

Laboratory Diagnosis of Tuberculosis in Resource-Poor Countries: Challenges and Opportunities

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INTRODUCTION

In many developed countries, tuberculosis (TB) is considered a disease of the past. However, the impact of this disease can be devastating even today, especially in those resource-poor countries suffering from high burdens of both TB and human immunodeficiency virus (HIV). In this review, we describe the status of conventional and recently introduced TB diagnostics, the newest tools that are under development, and issues involved in providing quality-assured (QA) TB laboratory services. This global awakening to TB’s devastating impact on vulnerable populations has been brought about in many countries by its deadly synergy with HIV. In response, the World Health Organization (WHO) has provided several key guidance documents for national TB control and laboratory programs for the use of light-emitting diode (LED) microscopes to improve the sensitivity of and turnaround time for the front-line assay, sputum smear microscopy, and for improv-

ing the accuracy of TB detection and drug susceptibility testing through the use of liquid culture and molecular line probe assays (LPAs). International and national laboratory partners and donors are currently involved in evaluations of new diagnostics that will allow the more rapid and accurate diagnosis of TB at point-of-care (POC) settings and also evaluations of alternative algorithms using new molecular tools for TB reference laboratories. Progress in the implementation of these initiatives is dependent on key partnerships in the international laboratory community and ensuring that quality assurance programs are inherent in each country’s national laboratory network.

BURDEN OF TUBERCULOSIS IN RESOURCE-POOR COUNTRIES

In 2008, there were an estimated 9.4 million new cases of tuberculosis (TB) globally, with most cases occurring in re-

TABLE 1. The 22 high-burden countries with 80% of the tuberculosis cases worldwide^a

Country	Burden of TB incidence (no. of cases/100,000 individuals/yr)	Global rank (by estimated cases)
Afghanistan	333	21
Bangladesh	246	5
Brazil	62	15
Cambodia	508	23
China	102	2
Democratic Republic of Congo	369	11
Ethiopia	356	7
India	168	1
Indonesia	285	3
Kenya	610	10
Mozambique	431	19
Myanmar	171	20
Nigeria	293	4
Pakistan	181	6
Philippines	296	9
Russian Federation	110	11
South Africa	948	5
Thailand	142	17
Uganda	411	16
United Republic of Tanzania	371	14
Vietnam	178	13
Zimbabwe	569	19

^a See reference 211.

source-limited or resource-poor countries in Asia (55%) and Africa (30%). Eighty percent of all cases worldwide occurred in the 22 high-burden countries, with an estimated 1.8 million deaths occurring among all cases in 2008, amounting to approximately 5,000 deaths every day (211) (Table 1).

One of the most important factors influencing the current TB epidemic in resource-limited settings is poverty, which is closely related to malnutrition, crowded living conditions, lack of access to free or affordable health care services, and dependence on traditional healers that can facilitate the transmission of tuberculosis (26). In addition to that, much of the deadliness of tuberculosis epidemics, especially in sub-Saharan Africa, has to do with the virulent synergy between HIV and tuberculosis (81). Of the 9.27 million incident cases of TB in 2007, an estimated 1.37 million (14%) were HIV positive; 79% of these HIV-positive cases were in the African region, and 11% were in the Southeast Asia region (186, 211). An estimated 1.3 million deaths occurred among HIV-negative incident cases of TB (20 per 100,000 population) in 2007. There were an additional 456,000 deaths among incident TB cases who were HIV positive. Infection with HIV-1 increases the risk of reactivating latent TB infection by 80- to 100-fold, and HIV patients who acquire new TB infections have higher rates of disease progression. Tuberculosis can occur at all points in the immunosuppressive spectrum of HIV disease, with variable presentations, and, particularly in high-burden countries, TB may be the first presentation of HIV disease.

Fatality rates are higher for HIV-TB-coinfected patients who are on anti-TB treatment but not antiretroviral therapy (16 to 35%) than for treated TB patients who are HIV negative (4 to 9%) (123). The highest death rates occur in coinfecting

patients with the lowest CD4 cell counts (1). Recently, multi-drug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) have had devastating effects on populations of HIV-infected individuals in developing countries, who ironically had good access to HIV care but died from some untreatable forms of TB ("living with HIV, dying of TB") (40, 53). Coinfection with TB has been associated with an increased replication of HIV-1, and the development of TB disease during HIV infection involves both a reactivation of latent TB infection (LTBI) and increasingly progressive primary TB infection (89, 95). Also, numerous drug-drug interactions exist between TB and HIV drugs (33, 193) and add to the complexity in managing these deadly epidemics so that, ultimately, each one fuels the other.

In addition, worldwide TB control suffers from the lack of a totally effective vaccine; the current vaccine, *Mycobacterium bovis* BCG, does not prevent pulmonary TB in adults but is still useful since it protects infants from severe forms of the disease (29). More effective vaccines developed in the future should focus on preventing the development of the disease in all populations and will require detailed knowledge on how to exploit host immune functions for the recognition of and protection against TB (29, 92).

Finally, extended treatment with multiple drugs is needed to effectively cure tuberculosis. The main reasons for this requirement are the hydrophobic cell envelope surrounding members of the *Mycobacterium tuberculosis* complex (MTBC) that serves as a permeability barrier to many compounds; the sequestered, nonreplicating subpopulation of TB that is affected by drugs only when the cells reemerge from dormancy; and the drug target or drug-activating enzymes in TB that are altered by mutation and result in a population of drug-resistant cells (141). The currently used four-drug 6- to 9-month therapy with rifampin (RIF), isoniazid (INH), pyrazinamide (PZA), and either ethambutol (EMB) or streptomycin (SM) is necessary to kill actively replicating TB, eliminate most persisting organisms, and inhibit the development of resistance. However, patients who are instructed to complete the extended regimen often stop taking the pills when they begin to feel better or are noncompliant due to the high pill burden or for other reasons. The irregular taking of antituberculars often results in persisting disease and the development of drug-resistant, multi-drug-resistant, and extensively drug-resistant TB, the other major influencing factors of the present epidemic.

Challenges in the Laboratory Diagnosis of TB

In high-incidence countries, TB control relies on passive case finding among individuals self-presenting to health care facilities, followed by either diagnosis based on clinical symptoms or laboratory diagnosis using sputum smear microscopy. Serial sputum specimens are required (one taken on the spot and the second brought in the following morning), which means that the people are asked to make repeated visits to the health care center for specimen delivery and collection of results. For many patients, the costs of repeated visits to health care facilities are prohibitive, and patient dropout is a significant problem. In addition, the sensitivity of sputum smear microscopy has been reported to vary (range, 20 to 80%), often depending on the diligence with which specimens are collected,

smears are made, and stained smears are examined (177). Also, TB smear microscopy is highly insensitive for HIV-coinfected individuals and for children due to the reduced pulmonary bacillary loads in these patients (56). In resource-poor countries, many smear microscopy laboratories are single-room and understaffed with poorly maintained microscopes, and some of these laboratories lack consistent sources of electricity and clean water. There are few opportunities for the training of staff and little staff capacity to handle high-volume workloads. Quality assurance programs including quality control and external quality assessments (EQAs) are often lacking. Thus, there is a critical need for new, sensitive, easy, and rapid point-of-care diagnostics and also for investments in laboratory infrastructure, quality assurance programs, and well-trained staff.

In more well-developed countries, TB diagnosis by smear microscopy is usually confirmed by culture, followed by the identification of the MTBC strain and drug susceptibility testing (DST). However, these assays require extended incubation times and are significantly more expensive than smears, requiring specialized equipment, highly trained personnel, and a reliable supply of water and electricity. Currently, TB culture laboratories in resource-poor countries often lack adequate infrastructure and have inadequate or outdated equipment and poor biosafety measures, with a scarcity of human and financial resources. Unfortunately, most of the resource-poor regions of the world with high burdens of TB have very few reference laboratories capable of reliably performing TB culture and DST.

Challenges of Accurate Laboratory Diagnosis of TB in HIV-Positive Patients

The lack of laboratory facilities makes the laboratory diagnosis of infectious diseases difficult in many parts of the African continent. As a result, clinical algorithms for syndromic management have been developed and evaluated; however, this approach has significant shortcomings, especially for HIV-infected persons. As mentioned previously, microscopic examination of sputum for acid-fast bacilli (AFB) is highly insensitive in HIV-positive individuals, since TB presents differently in coinfecting patients, with fewer organisms present in the lungs (56). Currently, WHO guidelines call for culture to be performed on sputum smear-negative HIV-infected patients with a clinical suspicion of TB; however, these tests are more expensive to perform and require upgraded infrastructure and skilled laboratory staff. Also, the WHO recommendation that two rather than three sputum examinations be used was generated largely for non-HIV-infected persons. However, this approach was evaluated in HIV-infected patients in Southeast Asia, and the incremental yield of microscopy was found to rapidly diminish to 2% after the second smear (119). Most importantly, those authors showed that liquid culture of at least two sputum specimens was highly sensitive in diagnosing most TB cases of HIV-infected persons. Those authors also documented that lymph node aspiration provided the highest incremental yield of any of the nonpulmonary specimens tested for TB (119). The recent WHO recommendation that countries with a high burden of TB develop laboratories for liquid culture would certainly improve the diagnosis of TB in

HIV-infected patients. This is even more critical in areas where resistant TB circulates, since culture and drug susceptibility testing would avoid the indiscriminate use of drugs and the further spread of resistant strains. Even with such impressive advances in the diagnosis of TB, especially in HIV-infected patients, most high-burden countries face a shortage of well-trained staff with extensive technical expertise according to the highest international standards.

CURRENT WHO-APPROVED METHODOLOGY FOR TB DIAGNOSTICS

The WHO Strategic and Technical Advisory Group for Tuberculosis

The WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) provides objective, ongoing technical and strategic advice to the WHO regarding TB care and control (http://www.who.int/tb/advisory_bodies/stag/en/index.html). Specific objectives of the STAG-TB are to provide the WHO Director General with independent evaluations of the strategic, scientific, and technical aspects of WHO's area of work in TB; to review progress and challenges in TB-related core functions such as policies, strategies, and standards; and to review and make recommendations on committees and working groups. The STAG-TB is comprised of 22 experts, representing ministries of health, national TB control programs (NTCPs), academic and research institutions, civil society organizations, communities and patients affected by tuberculosis, and professional associations. The STAG-TB reviews policy drafts and supporting documentation and may endorse the policy recommendation with or without revisions, request additional information and re-review the evidence in subsequent years, or reject the recommendation.

Guidelines for Sputum Collection, AFB Smear, and LED Microscopy

The current WHO international policy on TB case detection recommends the collection and examination of two sputum smears for the diagnosis of pulmonary tuberculosis (204). This change from three to two smears is based on a systematic review of 37 eligible studies (106) that quantified the incremental diagnostic yield of serial sputum specimens. The results indicated that almost 85.8% of TB cases were detected with the first sputum specimen. With the second sputum specimen, the average incremental yield was 11.9%, while the incremental yield of the third specimen, when the first two specimens were negative, was 3.1%. A study conducted in Kenya demonstrated that decreasing the number of smears examined for the detection of new pulmonary TB cases led to a reduction of patient visits to a clinic and the laboratory workload (16). The examination of only two smears could therefore alleviate the workload of laboratories, particularly in countries with a high microscopy workload, by one-third. It is also expected that the microscopic analysis of two sputum smear samples will improve case findings through enhanced quality of service, decreased time for diagnosis and initiation of treatment, and decreased number of patients dropping out of the diagnostic pathway. In addition, patients will spend less time at the diag-

nostic facility, which may save expenses for them and improve infection control measures. However, it is also cautioned that the reduction of the number of specimens examined for the screening of TB patients from three to two specimens should be recommended only in settings with a well-established laboratory network and a fully functional EQA program for smear microscopy including on-site evaluation and follow-up training for problem laboratories (6).

In addition, the WHO has recommended that conventional fluorescence microscopy be replaced by LED microscopy in all settings where fluorescent microscopy is now used and that LED microscopy be phased in as an alternative for conventional Ziehl-Neelsen (ZN) microscopy in both high- and low-volume laboratories (212). Also, countries implementing LED microscopy should address issues related to training requirements, especially for laboratory staff unfamiliar with fluorescent techniques; validation during the introductory phase; monitoring of trends in case detection and treatment outcomes; and introduction of adapted systems for internal quality control and external quality assessment.

Improving TB Culture

The WHO recommends a stepwise approach to introducing a liquid medium system for culture and DST in middle- and low-income countries (205). Additionally, the decision to implement a liquid culture and DST system should be based on need and be consistent with the NTCP's national plan for TB laboratory capacity strengthening and expansion. In most circumstances, the first priority would be to implement the system in a national TB reference laboratory (NTRL), assuming that the NTRL is currently performing the essential functions of supervising quality-assured (QA) microscopy of the laboratory network and performing TB culture and DST using solid media. The subsequent expansion of the liquid culture and DST capacity would logically be to include regional TB culture and DST laboratories. The extent of scale-up should be determined by need and the availability of funding and, again, should be consistent with a national laboratory plan. The WHO also reviewed noncommercial culture and DST methods and recommended that selected noncommercial culture and DST methods such as the microscopically observed drug susceptibility (MODS) assay and the nitrate reductase assay (NRA) (both described below) be used as an interim solution in resource-constrained settings while the capacity for genotypic and/or automated liquid culture and DST is being developed.

It was also recommended that all mycobacterial isolates should be identified to the species level at least to the extent that the MTBC isolate is differentiated from nontuberculous mycobacteria (NTM). When using liquid culture with a significantly reduced time to detection, it is also imperative that a rapid and affordable method of species identification be used. Standard biochemical tests or other methods requiring extended incubation times are not considered appropriate for the identification of the MTBC isolate because of the long turnaround time and the variability of reactions. Likewise, the presence of corded AFB grown in liquid media is suggestive of the presence of an MTBC organism but should not be used as the definitive identification method.

Detection of Multidrug-Resistant and Extensively Drug-Resistant TB

Multidrug-resistant tuberculosis (MDR-TB) is defined as resistance to both INH and RIF, and extensively drug-resistant tuberculosis (XDR-TB) is defined as MDR-TB with additional resistance to any fluoroquinolone and to at least one of three injectable drugs used for TB treatment: capreomycin, kanamycin, or amikacin (207). Based on surveys conducted in over 110 settings in the last decade, the WHO estimates that in 2007, nearly half a million TB cases were MDR, resulting in 130,000 deaths. In addition, estimates for XDR-TB were about 50,000, the majority of which were fatal. These figures are likely to significantly underestimate the problem, since drug susceptibility surveillance is either very limited or nonexistent in many of the countries with high burdens of TB. Thus, the importance of accurately diagnosing MDR-TB or XDR-TB has been the driving force behind the identification of newer, more rapid diagnostics to detect drug resistance in TB. TB culture and DST using solid media can take 6 weeks or more due to the slow growth of the MTBC organism. Reliable DST, including second-line drug testing, is recognized as a basic requirement of the Direct Observed Therapy Short Course Plus (DOTS-Plus) (207) treatment strategy. It is known that the intrinsic accuracy of susceptibility testing results (performed under the best circumstances) varies with the drug tested: it is most accurate for RIF and INH and less accurate for SM and EMB (97, 141). Second-line DST is not recommended when cultures are susceptible to first-line drugs, and only laboratories with experience and well-documented competency in performing first-line drug testing should perform second-line DST (207). Second-line drugs included in WHO guidelines are the aminoglycosides kanamycin and amikacin; the polypeptides capreomycin, viomycin, and enviomycin; the fluoroquinolones ofloxacin and ciprofloxacin; ethionamide; prothionamide; and *p*-aminosalicylic acid. Although several drugs are mentioned for a class, only one of them needs to be tested because of cross-resistance between members of that class.

