of providing more rapid test results. In contrast, molecular methods use novel technologies to detect the presence of *M tuberculosis* complex and genes conferring drug resistance. Evaluations of molecular assays have generally shown that these assays are of variable sensitivity for detecting the presence of *M tuberculosis* complex, and in particular are insensitive when used with smear-negative specimens. As a group, molecular assays have been shown to be of high sensitivity for detecting resistance to rifampin, but of variable sensitivity for detecting resistance to isoniazid.**

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Infections caused by *Mycobacterium tuberculosis* complex remain one of the most important global public health issues: there were 14 million prevalent and 9.4 million incident cases of tuberculosis (TB) in 2009, causing 1.7 million deaths[1] (Figure 1). Data from the same year indicate that "women account for an estimated 3.3 million cases (range, 3.1 million–3.5 million), equivalent to 35% of all cases."[1] Of the total cases, 1.1 million cases and 380 000 deaths occurred in persons infected with human immunodeficiency virus[1] (Figure 2). During 2008, there were an estimated 440 000 cases of multidrug-resistant TB (MDR-TB), or 3.3% of all new cases of TB, resulting in 150 000 deaths.[1] The highest rates of MDR-TB occur in 27 "high-burden" countries and regions, 15 of which are in the European region, with the highest incidence rates in China, Russia, India, and South Africa.[1] Extensively drug-resistant TB has now been confirmed in 58 countries.[1] Estimated TB incidence rates are highest in sub-Saharan Africa and in Southeast Asia, areas that also have high human immunodeficiency virus infection rates as well as inadequate access to health care in many areas. Recent diagnosis and treatment programs have been promising, as reported in the World Health Organization (WHO) 2010 annual report: "From 1995 to 2005, 49 million patients were treated. . .saving up to 6 million lives. This includes approximately 2 million lives saved among women and children. From 2010 to 2015, a further 5 million lives could be saved if current efforts and levels of achievement in TB control are sustained, including around 2 million women and children."[1]

In order for global TB control programs to be effective, particularly in these regions, improved diagnostic methods are needed. It is estimated, however, that the global case detection rate for TB is only 63%.[1] Access to improved TB diagnostics is of particular importance in areas where patients have infrequent or intermittent access to health care, sites where providers are not able to wait for results from reference laboratories before either withholding or initiating antituberculous therapy.

Despite this need for better diagnostic tests, until recently there has been little emphasis on developing new tests for the diagnosis of TB. From a global perspective, too many hospitals and clinics rely solely on sputum smears to make the diagnosis of TB without the ability to perform cultures or perform antimicrobial susceptibility testing (AST). Moreover, many laboratories use the same methods today that