Molecular Line Probe Assays

Systematic reviews and meta-analyses of the performance of molecular line probe assays (LPAs) compared to conventional DST methods showed that LPAs are highly sensitive ($\geq 97\%$) and specific ($\geq 99\%$) for the detection of RIF resistance, alone or in combination with INH (sensitivity, $\geq 90\%$; specificity, $\geq 99\%$), in isolates of *M. tuberculosis* and in smear-positive sputum specimens (7, 209). When RIF resistance alone was used as a marker for MDR, the overall accuracy for the detection of MDR was equally high at 99%. These results were confirmed by laboratory validation and field demonstration data from several countries, most notably in the large-scale demonstration project in South Africa, executed by the Foundation for Innovative New Diagnostics (FIND), the South African (SA) Medical Research Council (SAMRC), and the SA National Health Laboratory Service (NHLS) (7). Data from studies in South Africa also indicated the feasibility of introducing LPAs in high-volume public health laboratories. Detailed costing data from South Africa showed that by using the LPAs in routine diagnostic algorithms, the reduction in cost

amounted to between 30% and 50% compared to conventional DST methods. As expected, the cost was lowest when the LPA was applied directly to smear-positive specimens and highest when the assay was used on isolates from liquid primary culture. The introduction of these assays in screening and diagnostic algorithms could significantly reduce the need for sophisticated and costly conventional laboratory infrastructure, still vastly inadequate in most high-burden countries. Based on these studies, the use of LPAs has been recommended by the WHO (209) according to the following guidelines:

- The adoption of LPAs for the rapid detection of MDR-TB should be decided by the ministry of health (MOH) within the context of country plans for the appropriate management of MDR-TB patients, including the development of country-specific screening algorithms and timely access to quality-assured second-line anti-TB drugs.
- The performance characteristics of the LPA have been adequately validated for the direct testing of sputum smear-positive specimens and of isolates of the MTBC organism grown from smear-negative and smear-positive specimens. The direct use of LPAs on smear-negative clinical specimens is not recommended.
- The use of commercial LPAs, rather than in-house assays, is recommended to ensure the reliability and reproducibility of results, as in-house assays have not been adequately validated or used outside limited research settings.
- Any new or generic LPAs should be subject to adequate validation when first introduced into the field, that is, published validation studies, adequate data to allow systematic review and meta-analysis (including assessment of data quality), and results from field demonstration projects documenting feasibility and consistent performance equal to those of conventional methods and commercial LPAs.
- The adoption of LPAs does not eliminate the need for conventional culture and DST capability; culture remains necessary for the definitive diagnosis of TB in smear-negative patients, and conventional DST is required to diagnose XDR-TB. However, the demand for conventional culture and DST capacity may change based on the local epidemiological situation and the use of LPAs in country-specific screening algorithms.
- As current LPAs detect resistance only to RIF and/or INH, countries with documented or suspected cases of XDR-TB should establish or expand conventional culture and DST capacity for quality-assured susceptibility testing of second-line drugs based on current WHO policy guidance.

CURRENT STATUS OF TB LABORATORY DIAGNOSTICS AND DRUG SUSCEPTIBILITY TESTING

AFB Smear Microscopy

As stated above, 80% of all TB cases worldwide are from 22 high-burden countries, and in the majority of these countries, the diagnosis of TB relies primarily on the identification of AFB in sputum smears using a conventional light microscope. The sputum specimens are smeared directly onto the slides

(direct smears) and subjected to Ziehl-Neelsen (ZN) staining. Although all mycobacterial species are acid fast, this assay is highly specific for *M. tuberculosis* in countries where TB is endemic because of the high burdens of this disease (177). In spite of the high specificity, the sensitivity of the test has been reported to vary from 20 to 80% (177), and its usefulness is questionable for patients with reduced pulmonary cavity formation or reduced sputum bacillary load, such as children and HIV-coinfected patients. The sensitivity of the direct smear assay has been found to be dependent on staff that has been well trained so that sufficient time is spent on preparing, staining, and reading each smear, with a well-functioning EQA program in place. Although new technologies are under development, microscopy will likely remain the primary tool for the laboratory diagnosis of TB in resource-poor countries for the foreseeable future. Thus, the following two strategies for optimizing smear microscopy should continue to be explored.

Light versus fluorescence microscopy. A review of 18 studies with culture as the reference standard indicated that the sensitivity of conventional microscopy ranged from 32 to 94% and that the sensitivity of fluorescence microscopy ranged from 52 to 97%, with the fluorescent method being on average 10% more sensitive than light microscopy (176). For HIV-positive patients, two studies reported between 26 and 100% increases in the detection of AFB using fluorescence microscopy (177). Because fluorochrome-stained smears can be examined at lower magnifications, it takes less time to examine these smears than to examine smears stained with ZN stain and still results in a higher sensitivity and a similar specificity. The implementation of fluorescence microscopy has been hampered in the past because traditional fluorescence microscopes with short-lived mercury vapor lamps have been too costly for resource-poor countries. The current availability of newer, less-expensive microscopes with light-emitting diodes (LEDs) that can generate both light and fluorescence wavelengths should allow expanded feasibility studies under field conditions to determine the practicality of a more extensive implementation of fluorescence microscopy, including the need for training staff and the availability of LED microscopes and fluorescent stains. Characteristics of five commercial LED products currently available for TB diagnostics (Primo Star iLED [Carl Zeiss, Oberkochen, Germany], Lumin [LW Scientific, Lawrenceville, GA], ParaLens [QBC Diagnostics, Philipsburg, PA], FluoLED [Fraen Corporation SRL, Settimo Milanese, Italy], and CyScope [Partec, Grolitz, Germany]) have been described recently (113).

Direct versus concentrated smears. Fourteen studies that investigated the impact on microscopy results of sputum pretreatment with either bleach or sodium hydroxide followed by centrifugation were reviewed (177). With culture as the reference standard, those authors reported increases in sensitivity that ranged from 11 to 26%. In all studies, the sensitivity for concentrated smears was higher than that for direct smears, and in one study involving HIV-infected individuals, the sensitivity increased by 11% following processing with bleach and centrifugation (20). Also, the effect of passive sedimentation following treatment with either bleach or ammonium sulfate was assessed in eight studies that used culture as the reference standard. The increases in sensitivity were quite variable: in studies using overnight sedimentation, the increases over di-

rect smears ranged from 2 to 34%, while with shorter sedimentation times of 30 to 45 min, the increases ranged from 0 to 36%. From these studies, it appears that concentrating sputum by centrifugation following pretreatment with either bleach or sodium hydroxide was more consistent than passive sedimentation in increasing the sensitivity of the assay compared with that of direct smear microscopy. Another approach used in high-volume laboratories where the risk of infection for staff is increased due to large numbers of specimens being handled involves heating the sputum at 80°C for 1 h. This approach results in the killing of most organisms and in the digestion of much of the mucous present in the sputum prior to centrifugation (G. Coetzee, unpublished data). Further studies are being conducted to evaluate biosafety, training, turnaround times, and financial issues related to the implementation of a concentration step in AFB smear microscopy at point-of-care laboratories.

Sputum Collection and Processing Methods for TB Testing

Since quality test results require quality specimens, the accurate, rapid microbiological diagnosis of TB and other mycobacterial infections begins with proper specimen collection and rapid transport to the laboratory. To ensure the collection of the best possible specimen, the health care worker must be properly trained, and the patient must be provided with clearly presented and fully understood instructions for sputum collection. In addition, aerosol-producing procedures such as sputum production must be performed in a way that ensures the safety of the health care worker and others (27).

At present, the WHO recommends the testing of one morning specimen and one spot sputum specimen for the laboratory diagnosis of tuberculosis using AFB smear microscopy (204). Morning specimens are thought to be superior, since the mucociliary clearance of the lungs is lower during the night and the caliber of the bronchial tree is narrower. Therefore, sputum gradually accumulates in the airways during the night, and thus, the quality and yield of the morning specimen are improved. However, the collection of morning specimens may not be practical in resource-poor settings, since at least two visits from the patients are required. Therefore, recent studies examined the AFB smear positivity of two spot specimens taken the same day compared to one spot specimen followed by one morning specimen brought in the next day (16, 157). The two approaches were found to result in comparable diagnostic outcomes, although the quantity of bacilli seen was less with the spot specimens than with the morning specimens. However, the quantity and quality of the spot specimens can be significantly influenced by the patient being properly instructed and properly adhering to the instructions during collection. The WHO has recently indicated that "same-day diagnosis" or the "front-loading" method of specimen collection should be done according to a phased implementation plan with due consideration given to training, human resources, laboratory workload, infection control, and monitoring issues (212).

Most respiratory specimens contain microorganisms other than mycobacteria. Therefore, the specimens collected for growth detection and susceptibility testing of *M. tuberculosis* should be refrigerated if the transportation delay is expected or if the ambient temperature would encourage the growth of

contaminating organisms (111, 147). If specimens are not handled correctly, an overgrowth of rapidly growing nonmycobacterial contaminants may occur. The positive impact of refrigeration was shown by a recent study from Indonesia (104). When sputum specimens were refrigerated without preservatives for fairly long periods of time after collection at a remote location (median, 12 days; range, 1 to 38 days) and then transported to a reference laboratory without a cold chain, such as ice or cold packs (transportation time median, 4 days; range, 2 to 12 days), the specimens still yielded a high positive culture rate (94.4%) in a liquid culture system. Although a cold chain was not used during transportation in that study, keeping specimens cold during both storage and transport is suggested to lessen potential contamination and enhance the recovery of TB organisms.

In order to provide the best results for the detection of growth of *M. tuberculosis*, the volume of a sputum specimen should exceed 5 ml (84). Evidence from the past has shown that higher volumes of sputum collected over a period of time (overnight or 24 h) are more valuable for microscopic and growth detection of TB than the low volumes of sputum produced immediately upon request (3, 91). Although this approach may result in higher contamination rates, especially with the use of liquid media, the increase in the sensitivity of smear microscopy, growth detection, and molecular techniques may offset potential problems with contamination.

Before culture in the laboratory, clinical specimens from nonsterile body sites such as sputum must be subjected to a pretreatment involving digestion, homogenization, decontamination, and concentration. This procedure will eradicate more rapidly growing contaminants such as normal flora (other bacteria and fungi) while not seriously affecting the viability of the mycobacteria (84). However, the efficacy of these procedures is highly influenced by the time of exposure to the reagent used for decontamination, the toxicity of that reagent, the efficiency of centrifugation, and the killing effect of heat buildup during centrifugation. There is evidence that even the mildest decontamination methods, such as the widely used *N*-acetyl-L-cysteine (NALC)-NaOH method, can kill about 33% of the mycobacteria in a clinical specimen, while more overzealous methods can kill up to 70% of the mycobacteria (84).

Although the NALC-NaOH method has recently become one of the most widely used processing methods in many countries (the Mycobacterial Growth Indicator Tube [MGIT] system and LPAs are validated with this method) (205, 209), it is not the only processing method available for laboratories in the field. The main reason behind the wider use of this particular method is that it results in more stringent pH control than other processing methods and creates an environment that is less harmful to the organism and is indispensable for avoiding false fluorescence of the indicator used in the MGIT system (168). However, growth indicators of other liquid culture-based systems may not be as vulnerable to pH as the MGIT growth indicator, and therefore, different processing methods could be used. For example, when using the sodium dodecyl (lauryl) sulfate (SDS)-NaOH processing method, the sensitivity of both liquid culture and direct molecular testing was higher than that found with the NALC-NaOH method (149). In addition, because NALC is relatively expensive, the use of this method may not be affordable in some settings.

Sputum processing procedures involving centrifugation require manipulations in a biological safety cabinet (BSC) and, preferably, a refrigerated centrifuge with sealed safety cups and aerosol tight rotors. The purchase and maintenance of these instruments may be challenging for laboratories in resource-poor settings. Therefore, other simpler processing methods that do not require these instruments should be considered, especially for rural areas with limited funds, large sample volumes, and the potential for contaminated specimens due to the lack of refrigeration following collection or cold chain during transport. In the modified Kudoh method, sputum specimens are exposed to an equal volume of 4% NaOH and allowed to settle for 15 min, and the sediment is inoculated onto solid Ogawa medium, a modified Lowenstein-Jensen (LJ) medium with an acidic pH that partially neutralizes the strong alkali in the sediment (93). In a similarly simple approach, 4 ml of 0.124% chlorhexidine gluconate is added to each 1-ml sputum specimen and incubated for 18 to 20 h (usually overnight) (49). The sediment is then used to prepare smears and inoculate LJ slants. Preliminary data indicate that in addition to culture, molecular testing by the LPA is also possible after this pretreatment method (Á. Somoskövi, unpublished data). Since these methods do not require centrifugation and need very little manipulation with no more aerosol generation than during AFB smear preparation, they can be performed without biosafety level 2 (BSL-2) or BSL-3 requirements. In addition, large numbers of specimens can also be handled relatively quickly. However, the pH of these methods does not allow their use with liquid culture, and the passive sedimentation may negatively impact sensitivity. In spite of these issues, methods such as these may be suitable for district or peripheral laboratories where TB culture is needed for quality patient care, but because of infrastructure and equipment requirements, liquid culture is not possible. However, it is important to stress that once growth is obtained in a lower-level laboratory using these methods, the medium containing growth must not be opened or the isolates must not be manipulated on site but instead must be sent to a BSL-2 or BSL-3 reference laboratory for further testing.

Recently, two other novel specimen-processing methods that do require centrifugation were evaluated against the NALC-NaOH procedure. One study described the use of C₁₈-carboxypropylbetaine, while the second study applied a hypertonic saline-NaOH-based method (54, 133). Both studies reported a comparable or better sensitivity than that of the NALC-NaOH procedure, especially on smear-negative, culture-positive specimens. In addition, these novel methods were shown to be simpler and less expensive than the NALC-NaOH method.

As stressed throughout, the detection of *M. tuberculosis* through the growth of viable cells is highly dependent on the quality of specimen collection, storage, and transportation. In addition, shipments of specimens containing viable TB require special packaging and handling according to national and international biosafety standards. Alternatively, TB does not need to be viable for molecular test detection, and these assays can be used even if specimens are contaminated due either to long storage and transport or to local environmental factors (climate or higher occurrence of environmental mycobacteria, molds, or yeasts). A Whatman FTA card system (Whatman

Inc., Clifton, NJ) has been used to ship dried sputum samples to a referral laboratory by mail from geographically isolated locations for molecular testing (60). Sputum specimens from patients with tuberculosis were spotted onto FTA cards originally developed for preserving blood samples for HIV DNA molecular testing. Bacteria present in the sputum were tightly bound to the membrane. This enabled the extraction of amplifiable DNA from the bound cells. The sensitivities of the molecular testing in the referral laboratory by an in-house PCR were 100% for smear-positive, culture-positive specimens and 69% for smear-negative, culture-positive specimens. In a similar study, the GenoCard system by Hain Lifescience was evaluated by applying 100 µl of processed and homogenized sputum specimens, rather than unprocessed sputum, to the membrane of the card. The cards were then air dried and heat inactivated at 110°C for 15 min before being transported from Nigeria to Italy for molecular drug susceptibility testing using the line probe assay (115). Further work is essential to evaluate the sensitivity and specificity of this promising approach in field studies since, in addition to the ease of use and less handling of infectious materials, the automated extraction of the DNA can be performed with the dried sputum spots.

Direct Detection of TB in Patient Specimens

Direct nucleic acid amplification assays. Direct nucleic acid amplification (NAA) testing of diagnostic specimens can reduce the overall turnaround time for the laboratory diagnosis of tuberculosis by at least 2 to 4 weeks compared to conventional growth detection. The application of this technology has become part of the routine in many laboratories in low-prevalence, high-income countries (121). The most recent guidelines on the use of these tests from the U.S. Centers for Disease Control and Prevention (CDC) recommend that “nucleic acid amplification testing be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary tuberculosis” independent of smear microscopy results (28).

There are five commercial assays offering the capability to detect MTBC isolates in patient specimens. (Molecular assays that can provide information on drug resistance in addition to detecting MTBC will be described in a later section of this review.) The assays for the detection of MTBC isolates use either PCR (Amplicor PCR assay; Roche Molecular Systems), transcription-mediated amplification (Amplified MTD assay [Gen-Probe Inc.] and GenoType Mycobacteria Direct assay [Hain Lifescience]), strand displacement amplification (BD ProbeTec assay; Becton Dickinson), or loop-mediated isothermal amplification (LAMP; Eiken Chemical Co.). Table 2 lists the tests along with the reported sensitivity and specificity for smear-positive and smear-negative specimens (51, 75, 153, 197). According to a recent review and meta-analysis that was based on 125 separate studies, the pooled sensitivity and specificity of these tests were 85% (range, 36 to 100%) and 97% (range, 54 to 100%), respectively (101).

The newest addition to this list, the LAMP assay (Eiken Chemical Co. Ltd., Tokyo, Japan), is a novel, isothermal nucleic acid amplification method that potentially may be most readily adaptable to resource-limited settings. The assay applies autocycling strand displacement DNA synthesis targeting

TABLE 2. Commercial tests for the direct detection of *Mycobacterium tuberculosis* in processed sputum specimens^a

Direct NAA assay	Company	Method	% sensitivity (range)		
			Smear-positive samples	Smear-negative samples	All samples
Amplicor PCR	Roche Molecular Systems	PCR	90–100	50–95.9	91.3–100 (overall)
Amplified MTD	Gen-Probe Inc.	Transcription-mediated amplification	91.7–100	65.5–92.9	92.1–100 (overall)
BD ProbeTec	Becton Dickinson	Strand displacement amplification	98.5–100	33.3–85.7	98.9–100 (overall)
GenoType Mycobacteria Direct	Hain Lifescience	Transcription-mediated amplification	85.7–94.6	33.3–65.4	99.6–100
LAMP	Eiken Chemical Co.	Loop-mediated isothermal amplification	97.7	48.8	99 (overall)

^a See references 11, 51, 75, 101, 129, 153, and 197.

six regions of the *gyrB* and 16S rRNA genes. The advantages of this system are its simplicity, its rapidity (results can be available within 35 to 65 min), its high specificity, and its lack of need for costly instruments, and the platform can be used for organisms other than *M. tuberculosis* (75). Limited evidence on the accuracy of the LAMP assay from microscopy centers in Peru, Bangladesh, and Tanzania indicates a sensitivity of 97.7% for smear- and culture-positive sputum specimens and a sensitivity of 48.8% for smear-negative, culture-positive specimens (11). It was reported that technicians with no prior molecular experience could competently perform the assay after 1 week of training.

Nucleic acid amplification tests can be performed in 6 to 8 h, although for most tests, sputum specimens must already be processed (digested, decontaminated, and concentrated as described above). As for all laboratory work, these tests should be used only in laboratories that can ensure the competency of staff and provide sufficient reagents and controls to monitor the performance of the assays (129). Molecular testing, especially those assays that include the amplification of target DNA, is extremely susceptible to contamination from amplicons derived from positive specimens. Thus, there are strict infrastructure requirements that must be observed, beginning with the dedication of separate rooms for the three main steps of the procedure (209). Daily work should flow from the cleanest area, a room for reagent preparation, to a second room for DNA preparation, and, finally, to a third room for amplification and detection. Negative controls must be included to detect any potential amplicon contamination, and wipe tests should be performed monthly to ensure that work areas remain clean. The advantage of these methods is that *M. tuberculosis* can be rapidly detected and identified; however, *M. tuberculosis* cannot be ruled out due to issues related to sensitivity and inhibition. Costs can be kept to a minimum if testing is limited to specimens from the most high-risk patients based on proper clinical assessments and national testing algorithms based on public health policies.

Use of direct NAA assays for the diagnosis of tuberculous meningitis. Tuberculosis of the central nervous system is one of the most severe forms of human tuberculosis. The rapid and accurate laboratory diagnosis of tuberculous meningitis is of prime importance, since the smear is often negative, and cultures may take several weeks to grow, if at all. In two studies, the sensitivity of the manual Amplicor PCR and the automated

Cobas Amplicor PCR was 60%, and the specificity was 100%, for the detection of cases of definite and probable tuberculous meningitis (14, 15). Although the sensitivity was low, the Amplicor PCR was more sensitive than the combination of smear microscopy and radiometric growth detection. In three studies using slight modifications of the Amplified MTD assay, the sensitivity for specimens from patients with suspected tuberculous meningitis ranged from 83.0 to 93.8%, and the specificity ranged from 97.0 to 100% (30, 96, 148). The BDProbeTec assay was also applied for the rapid confirmation of tuberculous meningitis. In a study that examined 101 prospectively collected cerebrospinal fluid (CSF) samples, a sensitivity of 84.7% and a specificity of 100% could be achieved when a modified pretreatment procedure and a lower cutoff value were utilized (79).

Recently, an interesting approach was used to enhance the sensitivity of the GenoType Mycobacteria Direct test (131). The authors of that study subjected extrapulmonary specimens, including CSF specimens, to a short period of cultivation in a nutrient-rich broth-based system before molecular testing. The system was evaluated with 225 paucibacillary specimens from 189 patients suspected of having tuberculosis. Culture-enhanced molecular testing performed on incubation day 15 showed a sensitivity and a specificity of 88.6% and 100%, respectively, compared to the clinical and histological diagnoses used as “gold standards.” This approach allowed much more rapid results than waiting for up to 44 days for the detection of growth in liquid culture alone.

Bacteriophage methods for direct detection of *M. tuberculosis*. Bacteriophages that specifically infect and replicate in mycobacteria have been used for the direct testing of processed clinical specimens to indicate the presence of viable bacterial cells. A meta-analysis of 13 studies of *M. tuberculosis* bacteriophage-based tests concluded that the performance of these tests was not superior to that of smear microscopy (80). The phage-based systems were originally developed to offer a rapid and relatively low-cost diagnostic approach; however, the findings of those studies indicate that the reported sensitivity and specificity of these tests do not support their routine diagnostic use.

Growth Detection of TB in Culture

Culture is more sensitive than AFB smear microscopy for the detection of *M. tuberculosis*: while microscopy requires

approximately 5,000 to 10,000 AFB/ml of sputum for detection, culture can detect as few as 10 to 100 viable bacteria/ml (147). Although culture is not routinely performed in resource-poor settings except for purposes of surveillance, treatment failure, or detection of drug resistance, culture is increasingly recognized, as described above, as being critically important in detecting TB in HIV-positive individuals who often have low bacillary loads in sputum specimens (56).

Solid versus liquid media. While liquid media are preferred for the rapid initial isolation of mycobacteria (147, 205), most resource-poor countries depend on LJ egg-based solid medium for the detection of growth of MTBC isolates. LJ medium can be prepared locally, has good buffer capacity, has a shelf-life of several months when refrigerated, supports the growth of most mycobacterial species, and contains malachite green in the medium to inhibit the growth of most contaminants. However, there are disadvantages: LJ medium can vary from batch to batch in its ability to support the growth of TB depending on the quality of the eggs used, contaminants are not completely eliminated but only suppressed by the malachite green and can overgrow *M. tuberculosis* during subsequent testing, and difficulties have been noted in achieving consistent drug concentrations when using LJ media for susceptibility testing. In spite of these drawbacks, there are strains of the MTBC that will grow better or only on solid media, and thus, the CDC-recommended gold standard for the detection of TB is to inoculate at least one tube each of solid and liquid media (111, 147).

Liquid medium systems. The most commonly used liquid medium known to result in the better recovery and faster growth of mycobacteria is modified Middlebrook 7H9 broth (111, 147). This medium is labor-intensive to prepare and, in contrast with LJ medium, requires expensive ingredients. In addition to the broth base, the heat-labile growth supplement OADC (oleic acid, albumin, dextrose, and catalase) must be added following autoclaving in order to support mycobacterial growth. Also, because liquid medium is more prone to contamination with bacteria present in sputum specimens that may survive the decontamination process, a combination of antimicrobial agents, such as PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azolocillin), must be added to the liquid medium to suppress the growth of contaminants. This medium or this medium with slight modifications is used in both manual and automated liquid systems as described below.

MGIT system (Becton Dickinson) tubes containing Middlebrook 7H9 medium, OADC, and PANTA also contain an oxygen-quenched fluorochrome embedded in silicone at the bottom of the tube (168). During bacterial growth, the free oxygen will be utilized, and the fluorochrome will no longer be inhibited and will fluoresce when visualized under UV light. MGIT tubes may be read manually under a UV light or entered into an MGIT 960 instrument, where they are incubated and monitored for increasing fluorescence every 60 min. Growth can also be visually observed by the presence of small granules or flakes. The MB/BacT Alert system (bioMérieux) is an automated system that employs a colorimetric carbon dioxide sensor in each bottle to detect the growth of mycobacteria, and the VersaTREK system (Trek Diagnostic Systems) is an automated system that detects mycobacterial growth by automatically monitoring (every 24 min) the rate of oxygen con-

sumption within the headspace of the culture bottle. The Bactec 460 TB system (Becton-Dickinson) is a semiautomated system using a liquid medium with ^{14}C -labeled palmitic acid as a carbon source. The palmitic acid is metabolized by TB to $^{14}\text{CO}_2$, which is monitored by the instrument. The amount of $^{14}\text{CO}_2$ and the rate of the increase of gas production are directly proportional to the growth of the organism. While these systems use different means of detecting mycobacterial growth, they all have similar performances and operational characteristics. Advantages include recovery rates superior to those of conventional solid media (147) and, except for the Bactec 460 system, incubation and continuous monitoring in a closed system and electronic data management. However, contamination with normal flora or environmental organisms can be a problem in all these liquid-based systems, likely due to the enrichments added to the media. Moderate-to-heavy turbidity is most often a clear sign of contamination. Also, the automated systems have large footprints and constant power requirements due to incubation and continual monitoring of tubes and/or bottles within the instruments. While liquid broth systems have proven to be essential for strengthening TB diagnoses, care, and treatment in many countries (111, 168, 205), the purchasing of, maintenance of, and providing large rooms to safely accommodate these instruments can be extremely difficult for most public health laboratories in resource-poor countries.

Rapid Identification of Cultured *M. tuberculosis* Complex Isolates

Nucleic acid hybridization methods. The introduction of the AccuProbe (Gen-Probe Inc., San Diego, CA) nucleic acid hybridization kits represented a quantum leap in the rapid identification of organisms of the MTBC (results within 2 h, as soon as sufficient biomass is obtained following growth in culture) (111). Because DNA/RNA probe assays do not include an amplification step, these tests are not sensitive enough to be used directly on clinical specimens. However, DNA/RNA probes are usually capable of identifying *M. tuberculosis* in contaminated liquid cultures (depending on the extent of the contamination), given that they have a sensitivity and a specificity of nearly 100% when at least 10^5 mycobacteria are present (47, 219). However, the introduction of lateral-flow-based immune assays for the rapid identification of MTBC organisms from growth on solid or liquid medium has significantly diminished the use of DNA probes in resource-poor countries, since the probe assay requires more time, manipulation, contamination controls, and costly reagents and instruments.

Lateral flow assays. Lateral flow immunochromatographic assays have been developed for the rapid identification of members of the MTBC. There are three products that are currently available: the Capilia TB rapid diagnostic test (Taun Laboratories Inc., South Korea), the SD Bioline TB AG MPT64 Rapid test (Standard Diagnostics, Yongin, South Korea), and the BD MGIT TBc identification test (BD Diagnostics, Sparks, MD). These assays are based on the detection of the presence of the MTBC-specific protein MPT64 in culture isolates. Because the detection limit has been found to be approximately 10^5 CFU/ml, bacterial growth on solid or liquid medium is required prior to testing (137). Mouse monoclonal

anti-MPT64 antibodies are immobilized on a nitrocellulose membrane, and a second antibody recognizing another epitope of MPT64 (conjugated with colloidal gold particles) is used for antigen capture and detection in a sandwich-type assay (137, 208). Studies have demonstrated that the lateral flow tests provide results in 15 min and compare favorably with other conventional phenotypic or molecular methods (overall sensitivity of 98.6% and specificity of 97.9%) (208). The assay has been found to be rapid and easy using isolates grown in either liquid or solid medium, without a requirement for special equipment. However, since the tests are performed on viable cells, the tests require BSL-3 laboratory infrastructure and equipment, including a BSC and laboratory staff who are well trained in BSL-3 safety procedures. Therefore, further studies are warranted to determine if chemically inactivated or heat-inactivated isolates can reliably be identified with this assay so that these specimens could be tested outside the biosafety cabinet. Less than 100% sensitivity appears due to mutations in the MTP64 gene found by DNA sequencing in some isolates identified by conventional methods as belonging to the MTBC but negative in the lateral flow assays. In addition, some BCG strains are known to lack MPB64 antigen production (67, 127, 210).

Line probe assays. Recently, three commercial (but not yet approved by the U.S. Food and Drug Administration [FDA]) PCR-based kits have been made available for the identification of mycobacteria previously grown on liquid or solid medium. The Inno-LiPA Mycobacteria assay (Innogenetics NV, Ghent, Belgium) targets the 16S-23S DNA spacer region for the identification of mycobacteria, while the GenoType Mycobacterium CM (for the 14 clinically most relevant mycobacteria) and AS (for 16 further clinically relevant mycobacteria) assays (Hain Lifescience GmbH, Nehren, Germany) target the 23S rRNA genes (132, 166, 182). The methods from both companies are based on the hybridization of labeled amplicons (amplified by PCR from *M. tuberculosis* DNA present in patient specimens) to oligonucleotide probes arranged on a membrane strip. These systems are capable of the simultaneous detection and identification of MTBC organisms and other potentially pathogenic mycobacteria. A major advantage of this methodology is the possibility of the simultaneous detection of mixed mycobacterial or contaminated cultures that may not be revealed by lateral-flow-based rapid identification tests (132, 166, 182). The identification of mixed cultures is critical since drug susceptibility test mixtures inoculated with cultures of *M. tuberculosis* that are not pure may result in incorrect drug-resistant reports due to the growth of a contaminant or various species of nontuberculous mycobacteria (NTM).

DNA sequencing. While DNA sequencing of variable genomic regions offers a rapid and accurate identification of mycobacteria, this method is not yet practical for use outside academic settings in most resource-poor countries. DNA sequencing methods are based on the determination of species-specific nucleotide sequences, which are then compared to known quality-controlled sequences from in-house or commercially available databases (142). These assays are based on the amplification of one or more genes (16S rRNA genes, *rpoB*, *gyrB*, *hsp65*, *secA1*, the gene encoding the 32-kDa protein, or the 16S-23S rRNA gene spacer) (82, 85–88, 118, 142, 163, 165, 169, 185, 216). There are major advantages in sequencing *rpoB*

since the sequence data can be used to identify MTBC organisms and also NTM, and it can also detect mutations in *M. tuberculosis rpoB* that confer RIF resistance (173). Pyrosequencing, a technique based on the real-time sequencing of short sequences during DNA synthesis (sequencing by synthesis), has been described as a promising new tool for the rapid identification of mycobacteria not only with growth-positive cultures but also using processed smear-positive sputum specimens (35, 59, 65, 184). Although this method is easier to perform and less expensive than traditional sequencing, the shorter sequences are not as discriminating. By using automated sequencers and well-trained staff, sequencing assays can be completed and identification results can be reported within 1 to 3 days. However, the installation, maintenance, and running of automated DNA sequencing on a daily basis are currently too expensive and labor-intensive for use in resource-poor settings.

Differentiation of organisms within the MTBC. Each of the closely related members of the *M. tuberculosis* complex (MTBC) can cause tuberculosis in humans; however, most cases worldwide are caused by *M. tuberculosis*. The other primarily human pathogens, *Mycobacterium africanum* and *Mycobacterium canettii*, are seen less often and are more restricted to patients from sub-Saharan Africa (189). Variants most closely associated with animal hosts but known to also infect humans are *M. bovis* (from cattle), *Mycobacterium caprae* (from goats), and, in rare cases, *M. pinnipedii* (from seals) and *M. microti* (from field voles) (5, 34, 190). *M. bovis* BCG strains, used as live vaccines or for the treatment of bladder cancer, can sometimes cause tuberculosis in immunocompromised patients.

Over the years, the prevalence of pulmonary TB due to *M. bovis* in certain parts of Africa has been reported with increasing frequency, generating concern about the potential risk for HIV-infected persons. In Nigeria, the prevalence of pulmonary tuberculosis from *M. bovis* varies from 10% in the southern part of the country to about 15% in the more nomadic north (107). Similarly to most African communities, in Nigeria cow milk is often pooled and consumed without any form of pasteurization or purification (74). The risk of human infection with *M. bovis* in milk-consuming pastoral communities has been unknown due to a limited knowledge of the disease in cattle and the risks associated with milk consumption. However, a recent unpublished survey from the northern region of Nigeria compared isolates of *M. bovis* isolated from humans and cattle and found the isolates from both to be genetically identical. Of additional interest is the recent discovery of a new clonal complex of *M. bovis* named Africa 1, which has been found in high frequency in four West African countries, including Nigeria (124).