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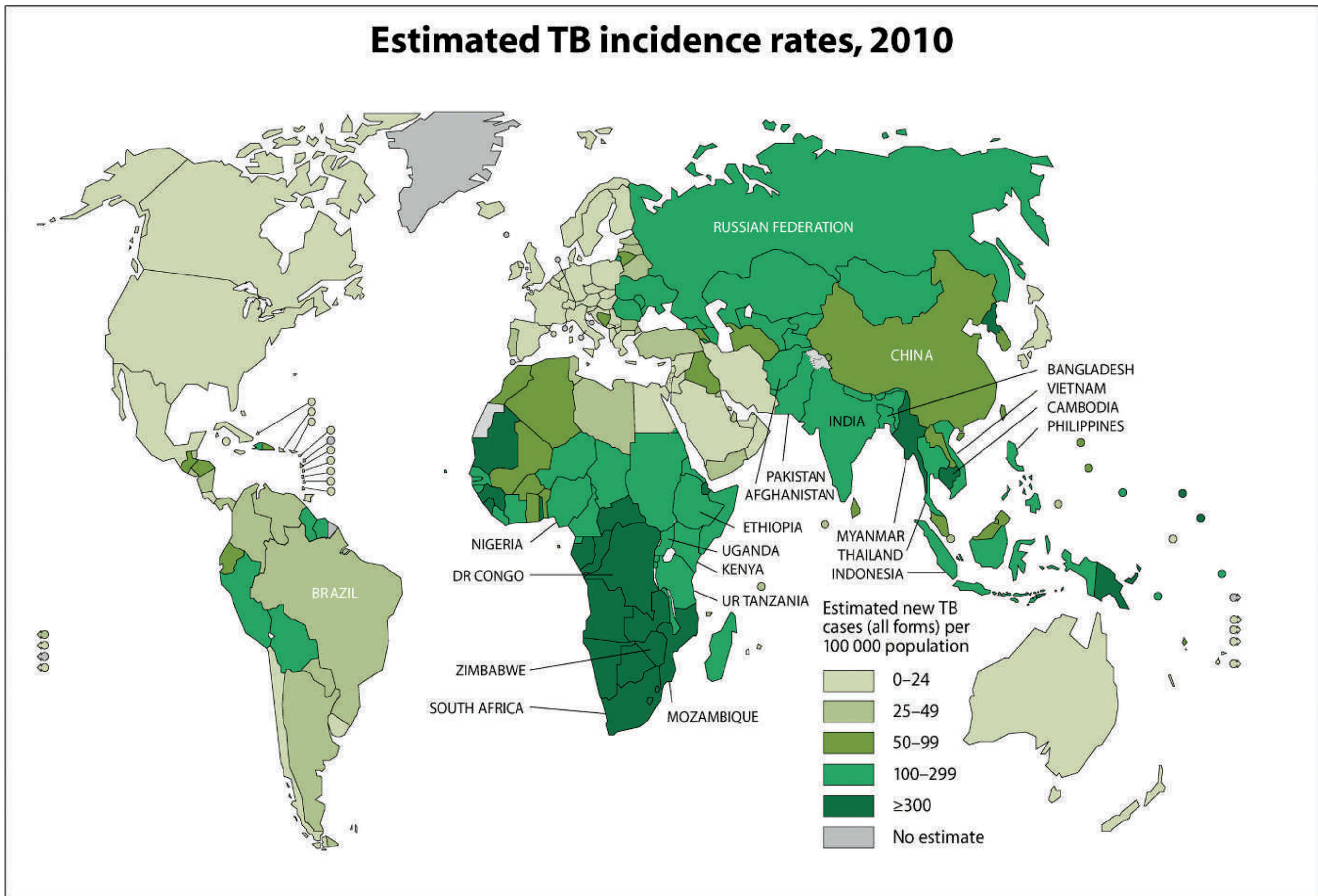

Figure 1. *Estimated tuberculosis incidence rates, 2010. Source: WHO Global Tuberculosis Control report 2011. Reprinted with permission from the World Health Organization.*

were in use a half century ago: conventional stains such as Ziehl-Neelsen or Kinyoun for staining sputum smears, egg-based solid media for culture, and solid media for AST. Although it is now more common for laboratories to use fluorochrome stains to stain smears and liquid-based media for cultures, these methods are not widely used in small hospitals or clinics because of the need for greater technical expertise and laboratory infrastructure. Far too many laboratories around the world do not even have access to these methods. Antimicrobial susceptibility testing is even more problematic, because it is difficult to perform well, the turnaround time is often measured in months, some drugs often show discordant results (particularly ethambutol), and AST for second-line drugs remains poorly standardized and not widely available. Thus, there is a pressing need for new methods that will allow both for the rapid detection of TB in patients and for AST to identify patients who are infected with resistant strains.

The WHO program called the Stop TB Strategy is based on a number of specific goals and objectives (Table 1).[1] As part of this strategy, 1 of the 6 key components is to "contribute to health systems strengthening based on primary health care," a component of which is to "upgrade laboratory networks." [1] More emphatically, the WHO report states, "One of the most important constraints to rapid expansion of diagnostic and treatment services for MDR-TB is laboratory capacity. Without greater capacity to diagnose MDR-TB, the number of cases diagnosed and treated will remain low. Diagnostic testing for drug susceptibility, or DST, among new cases of TB remains almost entirely confined to the European Region and the Region of the Americas." [1] A number of organizations have collaborated to develop, test, and implement new tests, including WHO, the Foundation for Innovative New Diagnostics, and the Centers for Disease Control and Prevention. These efforts are ongoing with the results of evaluations of some products already published. This review will summarize the current knowledge regarding new diagnostic tests for the detection of *M tuberculosis* complex in respiratory specimens, with or

Table 1. Six Components of the World Health Organization Stop TB Strategy
Pursue high-quality DOTS expansion and enhancement
Address TB-HIV, MDR-TB, and the needs of poor and vulnerable populations
Contribute to health system strengthening based on primary health care
Engage all care providers
Empower people with TB and communities through partnership
Enable and promote research

Abbreviations: DOTS, directly observed therapy, short [course]; HIV, human immunodeficiency virus; MDR-TB, multidrug-resistant tuberculosis; TB, tuberculosis; TB-HIV, tuberculosis human immunodeficiency virus coinfection.