Thus, the accurate molecular identification of species of the MTBC may help to guide public health and clinical decisions more effectively. However, routinely used phenotypic analyses cannot accurately be used for discrimination, but two molecular approaches have been found to differentiate the members of the MTBC: regions of difference (RDs) and single-nucleotide polymorphisms (SNPs) (19, 61). First, comparative genomic studies of the members of the MTBC revealed RDs that represented sequential losses of genetic material in the course of evolution from a common progenitor strain (19, 105,

122). The detection of the presence or absence of several RDs has been used to differentiate the members of the MTBC in patient isolates using PCR-based approaches (138, 194). More recently, a real-time PCR application that can further simplify the procedure was reported (154). The higher sensitivity of real-time PCR may allow the direct application of the assay for smear-positive clinical specimens. In addition to the RDs, DNA sequence analysis identified discriminatory SNPs in four genes (*oxyR*, *pncA*, *gyrB*, and *hsp65*) that differentiated certain members of the MTBC (61, 72).

The Genotype MTBC test (Hain Lifescience GmbH, Nehren, Germany) uses a combination of these two approaches to differentiate MTBC isolates. This line probe assay (LPA) is based on a combination of *gyrB* gene SNPs and the RD1 deletion of *M. bovis* BCG (105, 161, 162). As described above for the LPA, specific oligonucleotides targeting these regions are immobilized on membrane strips, and amplicons derived from a multiplex PCR bind to these probes during hybridization and are detected as specific patterns. The company literature indicates that the system will correctly differentiate *M. tuberculosis*, *M. africanum*, *M. microti*, *M. caprae*, *M. bovis*, and *M. bovis* BCG; however, *M. tuberculosis* and the rarely seen *M. canettii* cannot be differentiated. Recently, the test was validated for the direct testing of processed smear-positive clinical specimens (171). The assay is fast, with minimal technical requirements; uses the same platforms as those for other LPAs; and can be incorporated in a manual or automated manner into the routine of laboratories already using amplification procedures.

Immunodiagnosis of Tuberculosis

Serology. In the last decade, serology-based tests using formats well suited for resource-poor countries (performed without specialized equipment and with minimal training) have been successfully developed for many infectious diseases (e.g., HIV and malaria) (175, 179). In these assays, antigens are typically precoated in lines across a nitrocellulose membrane to which serum or whole blood samples are applied. Antigen-antibody reactions are visualized on the lines using anti-human antibody bound to substances such as colloidal gold. The tests take only minutes to perform. These technologies are very attractive candidates for the simple, accurate, inexpensive, and, ideally, point-of-care (POC) diagnosis of TB.

However, attempts to successfully develop sensitive and specific serological tests for the diagnosis of TB have been ongoing for decades, without a major breakthrough. There are currently over 40 rapid serologic TB tests (that use various antigenic compositions to detect patients' antibodies) available in many low- and middle-income countries. These tests differ in a number of features, including antigen composition, antigen source (e.g., native or recombinant), chemical composition (e.g., protein, carbohydrate, or lipid), extent and manner of purification of the antigen(s), and class of immunoglobulin detected (e.g., IgG, IgM, or IgA). The performance and reproducibility of 19 of these commercially available rapid *M. tuberculosis*-specific antibody detection tests were recently compared in a laboratory-based evaluation with 355 well-characterized archived serum samples aimed at identifying promising candidates for rapid TB diagnostics (210). In these stud-

ies, the sensitivity of the tests ranged from 1% to 60% (mean, 27%), and the specificity ranged from 53% to 98.7%, compared against a combined reference standard of mycobacterial culture and clinical follow-up. In general, tests with a high specificity (>95%) had a very low sensitivity (0.97 to 21%). The test performance was poorer for patients with sputum smear-negative TB ($P = 0.0006$ for sensitivity and specificity) and for HIV-positive patients ($P = <0.0001$ for sensitivity; $P = 0.44$ for specificity). Some products showed a high variability in lot-to-lot, run-to-run, operator-to-operator, and interreader comparisons. Twelve of the tests (63%) were rated as easy to use and therefore appropriate for use in primary health care settings in developing countries; however, none of the assays performed well enough to replace AFB smear microscopy.

A subsequent review of commercial serological tests for the diagnosis of TB currently sold and used in countries where the disease is endemic also concluded that there was a lack of evidence of effectiveness (175). Overall, commercial tests varied widely in sensitivity (10 to 90%) and specificity (47 to 100%). Accuracy was higher in smear-positive than in smear-negative specimens, and specificity was higher in healthy populations than in patients for whom TB disease was initially suspected and subsequently ruled out. There were insufficient data to determine the accuracy of most commercial tests in smear-negative patients, and none of the assays performed well enough to replace AFB smear microscopy. Also, there were no studies of commercial tests of sufficient quality to enable their evaluation for HIV-positive patients or for children, the groups for which these tests could be most useful. Thus, it was concluded that these tests must be considered to have little to no role in the diagnosis of TB at this time.

The choice of immune biomarkers for the diagnosis of TB has possibly been biased for decades by the panels of antigens and antibodies preselected for analysis. Progress in antibody detection has also been limited by the heterogeneity of host immunological responses to TB antigens. Furthermore, the profile of antigenic proteins of *M. tuberculosis* recognized by antibodies differs at different stages of infection and disease progression. Current research on new biomarkers is exploiting the availability of advanced technological platforms that allow an interrogation of all proteins that can be synthesized by viable TB organisms. It is expected that these new approaches can provide the basis for novel diagnostic biomarkers to more reliably diagnose active TB. Almost certainly, accurate diagnostic tests for TB will need to be based on a combination of biomarkers to increase their predictive value.

Gamma interferon release assays. One-third of the world's population is estimated to be infected with latent tuberculosis (LTBI). This infection will lead to active disease in 10% of these individuals during their lifetimes; however, if the infected individuals are immunocompromised (e.g., HIV infected), 8 to 10% of them will develop tuberculosis disease within a year (43). Therefore, it is imperative to accurately diagnose and treat patients with LTBI and also to predict who among the infected will develop the disease. The currently used tuberculin skin test (TST) is quite inexpensive and has been used worldwide for many years. The TST measures a delayed-type hypersensitivity response to purified protein derivative (PPD), a crude mixture of antigens from the members of the MTBC (and also NTM). Unfortunately, the TST has a low sensitivity

(e.g., in patients with either immune suppression or very advanced disease) and a low specificity (e.g., in BCG-vaccinated individuals or in NTM-exposed populations) (73). Also, the administration and reading of the TST require a certain amount of expertise that, when lacking, may result in erroneous interpretations.

The recent introduction of gamma interferon (IFN- γ) release assays (IGRAs) has provided an alternative test for the diagnosis of LTBI. Currently, two commercial assays are available, the Quantiferon-TB assay (Cellestis Ltd., Carnegie, Victoria, Australia) and the T SPOT-TB assay (Oxford Immunotec, Oxford, United Kingdom) (94, 109). These tests measure the IFN- γ release from T cells after stimulation by *M. tuberculosis*-specific antigens via an enzyme-linked immunosorbent assay (ELISA) or an enzyme-linked immunospot (ELISPOT) assay. The first-generation Quantiferon-TB assay is a whole-blood test that measures IFN- γ release to PPD with an ELISA; this test was approved by the FDA (109). The Quantiferon-TB Gold test is the enhanced form of the assay, which uses the *M. tuberculosis*-specific ESAT6 and CFP10 antigens instead of PPD. Subsequently, this test and the T SPOT-TB assay also received FDA approval (108). An even newer version of the test is the Quantiferon-TB Gold InTube assay, which entails simpler sample preparation and is further enhanced by the addition of the TB7.7 (p4) antigen. The T SPOT-TB assay, which requires the separation of peripheral blood mononuclear cells, detects IFN- γ release after exposure to ESAT6 and CFP10 with an ELISPOT assay. A major drawback of either method is that the incubation with the antigens must be initiated within 8 to 16 h following blood collection.

According to a recent systematic review of the performance of these commercially available assays as well as a significant number of in-house assays, IGRAs that use a cocktail of *M. tuberculosis*-specific antigens may offer several advantages over conventional TST (136). These advantages have higher sensitivity and specificity, better correlation with exposure to the MTBC organism, lower cross-reactivity with BCG and NTM, and the potential to identify individuals with LTBI who are at an elevated risk of developing active disease (136). However, in the absence of a true gold standard, the reliable determination of sensitivity and specificity is very difficult to predict. In addition, there is not yet adequate evidence for the accuracy of the IFN- γ tests for specific populations, including HIV-infected or other immunocompromised patients, children, extrapulmonary tuberculosis cases, MDR tuberculosis cases, or patients with NTM infections, or for monitoring patient responses to treatment (136, 160). Their cost-benefit is not well proven, although two recent studies indicated that IGRAs may be cost-effective and positively affect the control of the disease (39, 214). Therefore, the potential use of these assays in the clinical routine awaits further confirmatory studies, especially in high-incidence, resource-poor settings. It is also likely that IGRAs may be more appropriately performed in the routine clinical laboratory than in a TB laboratory.

NEW TECHNOLOGIES IN THE PIPELINE FOR DETECTION OF TB

Urinary antigen detection (LAM). A promising immune-based approach consists of directly detecting an *M. tuberculosis*

antigen, lipoarabinomannan (LAM), in urine. LAM is a heat-stable glycolipid specific to mycobacteria that is released by metabolically active bacteria, filtered by the kidney, and found in the urine of patients with active TB (36). LAM was originally detected in serum, but this test was limited by immune complex formation. Conveniently, urine is an easier specimen to collect than sputum and may be less variable in quality and safer to handle. Recent studies have evaluated commercially available tests that detect LAM in urine by antigen capture ELISA for the diagnosis of tuberculosis (98, 125, 158). The LAM ELISA sensitivity ranged from 38% to 50.7% for TB cases, with a specificity range of 87.8% to 89%, as confirmed by smear microscopy, solid culture, and/or liquid culture. The sensitivity was significantly higher for patients with HIV-related TB (62.0%) and for females (66.7%), whereas sensitivity for smear-negative patients was low (28%) for combined HIV-positive and -negative patients. The commercially available generation of LAM ELISAs has adequate specificity but sub-optimal sensitivity and does not appear to be useful as an independent diagnostic test to confirm or exclude pulmonary TB in either HIV-infected or noninfected patients. However, these assays could increase case finding if combined with smear microscopy and/or culture in settings of high HIV prevalence and could be of particular value in diagnosing TB in HIV-coinfected patients with CD4 cell counts of less than 100 cells/ml. The urine LAM assay also has a reasonably high negative predictive value, suggesting that a negative result could be used as evidence against active TB (36). Urine-based TB diagnosis is definitively attractive, and further work is needed to improve the LAM assay. There are several versions of new LAM ELISAs in development, including in-tube ELISA and dipstick methods. There is potential for further development and simplification, resulting in a lateral flow test. Lateral flow test implementation could significantly increase case finding through improved access to testing. The use of larger volumes of urine, alternative methods of extraction that avoid high temperatures, and stabilizing agents that can be added to urine at the time of collection may improve sensitivity. It is also important to evaluate other urine antigens that may have diagnostic accuracy (36).

Volatile markers. Breath analysis for the diagnosis of TB is an attractive alternative, especially since breath sampling is a noninvasive technique, which is totally painless and more agreeable to patients. It was well known in the past that patients harboring specific infectious diseases could liberate odors characteristic of the disease stage. Chromatographic techniques have identified a large number of volatile organic compounds (VOCs) in human clinical specimens that could serve as disease markers for the monitoring of disease stages. The use of smell in clinical diagnosis has been rediscovered over the last few years due to major advances in odor-sensing technology and artificial intelligence (21, 144). VOC patterns produced by *M. tuberculosis* from sputum samples were recently examined *in vitro* and *in situ* using an electronic nose based on a 14-sensor conducting polymer array (143). Using neural network analysis and cross-validation, the electronic nose successfully identified 100% of the TB cultures from among others and was able to discriminate between sputum containing either TB alone, *Mycobacterium avium*, *Pseudomonas aeruginosa*, or a mixed infection. Another set of breath

VOCs structurally similar to the most abundant VOCs observed for cultures of mycobacteria was able to distinguish between hospitalized patients whose sputum cultures were positive and those whose cultures were negative for mycobacterial infection (150). Breath testing with artificial-nose technology combined with multivariate analysis of data employing pattern recognition analysis could potentially provide a new method for the rapid, accurate, and noninvasive identification of patients at a high risk of active pulmonary tuberculosis and to distinguish between those with positive and those with negative sputum cultures. However, since these findings were derived from a comparatively small pilot study, confirmation will require additional studies with larger numbers of patients.

Bead-based methods for detection and identification. Most of the reported new methodologies which incorporate immunomagnetic beads to detect and identify bacteria still fail in routine clinical settings because they require extensive specimen processing, use complex measurement setups, or are not easily scalable for clinical demands. However, they constitute promising technological advances toward more sensitive and faster pathogen detection. In these approaches, beads are coated with monoclonal or polyclonal antibodies or with non-specific markers such as lectin and serve to capture or target bacterial pathogens, which are then concentrated and detected by different systems.

Microsens Medtech Ltd. (London, United Kingdom) developed a kit with paramagnetic beads coated with a chemical ligand that binds to mycobacteria. MTBC cells can be captured from the sputum of patients and then stained with auramine phenol for fluorescent microscopy. Although evaluation studies of the kit are still pending, this simple approach could enhance the sensitivity of microscopy by concentrating the sample, without the need for centrifugation. Magnetic micro-particles have also been coated with antimycobacterial polyclonal antibodies for the fast, efficient, and specific capture and concentration of mycobacteria. This technique performs well to concentrate mycobacteria from water samples, which can then be identified directly by PCR or other rapid techniques (90). More recently, core-shell magnetic nanoparticles coated with anti-BCG monoclonal antibodies were used to target *M. bovis* BCG cells spiked into human sputa (99). The superparamagnetically captured bacteria were concentrated into a microfluidic chamber and detected by a miniaturized nuclear magnetic resonance system, with a sensitivity as high as 20 CFU/ml of sputum detected in less than 30 min. With the potential for the rapid and simple operation of portable instrumentation, detection platforms using immunomagnetic beads, as reported previously (99), could be ideal point-of-care diagnostic tools, especially in resource-limited settings.

Simplified smart flow cytometry. With the demand to carry out CD4 assays on the multitudes of individuals requiring antiretroviral therapy, many efforts have been made to develop simple, cost-effective, quality-assured, sustainable, and industry-supported flow cytometry apparatuses and tests, referred to as "smart flow cytometry" (S-FC). S-FC has been successfully introduced in "resource-restricted" countries in Africa and the Caribbean for CD4⁺ T cell counting for patients with HIV/AIDS (76). Recently, S-FC has also been demonstrated to be compatible with antigen-specific cellular immune response assays that can help to rapidly diagnose active TB in both HIV-

negative and HIV-TB-coinfected individuals. Flow cytometric assays for the gamma interferon synthetic capacity of CD4 T cells work well even in a BCG-vaccinated population, are unaffected by disease site or HIV status, and show significant advantages over the ELISA (e.g., Quantiferon Gold) and the ELISPOT assay, particularly for patients who are coinfecting with HIV and TB (78). The S-FC technology could be of great utility in situations where sputum specimens are difficult to obtain or the sputum smear is negative, especially if combined with traditional microbiological methods (18, 77, 178).

Broad nucleic acid amplification-mass spectrometry. Advances in instrumentation (separation and mass analysis), ionization techniques, and bioinformatics have contributed to the successful applications of mass spectrometry (MS) for rapid and high-throughput pathogen identification and characterization (71). Electrospray ionization-tandem mass spectrometry (ESI-MS) has been successfully applied to the identification of clinical isolates of the MTBC based on their mycolic acid profiles (174). Other investigators coupled multilocus amplification of nucleic acid targets from pathogens with high-performance ESI-MS (PCR-ESI-MS) and base composition digital analysis to develop a system called the Ibis T5000 system (Abbott Laboratories, Abbott Park, IL) (45). This system has been designed to enable the simultaneous identification and quantification of a broad set of pathogens, including all known bacteria, all major groups of pathogenic fungi, and the major families of viruses, that cause disease in humans and animals along with the detection of virulence factors and antibiotic resistance markers. Recent studies confirmed that the system performs well to detect and identify a variety of bacterial and viral pathogens in clinical samples, including blood (46, 58). Analysis using the Ibis T5000 system provides detailed information that is analogous to that obtained using a microarray or parallel DNA sequencing instrument. This technological platform has the great potential to integrate the identification of multiple pathogens with costs for consumables that are comparable to those of current PCR-based molecular diagnostic methods (44). Its high-capital cost is, however, a main drawback at present, especially in resource-poor settings.

DETECTION OF DRUG RESISTANCE IN TB

In 2007, there were an estimated 500,000 cases of multidrug-resistant TB (MDR-TB), with 27 countries accounting for 85% of all such cases. The countries that ranked first to fifth in total numbers of MDR-TB cases in 2007 were India, China, the Russian Federation, South Africa, and Bangladesh. By November 2009, over 50 countries and territories had reported at least one case of extensively drug-resistant TB (XDR-TB) (206, 212). Drug resistance in *M. tuberculosis* occurs when resistant mutants, naturally occurring in the population, are selected by inadequate or interrupted drug treatment (141). Since drug resistance develops gradually, both resistant and susceptible cells will be present in the patient during the early stages of the development of resistance. Clinically significant resistance has been defined as *in vitro* growth in the presence of the critical concentration of the drug, which is equal to or greater than 1% of the growth in the absence of the drug (84, 198). The presence of this proportion of drug-resistant cells in the population indicates that therapeutic success is unlikely

TABLE 3. Critical concentrations for drug susceptibility testing of *Mycobacterium tuberculosis* in solid and liquid media^a

Drug	Critical concn (μg/ml)					
	7H10 solid medium	LJ solid medium	MGIT	VersaTREK	MB/BacT Alert	Bactec 460
First line						
INH	0.2, 1	0.2	0.1, 0.4	0.1, 0.4	1.0	0.1, 0.4
RIF	1	40	1	1.0	1.0	2
EMB	5, 10	2	5.0, 7.5	5, 8	2.5	2.5, 7.5
PZA	NA	NA	100	NR	NR	100
SM	2, 10	4	1, 4	8	1	2, 6
Second line						
Amikacin	4.0	NR	1.5	NR	NR	1
Capreomycin	10	40	2.5–3.0	NR	NR	1.25
Ciprofloxacin	2	2	1	NR	NR	2
Ethionamide	5	40	5	NR	NR	1.25
Kanamycin	5	30	NR	NR	NR	5
Levofloxacin	2	2	1.5–2.0	NR	NR	NR
Ofloxacin	2	2	2	NR	NR	2
PAS ^b	2	1	NR	NR	NR	4

^a See references 100, 111, 141, 168, 198, and 205. NA, PZA has not been validated for testing on solid media; NR, critical concentrations have not been established or reported.

^b PAS, *para*-aminosalicylic acid.

with the use of that drug. The critical concentration of a drug is the concentration that inhibits the growth of most wild-type cells within a population without affecting the growth of resistant cells that might be present. For some drugs, both a low concentration and a high concentration of a drug should be tested, either together or sequentially. If resistance is seen at the low concentration, then the higher concentration should be tested. This approach is especially important for INH, one of the most effective first-line drugs, since it is often more informative for patient management to test resistance at both concentrations. If the isolate is resistant to INH at the low concentration and susceptible at the high concentration, the Clinical and Laboratory Standards Institute (CLSI) recommends that the clinician should be notified “that patients infected with strains exhibiting this level of INH resistance may benefit from continuing therapy with INH” (31). This recommendation is meant to discourage the premature discontinuation of INH therapy, since INH has been found to be the best of the essential anti-TB drugs in preventing resistance in companion drugs due to its high bactericidal activity and its large therapeutic margin, which is unaffected by pH (50, 116).

Although most specimens collected from patients with tuberculosis show the presence of a single strain, patients can be infected with more than one strain of the MTBC during a given episode of tuberculosis. An average of 17% of the new tuberculosis cases in high-incidence settings can be multiple infections (159, 196); these can seriously confuse subsequent interpretations of drug susceptibility testing results as well as interpretations of epidemiological connections among patients (17). This possibility must be considered during interpretations of drug susceptibility test results.

Summary of Phenotypic Methods

Agar proportion. As stated above, clinically significant resistance to all antituberculosis drugs has been defined as *in vitro* growth, in the presence of the critical concentration of the

drug, that is equal to or greater than 1% of the growth in the absence of the drug (147). The agar proportion method uses the seeding of drug-free and drug-containing solid media with equal quantities of two dilutions of a standardized inoculum. Care must be taken to ensure that a homogeneous suspension of cells is used so that growths on the different media can be accurately compared. Countable CFU should be present for at least one of the dilutions on drug-free media. The number of separate, countable CFU found on drug-containing media compared with those of the same dilution on drug-free media is expressed as a percentage. Strains of TB showing growth on drug-containing media that exceeds 1% of the growth on drug-free media are considered resistant to that drug. The critical concentrations of drugs have been established for the most common media by utilizing the MICs for wild-type strains and differ based on which medium is used (Table 3). The most common medium for the agar proportion method in resource-limited countries is LJ medium; however, the CLSI considers this medium to be unsuitable for susceptibility testing due to uncertainty about the potency of drugs following inspissation and also because components present in the eggs or the medium may negatively affect some drugs (31). Both the CDC and CLSI recommend that Middlebrook 7H10 agar supplemented with OADC be used as the standard medium for the agar proportion assay (198).

Automated liquid broth systems. The liquid broth systems for TB growth detection described above also allow the testing of the susceptibility of members of the MTBC to the major primary drugs (INH, RIF, EMB, and, sometimes, PZA) and the secondary agent SM. For the MGIT system (Becton Dickinson), following inoculation with a homogeneous suspension of TB and incubation at 37°C, the fluorescence of drug-containing tubes is compared with that of the drug-free growth control. When using the Bactec MGIT 960 instrument, the ratio of the relative growth of the drug-containing tube and that of the growth control tube is determined by software

algorithms, and growth in the drug-containing tube equal to or greater than that of the growth control is reported as resistant. The VersaTREK system (Trek Diagnostic Systems Inc.) detects mycobacterial growth by the detection of pressure changes within the headspace above the liquid medium in a sealed bottle. An isolate is considered resistant if the time of detection is the same as, or up to 3 days after, the time when the control bottle has turned positive. The MB/BacT Alert instrument (bioMérieux) measures carbon dioxide release into the medium, detected by a gas-permeable sensor embedded at the bottom of the culture bottles. Resistance is reported if the drug-containing bottle is positive before or on the same day as the bottle containing a 1:100 dilution of the inoculum. The critical concentrations of first- and second-line drugs used in the systems are listed in Table 3. Of the liquid medium systems, only the MGIT and the Bactec 460 systems have been validated for second-line drug testing (100, 168, 198).

Quality control for DST. To ensure the accuracy of susceptibility testing results, it is good practice to confirm all drug resistances either by a second method or by a second laboratory. However, this confirmatory step should not delay reporting of the detection of resistance to the health care provider and to the TB control program. Quality control tests should be performed at least once a week in laboratories that perform tests daily or weekly or, if tests are performed less frequently, whenever a patient isolate is tested. *M. tuberculosis* H37Rv (ATCC 27294) or H37Ra (ATCC 25177) or another well-characterized strain that is susceptible to all standard antituberculosis agents should be used for quality control purposes. Because of safety concerns, it is not recommended that clinical isolates that are MDR or resistant to high concentrations of drugs be used for quality control.

Direct Culture and DST

Indirect susceptibility testing, described above, requires a primary culture of specimens and the isolation of *M. tuberculosis*, followed by drug susceptibility testing. The long turnaround time for the detection of MDR-TB has been shortened by the introduction of liquid culture; however, these systems may not be available in the regions of the world where the need is greatest. Recent work has evaluated novel rapid direct tests in which decontaminated sputum specimens are inoculated directly into drug-free and drug-containing medium or amplified by molecular methods for the detection of MDR-TB.

Microscopic observed drug susceptibility testing. The microscopic observed drug susceptibility (MODS) testing assay is a manual liquid culture technique that uses an inverted light microscope to detect microscopic colonies of *M. tuberculosis* grown in culture medium in sealed multiwell plastic plates (120). The characteristic corded growth pattern of members of the MTBC is used for identification. Sputum specimens previously digested, decontaminated, and concentrated are inoculated into drug-free and drug-containing media, followed by incubation. This approach allows direct susceptibility testing of INH and RIF. The MODS assay has been demonstrated in multicenter studies to be more sensitive and faster for the detection of TB in sputum than automated liquid culture systems and solid culture (7, 13, and 26 days, respectively). Because the MODS assay utilizes direct drug susceptibility test-

ing, the median time to detection of MDR-TB is also 7 days (208). Similarly to all TB culture systems, BSL-2 facilities are required for safe specimen processing using centrifugation and inoculation of cultures. BSL-3 facilities are required if the plates are opened for further testing, such as for the confirmation of the identification of TB. Laboratories using the MODS system require a functioning BSC, a safety centrifuge, an incubator, an inverted light microscope, and supplemented liquid media (Middlebrook 7H9 broth with OADC and PANTA). The laboratory staff needs practice to accurately differentiate the microcolonies of TB from those of other *Mycobacterium* species. Further studies are needed to adequately validate this procedure for safe use in the field.

Thin-layer agar. The thin-layer agar (TLA) technique uses a standard light microscope to simultaneously detect the growth of TB colonies and determine INH and RIF resistance directly from processed sputum specimens. Plates containing a thin layer of supplemented Middlebrook 7H10 or 7H11 solid medium either drug free or containing drugs are inoculated with sputum specimens, incubated, and examined microscopically on alternative days for the first 2 weeks and weekly thereafter. TB microcolonies can be detected in as little as 7 days, with results for DST between 10 and 15 days. Although faster than solid culture on LJ medium and relatively inexpensive compared to other culture methods, the TLA technique is not as fast or as sensitive as liquid culture. However, the specificity and predictive values for INH and RIF resistance have been reported to be 100% (164), suggesting that this assay would be a favorable alternative when liquid culture systems are not available. As with the MODS assay, BSL-2 (and possibly BSL-3) facilities are required, and the disposal of biohazardous material is needed, although solid media may be not as prone to biohazard spills or cross-contamination as liquid media.

Nitrate reductase assay (Griess method). The nitrate reductase assay (NRA) is a liquid or solid medium technique that measures nitrate reduction by members of the MTBC to indicate growth and to indicate resistance to INH and RIF (4). The medium is supplemented with potassium or sodium nitrate, which will be reduced to nitrite by actively growing *M. tuberculosis* bacteria and can be detected as a pink-purple color when a detection reagent (Griess reagent) is added to the tube. Since the NRA method uses nitrate reduction as a sign of growth, results are detected earlier than by examination of microcolonies on solid medium. However, the Griess reagent kills the organisms when added to the tubes, so multiple tubes must be inoculated if further testing is necessary. In addition, not all members of the MTBC reduce nitrate, so the presence of nitrate-negative AFB may require further testing.

Recently, a literature review and meta-analysis were conducted to analyze and compare the sensitivities and specificities of direct susceptibility testing by the NRA and the MODS test against conventional indirect susceptibility testing (24). The pooled sensitivities and specificities for the detection of resistance to INH were 94% and 100% for the NRA and 92% and 96% for the MODS assay, respectively. For the detection of resistance to RIF, the pooled sensitivities and specificities were 99% and 100% for the NRA and 96% and 96% for the MODS assay, respectively. The average time for all results was 23 days for the NRA (range, 18 to 28 days) and 21 days for the

MODS assay (range, 15 to 29 days) (24). Again, it was concluded that the direct testing of INH and RIF susceptibility of members of the MTBC has sensitivity and specificity comparable to those of indirect susceptibility testing.

Bacteriophage methods. Results of a recent meta-analysis analyzing data from 19 studies on RIF susceptibility testing of clinical isolates using bacteriophage methods revealed that while these tests have a relatively high sensitivity, the specificity is more variable (134), with false overdiagnosing of RIF-resistant tuberculosis. In addition, when performed directly on sputum specimens, the sensitivity and specificity of the tests were found to vary widely (2, 23). Therefore, such tests are currently not considered to be accurate enough for routine diagnostic applications.

Molecular Drug Susceptibility Testing Methods

Genetic studies have determined that in the MTBC, resistance to antimycobacterial drugs is the consequence of spontaneous mutations in genes encoding either the target of the drug or enzymes involved in drug activation. Resistance-associated random chromosomal mutations have been described for all first-line drugs (66, 218); however, no single genetic alteration has yet been found that results in the polyresistant or MDR phenotype. Rather, polyresistance or multidrug resistance develops through the sequential acquisition of mutations at multiple loci. Unfortunately, the multiple locations of the mutations for each drug and the fact that resistance to a particular drug can involve a mutation in more than one gene or in several distinct loci of the same gene have made the molecular detection of drug resistance a challenging task.

For example, INH resistance-associated mutations have been found in the mycobacterial catalase gene (*katG*) and in genes encoding enzymes that participate in the synthesis of the cell wall mycolic acids (i.e., *inhA*) (155). However, approximately 15 to 25% of INH-resistant isolates do not contain mutations in any of these regions, indicating that other sites must also be involved in resistance to this drug. Likewise, only about 70% of EMB-resistant clinical isolates of *M. tuberculosis* contain mutations in *embB*, the drug target involved in the biosynthesis of the cell wall component arabinan (139, 172, 218). RIF resistance is more easily assessed using molecular methods, since more than 96% of the RIF-resistant isolates of *M. tuberculosis* contain mutations in a well-defined, 81-bp (27-codon) central region of the gene encoding the beta subunit of RNA polymerase (*rpoB*) (8, 139, 218). However, mutations associated with RIF resistance can also occur in other regions of *rpoB* albeit less frequently (i.e., the V146F mutation in the N-terminal region) (63). Similarly, for PZA resistance, up to 97% of all PZA-resistant clinical isolates tested carry a mutation in the putative promoter region or the structural gene (*pncA*) that encodes pyrazinamidase (PZase), the enzyme responsible for the activation of the PZA prodrug (139, 172, 218).

The selection of the method used to detect mutations associated with drug resistance can be challenging. Since DNA sequencing of the amplified product not only detects but also identifies the specific mutation, this method serves as the gold standard. DNA sequencing also differentiates between mutations that result in an amino acid change and mutations that

are silent. However, the use of DNA sequencing for the precise detection of mutations is labor-intensive and requires a high level of staff expertise as well as expensive equipment. The majority of mutation detection methods that are practical for use in resource-poor countries with high burdens of TB do not use DNA sequencing. The validation of these assays has usually begun by testing for RIF resistance because of the clinical significance of resistance to this drug and its association with MDR-TB and also because close to 100% of resistance is due to mutations in the 81-bp region of a single target gene (218).

Line probe assays. The PCR-based reverse-hybridization line probe assay (Inno-LiPA Rif TB test; Innogenetics NV, Ghent, Belgium) has been developed for the rapid detection of RIF resistance (37, 173). The LiPA strip consists of 10 oligonucleotide probes: one is specific for the MTBC, five are partially overlapping wild-type probes that span the region at positions 509 to 534 of the *rpoB* gene, and four probes are specific for amplicons carrying the most common *rpoB* mutations (D516V, H526Y, H526D, and S531L) (37, 173). According to a recent review, the sensitivities of the LPA are above 95% for clinical isolates and 80% for smear-positive clinical specimens, with 100% specificity (102).