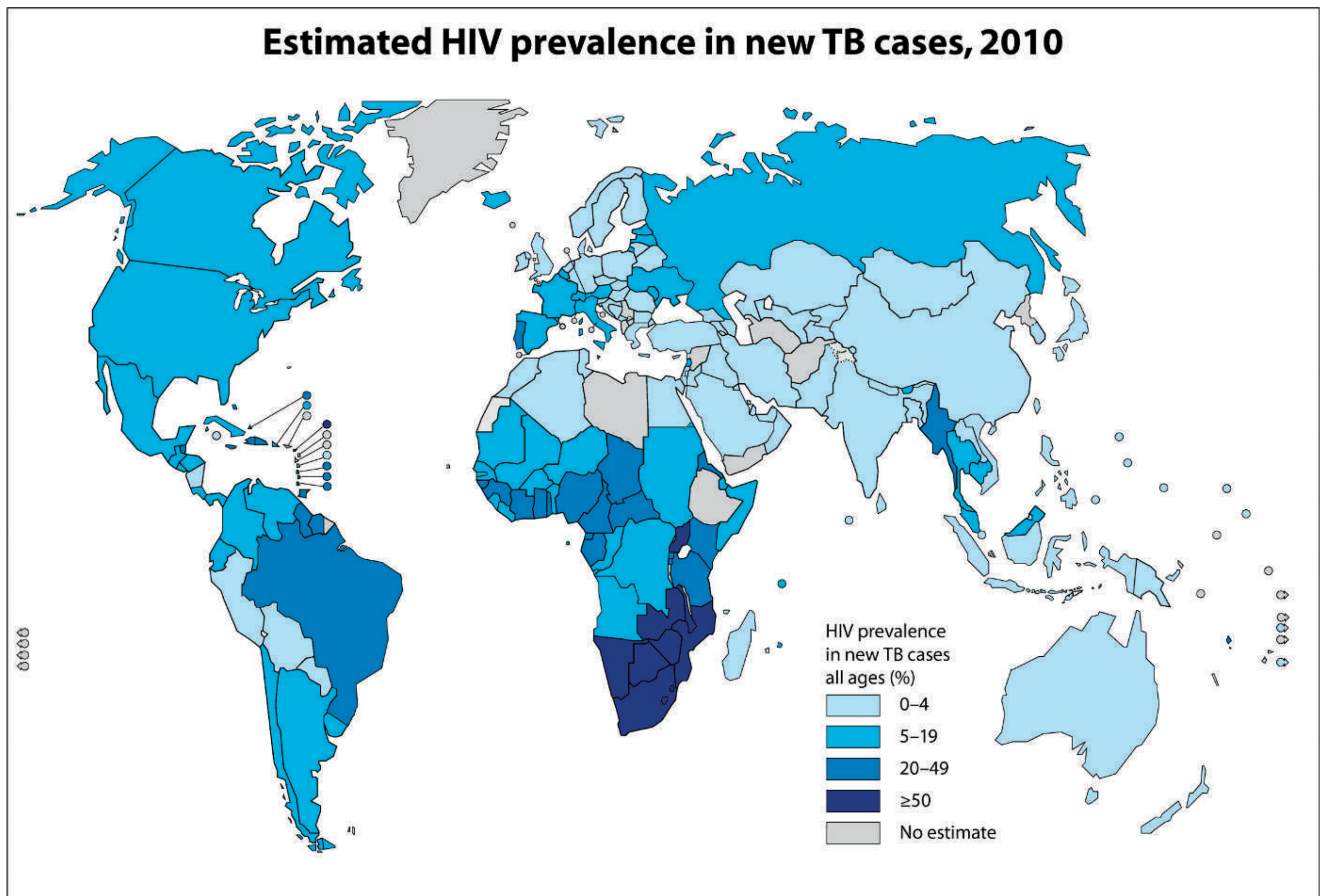

Figure 2. *Estimated human immunodeficiency virus prevalence in new tuberculosis cases, 2010. Source: WHO Global Tuberculosis Control report 2011. Reprinted with permission from the World Health Organization.*

without the simultaneous detection of genes conferring drug resistance.