Hain Lifescience (Nehren, Germany) has also developed a commercially available DNA strip assay. In a study using 103 clinical isolates of MDR-TB, the first-generation Genotype MTBDR test was capable of the simultaneous detection of RIF resistance-associated mutations in the 81-bp core region of *rpoB* (similarly to the LiPA assay) and the presence of INH resistance-associated mutations in codon 315 of *katG* (70). The sensitivities of this test were found to be 99% for *rpoB* and 88.4% for codon 315 of *katG*-associated mutations, with a specificity of 100% for both genes. This assay was also reliably applied directly to smear-positive specimens, with sensitivities of 84.2% for *katG* mutations in high-INH-resistant strains, 5.7% for *katG* mutations in low-INH-resistant strains, and 96.2% for *rpoB* mutations (170). However, that study also found that DNA sequencing analysis of *inhA* in isolates grown from these processed specimens was able to increase the capability of predicting INH resistance by 51.5% among strains with low INH resistance (170). This led the company to include *inhA* in the second-generation Genotype MTB DRplus assay, enabling the identification of an additional 11.6% INH-resistant strains compared to results obtained with the first-generation assay (68, 114). The routine performance of the Genotype MTB DRplus assay was evaluated with smear-positive specimens in a high-volume diagnostic setting in South Africa and was found to have sensitivities of 98.9% for the detection of RIF resistance, 94.7% for the detection of INH resistance, and 98.8% for the detection of MDR-TB (7). In a meta-analysis, the pooled sensitivity and specificity for the MTB DRplus assay were 98.4% and 98.9%, respectively (103). These tests could be used in an alternative algorithm to screen AFB smear-positive specimens and limit the performance of culture and DST only when the LPA result predicts resistance to either INH or RIF or both (MDR-TB). However, one has to keep in mind that this approach may be applied to only those settings that are screening for MDR-TB patients, since the assay does not detect resistances to other drugs.

Based on these findings, in 2008 the WHO endorsed the use of molecular LPAs for the rapid detection of MDR-TB in

at-risk patients at the country level (209). In 2009, the UNIAID-funded EXPANDx-TB program was launched by the WHO Global Laboratory Initiative (GLI), the Foundation for Innovative New Diagnostics (FIND), and the WHO Global Drug Facility to support the implementation of WHO-approved LPAs and liquid culture-based growth detection in high-burden countries for rapid MDR-TB detection among at-risk patients.

Most recently, the Genotype MTB DRsl assay was released by the manufacturer in order to support the rapid detection of fluoroquinolone, ethambutol, and aminoglycoside resistance by targeting the *gyrA*, *embB*, and *rrs* genes for the fast identification of patients with XDR-TB. One study with a limited number of drug-resistant isolates (63 strains) has shown sensitivities of 59% for EMB, 83.3% for amikacin, 86.8% for capreomycin, and 90.2% for ofloxacin (69). The assay was also evaluated with 64 processed smear-positive specimens, with sensitivities of 38% for EMB, 75% for amikacin, 87.5% for capreomycin, and 88.9% for ofloxacin. The unusually low sensitivity of the test for EMB may reflect the difference between "highly resistant" isolates selected from a repository versus clinical specimens and the shortcomings of the breakpoints and different performances of conventional DST for EMB with these strains (141). It is also important that this study did not evaluate the performance of the test on kanamycin-resistant strains and especially on strains resistant to newer fluoroquinolones (levofloxacin and moxifloxacin).

Only preliminary results are available for 24 isolates of newly diagnosed patients from the Ukraine tested with the RDB-2185 TB Resistance LPA test (Genome Identification Diagnostics GmbH, Strassberg, Germany), which is designed to detect resistance-associated mutations for INH, RIF, aminoglycosides, and fluoroquinolones. This assay is promising the prompt detection of MDR-TB and XDR-TB in one step. However, further data are necessary to validate the method, especially directly on clinical specimens (13).

Real-time PCR. The most rapid new method, real-time PCR, uses hybridization with fluorescence-labeled probes or molecular beacons during amplification; this hybridization can be followed by melting profiles to detect resistance-associated mutations (32, 38, 48, 151, 152, 181). Major advantages of real-time PCR are that the whole amplification mixture is analyzed for the presence of amplicons (versus LPAs, where only a portion of the amplified product is analyzed on the strips) and the reaction is running in a closed system, and therefore, the chance of contamination is significantly diminished.

Cepheid Inc. (Sunnyvale, CA) in collaboration with FIND developed the single-tube, molecular beacon-based, real-time PCR Xpert MTB assay for the detection of RIF-resistant *M. tuberculosis* isolates. The assay does not require sputum processing but can be used on chemically inactivated specimens directly. Therefore, it is simple, is less time-consuming (can be performed within 2 h), and does not require special expertise and biosafety requirements. The test uses self-contained cartridge fluidics and a walk-away instrument platform that may host from 1 to 16 cartridges. These features may enable a wide decentralization of the method compared to LPAs. Although the system requires minimal handling, and each of the 16

modules within the instrument may operate independently, the throughput of the system can be significantly lower than that of those molecular methods that use high-capacity conventional thermocyclers with a capacity for 96 tubes.

Recently, the Xpert MTB system was used to test 107 unprocessed sputum specimens, with reported sensitivities of 100% for smear-positive specimens, 84.6% for smear-negative solid culture-positive specimens, and 71.7% for smear-negative and solid-plus-liquid-culture-positive specimens (64). The specificity was 100% for all after correcting for conventional susceptibility testing errors. In a more recent larger study, 741 original sputum specimens that were positive upon culture were tested, with an overall sensitivity of 97.6% (12). The sensitivity of the testing of a single sample from culture-positive specimens was 92.2%, with increases to 96.0% when two specimens were tested and to 99.8% when three specimens were tested. Similarly, the sensitivity of a single sample for smear-negative, culture-positive specimens was 72.5%, with increases to 85.1% when testing two samples and to 90.2% when testing three samples. The overall specificities of the test to detect TB were 99.2% for a single test, 98.6% for two tests, and 98.1% for three tests. The test showed a 99.1% sensitivity and a 100% specificity for the detection of RIF resistance.

In spite of these encouraging results, this study has some limitations. The test is aimed for use in district and subdistrict levels of laboratories and health care facilities; however, the study was performed in central laboratories where staff have significant expertise in TB testing. At peripheral laboratories, technicians may not be proficient in liquid-handling procedures or instrument usage. Also, the sensitivity and specificity of the assay for TB detection should have taken into consideration the clinical diagnosis of the patients, including the radiologic findings, especially since 105 specimens from patients with negative culture results but clinical and radiological diagnoses of tuberculosis were not considered for further analysis. Such an analysis would have provided some field evidence of potential false-positive and false-negative results related to use of this test and also information on guiding the development of EQA programs.

Overall, the Xpert MTB system has been shown to provide excellent performance with lower biosafety requirements and simpler contamination control; however, the major drawback of real-time PCR-based methods is that the instrumentation and reagent costs at present are significantly higher than those of the LPAs or other array-based technologies. While recognizing major funding implications, in 2010 the WHO endorsed the use of this assay as an initial diagnostic test for suspected cases of MDR-TB or HIV-TB and as a follow-up test for microscopy on AFB smear-negative suspects in settings where MDR-TB or HIV is a lesser concern (213).

Array-based technologies. The recently developed QIAplex test (Qiagen) uses a target-enriched multiplex PCR to simultaneously amplify and detect 24 mutations in the *katG*, *inhA*, *rpoB*, *rrs*, *rpsL*, and *embB* genes associated with INH, RIF, SM, and EMB resistance (55). This assay targets several mutations using a Luminex 100 instrument-based suspension array for the detection of amplicons. At present, the system was evaluated with 196 clinical isolates and showed sensitivities and specificities of 85.4% and 96.1% for INH, 94.4% and 99.4% for RIF, 69.6% and 99.2% for SM, 50.0% and 98.8% for EMB, and

86.7% and 100.0% for MDR-TB, respectively. This system offers the rapid detection not only of MDR-TB but also of non-MDR-TB with resistance to any drug(s), an advantage for countries whose TB control programs require the identification and management of all cases of TB drug resistance within the country.

Another promising development is the use of oligonucleotide, or DNA, microarray platforms (chips), which contain a large collection of DNA capture probes. For hybridization to DNA chips, the DNA or RNA target in the sample must be amplified and labeled with a fluorescent dye for detection with a scanning fluorometer (112, 145, 167). Because microarrays have a capacity of hundreds of thousands of probes, a single chip can be used to identify the species of *Mycobacterium* and to detect mutations in all known target genes for antimycobacterial drugs. The potential of the DNA chip technology was demonstrated some time ago in two studies (57, 183). The GeneChip DNA probe array (Affymetrix, Santa Clara, CA) uses sequence databases for species identifications (16S rRNA sequence patterns) and for alleles involved in drug resistance. Epidemiological markers such as single-nucleotide polymorphisms (SNPs) or direct repeat regions could also be added to the array for tracing transmission links between strains. In two more recent applications, microarray methods were successfully used following multiplex PCR for the rapid detection of INH and RIF resistance and following an allele-specific PCR for the rapid detection of INH, RIF, and SM resistance (62, 180). With the potential to perform one-step testing of clinical specimens for mycobacterial identification, drug susceptibility, and genotyping, the DNA chip platform represents a promising, but not yet optimized, advance for the clinical mycobacteriology laboratory (191).

Advantages and disadvantages of molecular drug susceptibility testing. The major advantage of molecular methods is that they are fast, may be adapted for high throughput, and can be robust, with significantly fewer manipulations and technical skills needed than for growth detection and conventional drug susceptibility testing. These methods require mainly relatively simple-to-use equipment (LPAs, LAMP, and Xpert TB) that can also serve as a platform for testing specimens for other diseases or purposes (e.g., detection of MTBC isolates, other human pathogens, or mutations associated with drug resistance). In addition, molecular assays do not need viable bacilli; therefore, specimen transport conditions do not have as much impact on the test outcome as with culture, and specimens can be shipped by regular mail.

When using molecular assays to test clinical specimens (especially those suspected of containing TB), a BSL-2 laboratory is required, and when testing viable mycobacterial isolates, the preparation of the isolates for DNA isolation should be performed in a BSL-3 laboratory along with use of appropriate personal protective equipment (208, 209). Only after heat killing of the organism and DNA isolation can the sample be considered noninfectious and moved to the molecular laboratory. It is important to bear these prerequisites in mind when integrating molecular drug resistance testing for TB in existing molecular facilities that have been established for other diseases.

If organized on a one-on-one basis, the training required for establishing a laboratory staff member's proficiency in a particular molecular method can be accomplished within a few weeks. On the other hand, while it may be easy to perform the

test, it is often more challenging for trainees to become proficient in properly reading the molecular results, in interpreting the results correctly for the clinician and TB control, and in troubleshooting when problems arise. Specifically, the proper reading and interpretation of different LPA patterns have been found to be a major problem for even technically skilled laboratorians in the field. Confusion often arises if a wild-type band is missing but a corresponding mutation band is not present. It is likely that this banding pattern is the result of a mutation other than one of the common ones identified by the specific probes on the strip. However, there is a slight possibility that the pattern represents a silent mutation that does not result in an amino acid change (170). In this instance, it will be important for the interpretation of the molecular results to be considered in light of conventional phenotypic DST results. In fact, it is suggested that mutations observed with molecular methods should be confirmed with a phenotypic assay. Molecular mechanisms are not fully identified and understood for all drugs, and therefore, mutations in known targets may not be associated with phenotypic drug resistance in all cases (e.g., INH, aminoglycosides, and fluoroquinolones, etc.). Moreover, even if the known molecular mechanism is showing a high level of association with the respective phenotypic drug resistance (*rpoB* and RIF resistance, *pncA* and PZA resistance, and *katG* and INH resistance), the molecular test may not include probes for all possible mutations. For example, most molecular tests (including the LPAs, molecular beacons, or real-time PCR) are interrogating only the 81-bp hot spot of *rpoB* and only codon 315 of *katG*, while resistance-associated mutations may occur in other parts of these genes (173, 217).

However, in some instances, molecular tests may detect clinically relevant drug resistance more accurately than phenotypic tests. It was shown previously that certain mutations in the *rpoB* gene (Leu511Pro, Asp516Tyr, and Leu533Pro) showed slowly increasing growth indices in the presence of 2 mg/ml RIF in the Bactec 460TB system (140, 173). RIF-susceptible strains do not grow in the presence of this concentration of drug, while resistant strains grow much more rapidly. In addition, in contrast to drug-susceptible controls, these strains were shown to be resistant to RIF at 0.5 mg/ml, indicating elevated MICs. A recent report on highly discordant RIF susceptibility results during proficiency testing (PT) highlights the importance of determining the frequency of strains with mutations such as these and the shortcomings of conventional methods (188). The prevalence of these strains was 22% of all *rpoB* mutations in a recent systematic sampling from Hong Kong.

A critically important question is the clinical significance of predicting drug resistance by molecular methods. There are two important issues that require attention in this regard. The first issue is the detection limit of molecular tests when testing a heteroresistant (mixed wild-type and mutant) population of cells. In the beginning of the development of drug resistance in a member of the MTBC, only a relatively small proportion of resistant bacteria are present in the total population. Drug-resistant tuberculosis will influence the treatment outcome of the patient only if the amount of these strains reaches a clinically significant proportion, previously described as being 1% of the population when performing the agar proportion method. However, the ability of molecular tests to detect a clinically significant proportion of resistant cells in the patient

specimen has not been properly addressed. Evaluations of these tests have focused primarily on the analytical sensitivity (total amount of DNA detected). Recently, the detection limit of the Inno-LiPA Rif LPA was assessed with mixtures of DNA from RIF-susceptible and -resistant strains and compared to the detection limit with an in-house multiplex PCR-based macroarray (192). The Inno-LiPA Rif LPA was able to detect 20% RIF-resistant DNA in the presence of 80% wild-type DNA, while the macroarray was able to detect 2% resistant DNA. In a similar experiment, where heat-killed mixtures of RIF-susceptible H37Rv and H37Rv *rpoB* mutants were mixed at different ratios (from 0 to 100% resistant cells), the MTB DRplus LPA was able to detect as few as 5% resistant cells (A. Somoskovi, Z. Rey, and K. Tholen, unpublished data). In a similar study, the Xpert TB system was used for the detection of RIF resistance in a mixture of DNA from susceptible and resistant strains. Resistance detection was found to be dependent on the particular mutation and required between 65% and 100% of the mutant DNA to be present in the sample for a 95% certainty of detection (10). The lack of an ability to detect lower levels of resistant cells in a population may limit the use of this test for the detection of the early stages of development of RIF resistance.

The second issue relates to the ability of molecular methods to provide information on the level of resistance, particularly to INH. As described above, the MTB DRplus LPA includes two genetic regions associated with resistance to INH, *katG* and *inhA*. There is general agreement that mutations in *katG* tend to confer high-level resistance to INH, while mutations in *inhA* or its promoter region generally confer low-level INH resistance (155, 195, 218). Thus, identifying and reporting whether the specific mutation was detected in *katG* or *inhA* would provide information to the clinician as to whether the patient would benefit from continuing treatment with a higher dose of INH. It is essential not to discontinue INH unnecessarily, especially since INH has potent bactericidal activity that rapidly reduces the infectivity of newly diagnosed cases (50, 116, 195).

QUALITY ASSURANCE SCHEMES FOR TB DIAGNOSTICS

National External Quality Assessment Programs for AFB Smear Microscopy

To ensure the quality of AFB smear microscopy performed at each testing site, it is essential for each country to provide and support a functioning external quality assessment (EQA) program. EQA programs ideally include three components: blinded rechecking of smears, site supervision, and external proficiency panel testing (6); however, the establishment and administration of these programs in resource-poor countries are often challenging. The American Society for Microbiology (ASM) has worked in Botswana in collaboration with the CDC/BOTUSA and the Botswana MOH to provide technical assistance for the development and presentation of a training program for AFB smear microscopy and to strengthen their existing national EQA scheme (NEQAS). In Botswana, the TB laboratory network consists of approximately 45 smear microscopy laboratories located at private, government, mine, and missionary hospitals. The ASM worked with the national TB

reference laboratory (NTRL) EQA unit to finalize all technical and systems procedures, forms for reporting and feedback, and analysis templates for proficiency panel testing, supervisory visits, and blinded rechecking.

The Botswana NTRL EQA unit staff members were trained in the above-described procedures and developed a NEQAS Annual Plan for 2008 to 2009. The plan objectives were to distribute one round of panel testing to all laboratories, resume annual supervisory visits to all laboratories, and set up a program of blinded rechecking for AFB slides. In 2008 and 2009, more than 95% of the laboratories sent back results for the proficiency panel testing, and the percentage of laboratories receiving a passing score (>80%) increased from 73% to 100% during the 2-year period so that all laboratories achieved a passing score in 2009. Although supervisory visits did not occur at all laboratories, the EQA unit did visit more than twice as many laboratories in 2009 compared to 2008. The new blinded rechecking program was also successfully piloted at five facilities during 2009. The increase in proficiency test scores indicates that these EQA components, supplemented with refresher training of laboratory technical staff and supervisors, have positively impacted the quality of AFB smear microscopy performance in Botswana. Challenges such as shortages of staff for the NTRL EQA unit, supply procurement issues, and a need for better coordination with the Botswana NTCP highlight the importance of collaboration among key stakeholders and partners.

In 2008, the national public health reference laboratory (NPHRL) in Port-au-Prince, Haiti, was designated by the MOH to establish a national EQA program for AFB smear microscopy, with the ASM invited to assist the NPHRL with the implementation of the blinded rechecking component. The first step was a national situational analysis of the TB laboratory network, which the NPHRL performed with ASM assistance. The thorough evaluation of 238 smear microscopy laboratories allowed a proposed redefinition of the network, since some laboratories were noted as having very low to no activity. Following the evaluation, an action plan for introducing blinded rechecking was developed, and the roles of the NPHRL and 10 geographical laboratories selected to serve as intermediate reference laboratories were defined.

Since 2008, the ASM has presented two didactic and practical training events at the NPHRL to sensitize select Haitian laboratorians to the requirements for implementing blinded rechecking and has worked with the NPHRL to pilot blinded rechecking at eight microscopy centers in Haiti's western region. The blinded rechecking pilot consisted of collecting approximately 600 routine smears from the eight centers during 2009 and verifying 10% of any discordant smears. The pilot results, which included six of the eight centers, revealed that 67% of the laboratories had achieved a passing score. Corrective actions consisting of examining staining procedures and retraining were implemented in 2009. Recently, the Massachusetts State Department of Public Health Laboratory was assigned to function as Haiti's WHO supranational TB reference laboratory (SRL). The SRL and ASM are working to coordinate activities to improve EQA for AFB smear microscopy in Haiti, with expansion of the EQA process to the national level as the next step.

Indicators To Monitor the Quality of TB Culture

There are limited opportunities for TB culture and drug susceptibility test EQA in resource-poor countries, although the WHO SRL network does have a DST proficiency testing program for its member laboratories. Difficulties in expanding this service globally involve labor-intensive preparatory work and the high cost and strict packaging requirements for shipping drug-resistant isolates of TB. To overcome this lack of available EQA, performance indicators were recently implemented for mycobacterial culture as a means of internal quality assessment in three laboratories in Thailand (110). The study was a demonstration project to enhance the surveillance and treatment of TB in Thailand by the Thailand TB Active Surveillance Network, in collaboration with the Thai Ministry of Public Health, the Bangkok Metropolitan administration, Japan's Research Institute for Tuberculosis, Thai local health authorities, and the CDC. The following monthly indicators were found to be essential:

- Monitor date collected and date received in the laboratory. The timely submission of specimens will positively impact specimen integrity.
- Determine the percentage of AFB-positive smears reported and the percentage of smear-positive and culture-positive as well as smear-negative and culture-positive specimens. Rates of smear positivity among those suspected of having TB generally range between 5 and 20%, and fluctuations from an established baseline percentage should be investigated.
- Determine the percentage of specimens reported as being contaminated. Contamination rates of 3 to 5% for solid media and less than 10% for liquid media are generally considered acceptable. Higher contamination rates may indicate incomplete processing, while rates of less than 3% may indicate overly harsh processing procedures. Another important indicator is whether the contamination is seen on solid or liquid media or both.
- Determine the turnaround time for smear, culture, and DST. The timely reporting of results is essential to ensure the prompt initiation of therapy for the benefit of the patient and to reduce disease transmission. Standard turnaround time indicators should be based on the methods in use in each laboratory.

The results from the study in Thailand demonstrated that the monitoring of standardized indicators provided credibility to the laboratory results, ensured the optimization of the methods used for TB smear and culture, and assisted managers in identifying staff training needs (110).

Quality Assurance of Molecular/LPA Testing

The monitoring of the performance of molecular testing should be based minimally on appropriate internal quality control but preferably on a quality assurance program. The internal quality control of molecular testing should address performing adequate testing and contamination controls, regular monitoring and documentation of reagent performance (lot-to-lot testing), routine temperature check of temperature-controlled instruments, and periodic maintenance and calibration

of equipment. Note that for many tests, laboratories must prepare their own controls from positive cultures. This may lead to differences between laboratories and difficulties in comparing results since obtaining standard ATCC control strains is often challenging for laboratories in the field. The GenoType MTB DRplus and DRsl and the Cepheid Xpert Rif systems contain internal controls for amplification; however, the target sequences are not from *M. tuberculosis*. Controls are also needed for the specimen preparation part of the procedure, and thus, there is a need for an appropriate control capable of monitoring the test performance from specimen preparation to detection. Therefore, it is a good laboratory practice to include one negative control that, together with the clinical specimens, is subjected to DNA extraction and all subsequent steps of the procedure. A second negative control should be included in the amplification step to detect any contamination during reagent preparation and from that point on.

Also, the testing of sentinel controls is a very simple and useful approach for detecting amplicons or other aerosols that may potentially contaminate laboratories. Three or more tubes can be filled with 100 μ l of sterile distilled water and placed in different areas of the laboratory with their caps open. After a period of time, the tubes can be collected and tested by the routine molecular assay in use in the laboratory. Any positivity would indicate crossover contamination in the laboratory. It is suggested that a quarterly sentinel test be done in all three areas of the molecular laboratory.

In addition to testing controls, molecular laboratories need to establish and regularly monitor quality indicators using up-to-date paper-based or electronic laboratory registries. Indicators to be monitored include turnaround time from specimen receipt to reporting of results, the number of successful and unsuccessful amplifications, the presence of particular mutations, the number of specimens with mixed drug-resistant and -susceptible patterns, and a list of specimens tested in each batch or by the same technician. An inherent problem is that these or other quality indicators have not been standardized and implemented in the routine even in countries with low incidences of TB. Further studies on the refinement and validation of the quality indicators are warranted.

External quality assessment is the second pillar of quality assurance. At present, there is no well-established EQA program for molecular testing for TB. However, recently, the FIND India office has developed a simple proficiency testing scheme for LPA (156). In this scheme, proficiency is assessed on the basis of the random blinded testing of 50 smear-positive specimens in duplicate. Testing sites are required to demonstrate a higher-than-95% concordance on blinded duplicate specimens, a lower-than-10% proportion of unsuccessful amplification, and no contamination with the negative control. This simple approach allows the identification of any basic problems in regard to proficiency and also to measure the efficacy of corrective actions. A similar evaluation may also be performed by using well-characterized, noninfectious DNA panels that would include the following: at least one pansusceptible strain, one RIF-monoresistant strain with a common *rpoB* mutation, one RIF-monoresistant strain with a less common *rpoB* mutation, one MDR strain, one common *katG* mutant INH-monoresistant strain, one common *inhA* mutant INH-monoresistant strain, and one NTM strain.

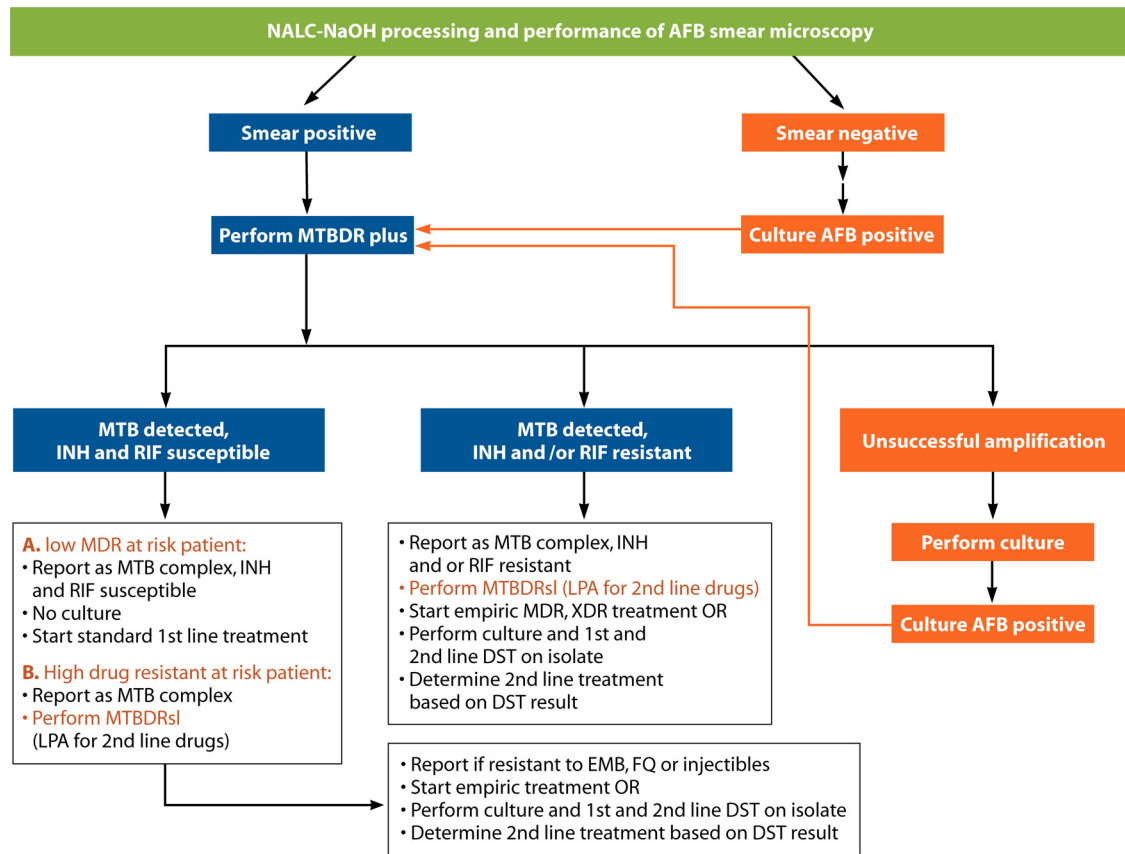


FIG. 1. Basic diagnostic algorithm to link the molecular line probe assay with solid culture- and liquid culture-based growth detection and susceptibility testing. AFB, acid-fast bacilli; DST, drug susceptibility testing; EMB, ethambutol; FQ, fluoroquinolones; INH, isoniazid; LPA, line probe assay; MDR, multidrug resistant; MTB, *Mycobacterium tuberculosis*; MTB DRplus, LPA for *M. tuberculosis* and resistance to RIF and INH; MTB DRsl, LPA for second-line *M. tuberculosis* drugs; RIF, rifampin; XDR, extensively drug resistant.

With the LPAs it is also important to assess the interpretation of the strip patterns. For that, either a paper- or Web-based test can be developed using LPA strip image repositories. It is important to test the proficiency of interpretation not only with strips with clear banding patterns but also with strips with weak or inconclusive results by the blinded rechecking of strips by either a second examiner or a supervisor, similarly to that performed for AFB smear microscopy. Since only the captured images of the strips need to be submitted for rechecking, data collection and submission to supervisory centers would be facilitated.

ALGORITHMS FOR RAPID TB DIAGNOSIS AND DETECTION OF DRUG RESISTANCE

As discussed above, several novel or nonconventional methods have been introduced to detect and identify MTBC isolates in recent years. In spite of all these efforts, there is still no single test that can stand alone for the laboratory diagnosis of TB. Thus, to obtain the best performance of laboratory tests and generate complete and rapid information on a particular patient, it is very important to link complementary techniques in an adequate laboratory diagnostic algorithm. The MOH, NTCP, and NTRL should work together to develop a national algorithm, with the first step being the determination of the

target patient population. Diagnostic algorithms in industrialized countries usually aim to identify any patient with tuberculosis, while with limited resources and a higher incidence of HIV-associated and drug-resistant tuberculosis, it is necessary to focus on those patients that are at risk of infection with drug-resistant TB, MDR-TB, or XDR-TB (e.g., treatment failure or default, HIV-infected cases, or contacts of drug-resistant cases). However, it is very important that clinicians use an adequate clinical screening approach to identify such patients (25). Otherwise, laboratory services will be overburdened with unnecessary testing, and the clinical significance of laboratory results may be negatively influenced (i.e., high rate of drug-susceptible results for those suspected of having a “drug-resistant” infection).

Figure 1 shows an example of an algorithm using WHO-approved tests for the rapid laboratory diagnosis of patients suspected of having MDR-TB. Based on clinical evaluation, specimens from patients suspected of having MDR-TB are first tested by AFB smear microscopy. If the result is positive, then LPA testing can be used to confirm the presence of the MTBC isolate and identify resistance to INH and/or RIF. If molecular testing indicates resistance, there can be two options. The first option is to start empirical treatment and monitor its efficacy by performing culture 3 months later. In cases where the cul-

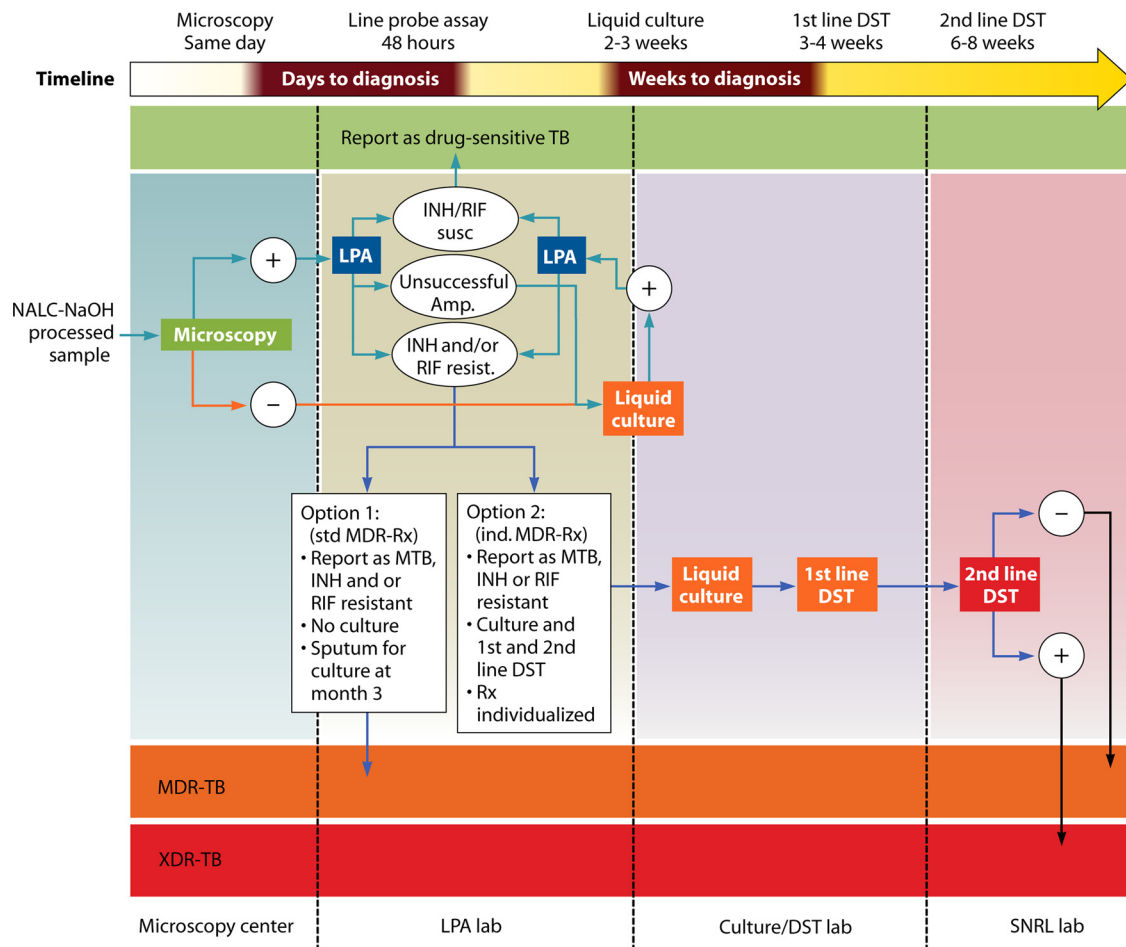


FIG. 2. Synchronization of a basic diagnostic algorithm (linked diagnostic tests) with different levels of diagnostic services. DST, drug susceptibility testing; INH, isoniazid; LPA, line probe assay; MDR, multidrug resistant; MTB, *Mycobacterium tuberculosis*; MTB DRplus, LPA for *M. tuberculosis* and resistance to RIF and INH; RIF, rifampin; SNRL, supranational reference laboratory; XDR, extensively drug resistant.

ture after 3 months of therapy is positive (indicating treatment failure), then second-line conventional DST can be performed. The second option is to immediately carry out rapid liquid-based second-line DST and determine an individualized regimen based on the results. Although not yet WHO approved, at this point the use of the Hain MTB DRsl assay for second-line molecular testing may provide additional information in order to rapidly identify XDR-TB patients for immediate respiratory isolation and treatment optimization. This assay may also help to identify resistance in non-MDR-TB or non-XDR-TB patients, since it can provide information on fluoroquinolone, aminoglycoside, and EMB drug resistance. Several resource-poor countries target the identification and treatment not only of MDR-TB and XDR-TB but also of patients with strains resistant to other drugs. If initial LPA results indicate the presence of an INH- and RIF-susceptible strain, then standard first-line treatment may be started to shorten the turnaround time of treatment initiation and save culture resources. However, the sensitivity of the LPA may be lower for low-INH-resistant strains. In this case, the second-line LPA may be useful if a country's national TB policy targets the identification and treatment of cases resistant to any drug, although only

patients who are at risk for drug resistance should be targeted for testing.