NONMOLECULAR METHODS

Microscopic Observation Direct Susceptibility Assay

The most thoroughly evaluated of the nonmolecular methods is the microscopic observation drug susceptibility (MODS) assay.[2–6] This method is based on the use of microtiter plates containing Middlebrook 7H10 liquid medium, with wells for controls as well as growth and AST of any isolates that grow. Specimens are processed prior to inoculation and the microtiter plates inoculated and incubated. Rapid detection of growth is by low-power microscopic examination of plates. Preliminary identification of isolates is based on their growth rate and the presence or absence of cording. Growth in wells containing antimicrobial agents in various concentrations is used to demonstrate resistance to those agents; lack of growth is used to demonstrate drug susceptibility. The method has been evaluated in a number of field trials, with a reported sensitivity for the detection of *M tuberculosis* complex of 87% to 98%.[7] It should be noted, however, that these clinical evaluations compared MODS against a variety of gold standards, so the true diagnostic sensitivity and specificity remain undefined. For detection of low-level isoniazid resistance the method is reported to be approximately 98% sensitive and 96% specific; for detection of high-level isoniazid resistance the method is only approximately 90% sensitive but is approximately 99% specific.[7] For detection of rifampin resistance, the MODS assay is approximately 98% sensitive and 99% specific.[7]

Although the MODS assay has been evaluated to a greater extent than other rapid nonmolecular methods, it is not widely used. The reasons are that use of the method still requires an adequate laboratory infrastructure and training of technical staff, and that the method is not yet fully standardized. Use of this assay may be limited to laboratories that already have experience with performing mycobacterial cultures and for which the transition to using the MODS assay would be relatively easy. For laboratories without existing capacity to perform cultures, use of the MODS assay may not be practicable.

Light-Emitting Diode Microscopy

A more recent method for the detection of mycobacteria in smears is not a diagnostic assay per se, but rather is the use of light-emitting diode microscopy in place of either conventional light microscopy or conventional fluorescent microscopy.[8] Specimens are processed as for conventional microscopy and then examined using a light-emitting diode microscope. The available data indicate that light-emitting diode microscopy is equally sensitive when compared with conventional fluorescent microscopy. It has the advantages of being less expensive than conventional fluorescent microscopy and eliminating the need for a mercury-based

light source.[8] The WHO has endorsed use of this technology, but widespread use of microscopes with light-emitting diode capability would require a substantial investment of resources.

MDR-XDRTB Colour Test

A nonmolecular method under development by the Foundation for Innovative New Diagnostics and partners is the MDR-XDRTB Colour Test.[7,9–11] This method is conceptually simple: in some ways it is a solid medium variation of the MODS assay. In contrast to MODS, which uses liquid medium in microtiter plates, the MDR-XDRTB Colour Test uses thin-layer agar technology on a petri dish divided into 4 quadrants. The 4 quadrants include one agar quadrant without antimicrobial agents to detect mycobacterial growth, a second containing agar with isoniazid, a third containing agar with rifampin, and a fourth containing agar with ciprofloxacin. As designed, sputum specimens would be collected directly into specimen containers that are partially filled with a disinfectant transport medium. The sputum/transport medium mixture can then be applied directly to the 4 quadrants of the petri dish without further processing. To date, there are only very limited data regarding the performance of this method.[7,9–11] Although the MDR-XDRTB Colour Test is conceptually simple, and has the potential to be easy to use and inexpensive, field use would require adequate laboratory infrastructure. Therefore, as with the MODS assay, use of this assay might be limited to clinics or laboratories that already have the experience and infrastructure necessary to perform mycobacterial cultures.

Colorimetric Assays

This approach to detecting drug resistance in strains of *M tuberculosis* was first described in 1998 and has been evaluated in a series of studies since then.[12–17] The method is not used to detect the presence of *M tuberculosis*. When growing, *M tuberculosis* bacilli convert a yellow dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, to a purple color. As bacilli grow and metabolize the dye, the color change can be detected visually or by spectrophotometric analysis. When compared with conventional AST the method appears to work well for detecting resistance to isoniazid, rifampin, ethambutol, and streptomycin.[14–17] A standardized, commercial assay is not yet available, and would require some minimal laboratory infrastructure. As a result, it is not yet clear whether this method will gain acceptance as a routine diagnostic test.

Phage Amplification Assays

Another nonmolecular method that has been evaluated in field trials is based on phage amplification technology. Phage amplification assays are based on the formation of plaques that indicate growing bacterial cells; if the number of plaques on a plate containing a drug decreases by a certain amount relative to a control culture, then the isolate is considered to be susceptible to the drug. Conversely, if the number of plaques does not decrease the isolate is considered to be resistant. These assays are potentially useful for the detection of drug resistance, as well as for detecting the presence of mycobacteria.[18,19] One commercial assay, the *FASTPlaque*TB Assay (Biotec Laboratories Ltd, Ipswich, United Kingdom), has been evaluated in field trials, where it has been shown to reliably detect the presence of *M tuberculosis* as well as rifampin resistance.[18,19] It appears to be less sensitive than some other methods, particularly for detecting mycobacteria in smear-positive specimens.[18] Compared with other technologies, phage amplification assays may require too much technical expertise to be useful outside of reference laboratories.

Other Nonmolecular Methods

During the past decade, a number of modifications of existing methods have been developed either to improve the turnaround time for test results, to improve diagnostic sensitivity, or to modify methods so that they can be used in resource-limited areas.[20–25] Only limited data are available regarding the performance characteristics of any of these methods. Part of the reason for this is that, as a group, these are not commercial systems that can be readily evaluated in controlled clinical trials. This same factor is likely to limit their clinical use: laboratories in resource-limited areas are more likely to rely upon commercial systems that can be purchased, shipped, stored, and monitored more carefully.

MOLECULAR METHODS

Line Probe Assays

Line probe technology has been available for almost 15 years and has been used for a number of different purposes in diagnostic testing. The technology, although not automated, is a type of molecular assay that has the appeal of providing detection of specific gene markers without the need for a sophisticated laboratory infrastructure. The technology is straightforward: (1) extraction of DNA from respiratory specimens or from mycobacteria isolated in culture; (2) amplification of nucleic acid sequences using polymerase chain reaction; (3) hybridization of amplified nucleic acid sequences to a variety of oligonucleotide probes that are immobilized in lines on a solid strip, and (4) colorimetric development to mark the nucleic acid probe lines on the immobile strip.[26] The technology has been evaluated for its use in detecting *M tuberculosis* in respiratory specimens, as well as for detecting drug resistance. Based on the results of these evaluations, the WHO has endorsed use of these assays in TB control programs.[26] To date, 2 line probe assays have been developed and evaluated for clinical use.

The first of these assays is the INNO-Lipa Rif.TB (Innogenetics NV, Ghent, Belgium).[27–31] A number of field trials have been conducted to evaluate the performance characteristics of this assay, which have been summarized in a recent meta-analysis.[31] Overall, the assay has a diagnostic sensitivity of approximately 80% and a specificity of 100% for detecting *M tuberculosis* complex in respiratory specimens.[31] The assay has a sensitivity of 80% to 100% for detecting rifampin resistance.[31] The variation in the reported sensitivity for detecting rifampin resistance has yet to be fully explained, but likely is due to the multiplicity of study designs and the various gold standard assays against which the line-probe assay was compared. The INNO-Lipa Rif.TB assay does not test for isoniazid resistance.

The second line-probe assay is the Genotype MTBDR*plus* assay (Hain Lifescience, GmbH, Nehren, Germany).[32–42] Conceptually similar to the INNO-Lipa Rif.tb assay, the MTBDR*plus* assay has been reported to have a diagnostic sensitivity of approximately 94% for detecting *M tuberculosis* DNA in smear-positive respiratory specimens.[33] It is of interest that most clinical evaluations of this device have focused on its use for susceptibility testing, not detection of

M tuberculosis, so the performance characteristics for the latter use are less well defined. For detecting rifampin resistance, the Genotype MTBDR*plus* assay shows a diagnostic sensitivity of approximately 98% with a specificity of approximately 99%.[41] In contrast, for detecting isoniazid resistance the diagnostic sensitivity is only approximately 84% with about the same specificity (approximately 100%).[41] The reason for the lower sensitivity in detecting isoniazid resistance compared with detecting rifampin resistance isn't completely understood, but may, in part, be that the assay tests for fewer genes conferring resistance to isoniazid resistance compared with the number of genes that can be detected conferring resistance to rifampin.

Because of the difficulty in transporting sputum specimens in many rural areas of the world, one novel approach is to ship sputum smears on slides to a central laboratory for testing. A recent evaluation of the MTBDR*plus* assay for detection of drug resistance showed that use of a multiplex polymerase chain reaction amplification step as part of extracting DNA from slides resulted in a sensitivity of detecting isoniazid resistance of approximately 80%, and a sensitivity of approximately 98% for detecting rifampin resistance, and a sensitivity of approximately 83% of detecting MDR-TB.[42] The specificities were approximately 98% for rifampin, isoniazid, and MDR-TB.[42] This study did not evaluate the ability of the assay to detect the presence of *M tuberculosis* in the specimens, because all of the smears were positive.[42] Further studies for the ability of this method to improve detection of *M tuberculosis* in smear-negative specimens would be of interest to TB control programs. Nonetheless, the study does present one approach to improving access to diagnostic laboratory tests, namely using smears as their own stable and inexpensive transport medium.

Another version of the MTBDR assay has been developed, the MTBDR*sl*, designed to detect resistance to second-line antituberculous drugs.[43–45] Evaluations of this assay have shown variable results. In the most recent evaluation of this assay it was compared against DNA sequencing and was found to accurately detect resistance to amikacin and fluoroquinolones (although only ofloxacin was tested by conventional susceptibility testing).[45] In contrast, the method was not as sensitive for detecting resistance to kanamycin or ethambutol, and had a poor predictive value for detecting resistance to capreomycin.[45] Because of the worsening global burden of MDR-TB and extensively drug-resistant TB, methods that can be used to test for resistance to second-line antituberculous drugs should receive emphasis for research and development as well as for clinical trials.

Automated Nucleic Acid Amplification Tests

Only 1 automated method for amplifying and detecting nucleic acids has been developed, the Xpert MTB/RIF (Cepheid, Sunnyvale, California).[46–53] This method is designed to be a fully automated, self-enclosed system that eliminates the need for most of the laboratory infrastructure needed for nucleic acid amplification testing. The method has undergone limited field testing, with the results of those tests showing that the sensitivity of detecting *M tuberculosis* DNA in smear-positive specimens is 98.2%, with a sensitivity of detection *M tuberculosis* DNA in smear-negative specimens of 72.5%.[48] The reported specificity for detecting *M tuberculosis* DNA is 99.2%.[48] The assay, like the INNO-Lipa Rif.tb assay, does not detect resistance to isoniazid but only to rifampin. The first published field trials indicated that, for this purpose, the sensitivity of the assay is 97.6%.[48] A subsequent evaluation comparing the Xpert MTB/RIF assay against a laboratory-developed IS6110 polymerase chain reaction assay showed that it has high sensitivity (100%) for detection of *M tuberculosis* in smear-positive respiratory specimens, but again much lower sensitivity when used with smear-negative respiratory specimens (57%) or smear-negative nonrespiratory specimens (37%).[49] Another comparison of the Xpert MTB/RIF assay against the commercially available Amplified Mycobacterium Tuberculosis Direct assay again confirmed a high sensitivity (85.6%) when the assay was used with smear-positive, culture-positive respiratory specimens, but a much lower sensitivity (59%) when used with smear-negative, culture-positive respiratory specimens.[50] A recent evaluation of the system in a population of patients in an area with a high prevalence of human immunodeficiency virus infection showed similar results: when compared with cultures, Xpert MTB/RIF had a sensitivity of 95% with smear-positive specimens but only 55% with smear-negative specimens.[51]

Another recent evaluation of the Xpert MTB/RIF assay reported the performance characteristics when the assay was used with induced sputum specimens obtained from children with suspected pulmonary TB in Cape Town, South Africa.[52,53] Because sputum specimens obtained from children often are smear negative, the diagnosis of TB may be difficult using conventional methods. In this study, which used microbiological cultures as the gold standard, the sensitivity and specificity of the Xpert MTB/RIF assay on a single induced sputum specimen were 58.7% and 99.4%, respectively, which increased to 75.9% and 98.8% when 2 induced sputum specimens were tested. All of the smear-positive specimens yielded positive test results; the increased sensitivity was due to higher detection of smear-negative cases, which increased from 33.3% to 61.1% sensitivity. Detection of rifampin resistance was evaluated in a subset of specimens, in which Xpert MTB/RIF yielded susceptible results in 70 of 74 susceptible specimens correctly, the other 4 specimens yielding indeterminate test results. For the 3 rifampin-resistant specimens, Xpert MTB/RIF detected 2 and gave an indeterminate test result on the third. However, the number of specimens tested for rifampin resistance in this study was small, precluding any definitive conclusions as to the performance characteristics in this patient population.[52]

Loop-Mediated Isothermal Amplification

Loop-mediated isothermal amplification (Eiken Chemical Company, Tokyo, Japan) is a novel method for amplifying DNA that generates sufficient quantities of nucleic acid for visual detection by use of fluorescent labels.[54–60] Because of the simplicity of the technology, with the resulting potential use in field situations, soon after the technology was introduced a number of investigators began using it to detect *M tuberculosis* DNA. As summarized in a recent review,[54] to date there have been 6 evaluations published regarding the performance of loop-mediated isothermal amplification for this purpose. The first of these was a proof-of-concept study that allowed for further modifications and improvements of the assay so that clinical trials could be conducted.[55] The first clinical trial showed a diagnostic sensitivity of 97.7% in smear-positive, culture-positive specimens, but only 48% sensitivity in smear-negative, culture-positive specimens.[56] Subsequent studies have

Table 2. Characteristics of an Optimal Rapid Diagnostic Test
Simple technology
Easy to train users
Easy to interpret
Reproducible test results
No need for electricity
No need for refrigerated storage
Rapid

Data derived in part from Murray CK et al. Update on rapid diagnostic testing for malaria. *Clin Microbiol Rev*. 2008;21:97–110.[63]

confirmed the high diagnostic sensitivity of the method in smear-positive, culture-positive specimens.[58–60] It should be noted, however, that loop-mediated isothermal amplification is not a commercial assay but rather a molecular method, and that each of the published studies used modifications of the method using different targets and study designs. Until there is a commercial assay based on the loop-mediated isothermal amplification method, widespread use of is unlikely because most resource-limited health care systems cannot develop, validate, and implement laboratory-developed molecular assays.

Oligonucleotide Microarray

This technology allows for the simultaneous detection of many nucleic acid sequences in a sample. The technology allows for detection of nucleic acid sequences of interest, which for *M tuberculosis* could mean either detection of conserved sequences to identify the presence of the bacterium, or detection of other sequences to detect microbial genes that confer drug resistance. Only 1 commercial assay based on this technology has been evaluated, the TB-Biochip (Engelhardt Institute of Molecular Biology, Moscow, Russia).[61] In a small study that compared the TB-Biochip assay with conventional AST, the microarray was found to have a diagnostic sensitivity of 80% when used to detect resistance to rifampin.[61]

CURRENT ADVANTAGES AND DISADVANTAGES OF RAPID DIAGNOSTIC ASSAYS FOR TB

The current rapid diagnostic assays for TB are potentially major advances in the diagnosis and treatment of TB, but as a group have both advantages and disadvantages compared with conventional tests to detect and identify TB, or to detect drug resistance.[62] For TB control programs, the questions will be (1) whether any of these methods are sufficiently better than conventional testing to justify the investment to use the new tests, and, if so, (2) which test would be best in a given setting. There is some published information regarding what might be considered an ideal or optimal rapid diagnostic test for infectious diseases. As summarized by Murray et al[63] and shown in Table 2, an ideal rapid diagnostic test needs to be more than just rapid. The most important requirement, as with any laboratory test, is that rapid tests need to have performance characteristics that are acceptable for their intended use. Rapid tests without adequate performance characteristics will be of little use in TB control programs. If that criterion is met, the other characteristics are more pragmatic: ease of use, ease of interpretation of test results, supply chain issues, and a requirement for minimal laboratory infrastructure.

Table 3. Rapid Nonmolecular Tests: Advantages and Disadvantages
Advantages
Build on existing infrastructure and technology
Conceptually simple methods
Easy to manufacture and distribute
Easily interpreted by clinical staff
Inexpensive
Disadvantages
Unlikely to substantially improve TB control efforts because of slow turnaround time for results
Methods have yet to be standardized
Relatively more difficult to standardize
Few published controlled clinical trials

Table 4. Rapid Molecular Tests: Advantages and Disadvantages
Advantages
More rapid than nonmolecular tests
Potential for high sensitivity and specificity
Can be manufactured in large quantities
Decreased cost
Standardization of field use
Require less training and infrastructure compared with conventional cultures and susceptibility testing
Conceptually simple methods
Easy to manufacture and distribute
More rapid definitive test results
Relatively easier to standardize
Disadvantages
Cost
Do not eliminate need for cultures
Test limited number of drugs for resistance
Require laboratory infrastructure that can accommodate molecular testing
Work better with smear-positive than with smear-negative specimens

The advantages and disadvantages of nonmolecular and molecular rapid tests for TB are summarized in Tables 3 and 4. The most important advantage relates to the rapidity of obtaining test results so that treatment and control efforts can be started at the time of a patient visit, a feature that is of critical important in many resource-limited areas. Second, the ability to simplify TB laboratory training and infrastructure requirements by use of simple, standardized assays would be valuable in many settings where the infrastructure for conventional TB testing does not exist. Third, use of some rapid tests could facilitate case reporting and other epidemiologic information, important requirements for effective TB control programs. Last, when viewed from the perspective of the overall health care system, use of rapid tests could potentially decrease costs for the system as a whole. However, because many assays have yet to be used on a large-scale basis, it is not yet clear if these potential advantages can be realized.

The disadvantages of these assays, although they can be mitigated to some extent, should not be overlooked. First, at this time none of the rapid diagnostic assays has the performance characteristics to replace conventional cultures. Although many of them are highly sensitive when used with smear-positive, culture-positive respiratory specimens, the molecular assays in particular show much lower sensitivity when used with smear-negative, culture-positive specimens. Second, none of the rapid assays offers AST beyond isoniazid and rifampin, with the exceptions of the MDR-

XDRTB Colour Test and the MTBDR*sl* assay designed to test for resistance to second-line drugs. Neither of the latter 2 assays, however, has been evaluated extensively. Third, rapid molecular assays still require some laboratory infrastructure that often is unavailable in the very places that the assays are needed most. Fourth, supply chain issues will exist for some of these assays, which may require refrigerated transportation and storage, or for instrument-based systems a need for ongoing technical support. Last, the cost of these assays remains an issue, because what may appear to be an inexpensive assay, when combined with the need for adequate laboratory infrastructure and training, may still not be affordable in some settings.

COST EFFECTIVENESS OF RAPID METHODS

The cost-effective use of rapid methods is dependent on many factors other than the direct cost of the individual rapid test.[64] In the long run, cost-effectiveness will be determined by the effect the test has on clinical outcomes that are part of TB control programs: if outcomes are improved substantially, and the incidence of TB cases decreases, then a relatively expensive test will be cost-effective. However, because the cost of any necessary laboratory infrastructure can be high, a rapid test that yields only marginal improvements in outcomes may not be cost-effective if it is expensive to implement and perform on an ongoing basis. To date there are only minimal published data regarding the cost-effectiveness of rapid methods as part of TB control programs, with almost no data regarding which methods are the most cost-effective in different settings, particularly for children.[53]

SUMMARY

As with any diagnostic test, there will be advantages and disadvantages to any category of rapid method as well as for any specific assay. For rapid tests designed to be used in TB control programs, it should be emphasized that no existing method can be used to replace conventional cultures or AST. Moreover, the coordinated use of rapid methods in TB control programs will be critical in order for them to be cost-effective or for their potential impact to be realized. As a corollary to this principle, different methods are likely to have different niche roles in TB control efforts, or, put another way, no single method is likely to be the optimal method in every situation. To date, there are relatively few controlled trials comparing the existing rapid methods against conventional methods in various field settings, even fewer controlled trials evaluating rapid methods as an integral part of TB control programs, and essentially no controlled trials comparing rapid methods against one another. What is known with some certainty is that, as a group, molecular methods are more rapid compared with nonmolecular methods, but that molecular methods have inadequate diagnostic sensitivity when used with smear-negative specimens. Overall the existing rapid methods for the detection of *M tuberculosis* complex in respiratory specimens, and for detecting drug resistance, meet some but not all of the requirements of an ideal rapid diagnostic test. Nonetheless, if used correctly as part of a comprehensive TB control program they have the potential to substantially improve those programs. This is needed particularly in Africa, where the social and economic factors often limit access to health care for women and children even more than for adult men. More research into health disparities is needed in Africa, but there are data to suggest that women and children are at particular risk for infectious diseases. This is understood clearly for some infectious diseases such as malaria, but there is evidence that women and children are at risk for other diseases. In 1 recent report, female sex was an independent risk factor for acquiring extensively drug-resistant TB in KwaZulu-Natal, South Africa.[65] Programs to diagnose and treat TB, including use of rapid diagnostic tests, need to place more emphasis on the health of women and children.

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