At present, the LPAs are approved for smear-positive specimens only. Therefore, specimens of smear-negative at-risk patients must be cultured in liquid medium. Positive isolates can then be tested by the LPA to further shorten the turnaround time. Smear-positive specimens with inconclusive results in the LPA should also be inoculated into liquid culture as well. As described above, smear-negative TB is more common in HIV-infected patients. The rapid molecular diagnosis of this arm of the algorithm may be expedited by the use of the Cepheid Xpert MTB/RIF real-time PCR system, since it has a higher sensitivity for these specimens (12). However, similarly to LPAs, molecular resistance detected by this method will require access to confirmatory testing by second-line molecular LPA and more conventional DST capacity.

The development of a laboratory diagnostic algorithm requires a further step, namely, adapting this linked system to the different levels of the existing diagnostic network and determining which tests should be performed at a particular level of service (Fig. 2). This requires that the laboratory network be integrated, including the establishment of a reliable specimen

collection, transportation, and laboratory information management system (LIMS). In such a system the initial testing results should direct the next necessary step to be taken (additional specimen collection, specimen forwarded for further testing, and therapy initiation), and the determination of subsequent diagnostic test selection and location should follow to avoid delays and ensure system efficacy. For example, if two sputa are collected at a microscopy center and one or both of them are AFB positive, one can be forwarded for molecular LPA testing (Fig. 1). If the LPA detects a susceptible strain, the result should be immediately provided to the microscopy center, and the treatment provider should begin first-line drug treatment as soon as possible. If the LPA predicts drug resistance, the report should contain instructions to submit an additional specimen to a higher level of the laboratory system for conventional DST (Fig. 2). The projection of the algorithm to the diagnostic network should also reflect expected turnaround times for any testing and related reporting. In the near future, simpler and more sensitive molecular assays in the pipeline, such as the Xpert MTB/RIF system, will allow the implementation of similar but smear-independent algorithms in a more decentralized fashion.

CHALLENGES/OPPORTUNITIES FOR PROVIDING QUALITY TB LABORATORY SERVICES IN RESOURCE-POOR COUNTRIES

Establishment of a National Laboratory Policy and Strategic Plan

National laboratory systems (ideally composed of a tiered network of diagnostic laboratories and a national public health reference laboratory that includes the TB reference laboratory) must be capable of providing accurate, timely, and cost-effective testing that is in line with each country's programmatic goals and available clinical interventions, as determined, supported, and coordinated by the MOH. The development and implementation of a national laboratory policy and strategic plan are important steps to enable countries to work with partners and donors to define specific objectives, set standards, and make decisions on allocating funding and personnel for sustainable laboratory services (128). The entire process involves the following steps: engagement of the country and laboratory partners in discussions; formation of coordinating and technical committees; development, budgeting, and implementation of the national laboratory policy and strategic plan; and, finally, reviews and evaluations of progress. Laboratory infrastructure, test menus, technology, platforms, and commodities should be standardized in each country. This approach requires strong leadership and coordination by the local ministries involved in health and procurement and includes many benefits, such as reduced procurement costs for commodities, easier implementation of quality assurance programs, and integration of testing using shared equipment. For example, partners could work together in developing specialized facilities that would accommodate instruments for molecular diagnostics of TB, HIV, and other diseases rather than building separate facilities dedicated to only one disease. As stressed throughout the manuscript, the effective detection and treatment of TB require accurate and timely laboratory diag-

nostics. To achieve this goal, clinical laboratory services have to be supported by the MOH and must have a policy that establishes minimal laboratory standards and a well-developed, well-organized, and well-managed laboratory network to reach different affected populations where they live.

Tiered laboratory network. In resource-limited settings, a tiered laboratory network provides a flexible and cost-effective model for providing clinical services to patients at different levels. Typically, a network is composed of laboratories at three levels, each one defined by the types and complexity of testing performed, based on the availability of appropriate personnel and resources.

- The interconnected peripheral-level laboratories are located outside the country's main population centers at district and/or local levels. This level serves as a focal point for TB case detection by performing AFB smear microscopy and perhaps other integrated laboratory testing and refers more complex tests for TB, e.g., liquid culture or DST, to a higher-level laboratory.
- Intermediate-level laboratories perform at least AFB smear microscopy and more complex integrated laboratory testing. They should be responsible for the provision of EQA for AFB smear microscopy and HIV rapid testing performed at the lower-level (peripheral) laboratories.
- At the central or national level, as an integral part of the national public health laboratory, the national TB reference laboratory (NTRL) provides and coordinates all TB clinical laboratory services, including the surveillance of MDR-TB. The NTRL is responsible for the coordination of AFB smear microscopy training and EQA for the network. Also, the NTRL often conducts operational research and liaises with WHO supranational reference laboratories (SNRLs) for collaboration and EQA.

Country-specific diagnostic priorities as outlined by the MOH in the national laboratory policy must always be a key consideration in designing a national laboratory network (128). The country's size must be considered in determining the number of levels within a network, as the network structure greatly affects its governance. For example, for some countries a two-tiered network, with a simple referral system, might provide fewer logistical challenges and prove more effective than a three-tiered network. Alternatively, an additional peripheral level where only specimen collection and preliminary processing are done might also be considered. The ability to perform testing of clinical specimens, including those from patients suspected of having TB, depends heavily on a good referral and reporting system. A well-designed national tiered system of laboratories provides a flexible mechanism for developing such a system. This is especially crucial for the lower-tiered peripheral laboratories. An approach that has been successfully used in South Africa to define and map out the laboratory referral network is through the use of global positioning system (GPS) devices. In addition, once the peripheral laboratories have been identified, specimen referral systems can be coordinated nationally, as has been successfully piloted in Uganda through Posta Uganda, the government postal system.

Coordination with the national TB control program. A well-organized laboratory network should be a crucial component of each country's NTCP. Unfortunately, this is not the case in

many countries, where there is little or no communication between staff in the NTCP and staff in the NTRL. This is often due to the lack of understanding and acknowledgment of the important role of the TB laboratory network within the NTCP. Bridging this gap requires substantial leadership and political support from the MOH, the ministry with responsibility for the direction and program management of both the NTCP and NTRL. In this regard, some useful country-level integration activities are as follows:

- A senior laboratory staff member should be included in the development and implementation of national laboratory and public health strategic planning.
- National laboratory officers with decision-making authority should be appointed within the MOH and the NTCP.
- The NTCP should include representatives from the NTRL during all planning and budgeting meetings.
- TB control activities should be coordinated with laboratory activities.

From the MOH perspective, it is important to have the “big-picture” outlook during strategic planning. The design, planning, implementation, and management of the NTRL, and any TB laboratory network in general, must be considered during the early stages of program development. It is considerably more difficult and costly to make adjustments after the network is already in place. The MOH must provide leadership and creative guidance in addressing key operational aspects of the NTCP, including how it can best collaborate with the NTRL.

Defining priorities. Although there are global reference documents, including those of the WHO, to guide countries in strengthening their TB laboratory network, defining national priorities and objectives during the development of the national laboratory policy and strategic plan must be step 1. However, the goal should not be to replicate some ideal existing model. Rather, each country should define its own priorities and needs according to its available resources and epidemiological setting as long as requirements for successful patient care and treatment form the basis of the program. Key questions to consider may include the following:

- What are the top technical and/or managerial priorities for the NTRL?
- What practical steps can the NTRL take to ensure proper training/mentoring for quality TB case detection at the point-of-care level?
- How can the NTRL work with the NTCP to leverage resources by integrating laboratory services for TB with those of other key public health programs, such as those for malaria and HIV/AIDS?

In many countries, vertical funding of public health programs (TB, malaria, and HIV/AIDS) makes it challenging to share and/or exchange relevant information about laboratory and clinical services. Coordination between key public health programs can fulfill a vital advocacy role, raising awareness among partners and donors of the need to strengthen laboratory services. Ideally, these programs should work closely to strengthen their national laboratory network to support all diseases. Currently, efforts are being made to address issues related to the integration and harmonization of laboratory

services. For example, the WHO, through its Stop TB program (http://www.tbevidence.org/documents/rescentre/books/GLI_Roadmap_2010.pdf), the Global Laboratory Initiative (GLI), and the New Diagnostics Working Group, has developed a roadmap to guide member countries in strengthening their laboratory capacity for TB diagnostic services. The WHO programs promote the strengthening and scale-up of laboratory services to respond to the diagnostic challenges of TB-HIV coinfection and MDR-TB through the following efforts:

- Global policy guidance on laboratory technology and best practices.
- Laboratory advocacy and resource mobilization.
- Laboratory capacity development and coordination.
- Integration of laboratory networks.
- Laboratory standardization and quality assurance.
- Coordination of technical assistance.
- Roadmaps for skill shifting by laboratory staff.
- Knowledge sharing.

Leveraging of technical resources. In countries with no existing or a weak TB laboratory network, it is critical to optimize technical resources from all sectors. All countries should cast a wide net to identify the best technical base possible, both inside and outside their NTCP/MOH. For TB diagnostics and related clinical services, expertise can be greatly leveraged by collaborating with national academic and research laboratories in both the public and private sectors. It will be imperative that the nature and scope of the partnerships be established from the beginning, with formally defined roles and responsibilities. It is also important to establish links with international laboratory networks. Ideally, each NTRL should be connected to a WHO SNRL, from which it receives training and to which the NTRL is accountable in terms of technical proficiency.

Establishing information flow through LIMSs. A laboratory information management system (LIMS) is the backbone for the management of laboratory data on samples, instruments, results, and quality indicators. Therefore, the LIMS can also support accounting and accreditation functions. The development of software that can meet these tasks is a highly complex and demanding task and particularly so for resource-limited and high-HIV- and high-TB-prevalence settings, where budgets are limited, expertise in information technology is hard to come by, and internet access is still limited and relatively expensive. Therefore, laboratory and program managers need to think carefully about their needs and priorities in order to make a decision on the implementation of a LIMS. For example, while open-source software for LIMS is available (Bika Lab Systems, Cape Town, South Africa), there are costs associated with implementation and maintenance. Furthermore, in addition to supporting traditional laboratory-related functions, a LIMS should also support effective case management for TB and HIV by the following: (i) directing the stepwise completion of complementary laboratory tests at different levels of the diagnostic network and (ii) initiating adequate therapy as soon as supporting decision-making information is available. As described above in the section on diagnostic algorithms, this requires close collaboration between clinicians, laboratorians, and the commu-

nity to ensure that the appropriate laboratory exams are ordered, that results are quickly available, and that they are acted upon for clinical decision-making.

Decisions on the implementation of a LIMS should not be taken in isolation but in the context of the other needs of TB, HIV, and other disease control programs and with opportunities for input from the other stakeholders, including clinicians and patients, who will also be impacted by the use of the LIMS. TB and HIV disease control programs also have their own data requirements for patient management and program reporting (200, 201), and various software suites have been developed (201) to overcome the inherent inefficiencies in the managing of a paper-based system, as described elsewhere previously (52, 126). Tracking of patient screening, clinical status, the prescribing and usage of first- and second-line regimens, and follow-up testing in addition to timely access to laboratory results and quality indicators are some of the additional tasks that are needed to effectively manage these two diseases. Another challenge arises from the various donor reporting requirements for the TB-HIV epidemic (202), and for many countries that have multiple donors supporting related programs, solutions are needed to support these needs and to enable frontline health care workers and program managers to focus their efforts on the needs of patients and the community rather than compiling and generating reports. The recent explosive growth in the use and availability of cell phones also offers exciting opportunities to leverage the wide coverage of cell phone networks in resource-limited settings to extend the reach of software and electronic-based data management to meet diagnostic and patient management needs (<http://mobileactive.org/>) (187).

Establishing Safe Laboratory Environments

In order to provide quality laboratory services, one must start with the basic laboratory infrastructure. TB presents additional challenges, one of which, safety issues, is related to personnel, equipment, and procedures and should be among the top priorities for laboratory management. In reality, this is seldom the case: biosafety is often neglected or not regarded as something to be taken seriously due to a lack of recognition of related risks. This is reflected in the lack of or inadequate supplies of basic protective equipment (clothing, gloves, and respirators, etc.) as well as their proper use in many laboratories, including the NTRL. This can in part be attributed to the high cost of biosafety equipment. Another important contributing factor is the lack of effective quality management systems.

Laboratory staff. Safety equipment greatly adds to but can never replace appropriate procedures. A core function of any laboratory training must always include the promotion of good laboratory practices and microbiological techniques according to international standards. Laboratory staff members in many resource-limited countries usually are not given adequate training on the safe handling of specimens and TB isolates and are often unaware of the dangers of working with these biological products. Also frequently lacking in the quality assurance scheme is the continual monitoring of the safety performance of the staff, including a log book of accidents and preventive measures. In addition, there should be adequate surveillance to safeguard and monitor laboratory staff for oc-

cupationally acquired diseases (i.e., TB status). The responsibility for a safe work environment must be assumed by those in management positions. The following key activities can be implemented to achieve this objective: periodic health check-ups, the exclusion of highly susceptible workers (e.g., pregnant women or immunocompromised persons) from hazardous work, and the provision of effective personal protective equipment and procedures.

The monitoring of the health status of laboratory workers is particularly challenging in many resource-limited settings, as standardization is not fully practiced and standard operating procedures (SOPs) are often not well developed and/or implemented. In many settings, SOPs are viewed as merely a documentation tool rather than as practical job aids for laboratory workers.

Laboratory design. As mentioned above, biosafety must begin with the proper design of TB laboratories according to international biosafety standards. In resource-limited settings, there is very limited expertise in biosafety level 3 design and construction. With the adoption of TB liquid culture and DST, many facilities often require costly upgrades that are beyond the available resources of the institutions. Furthermore, existing equipment is often not used properly or not well maintained. For example, common biosafety problems seen in many laboratories are related to the misuse of biosafety cabinets (BSCs), including the following: the location of BSCs in high-traffic areas, crowding of work areas inside BSCs (paperwork or other materials), HEPA filters not being changed periodically (or never changed), and inappropriate installation and certification of BSCs.

The significant influx of funds by large programs such as the Global Fund against AIDS, TB, and Malaria (GFATM) and the U.S. Presidential Emergency Plan for AIDS Relief (PEPFAR) have improved this situation in many countries. With the advent of those and other donor programs and new WHO guidelines (205), the number of BSL-3 facilities for liquid culture has grown and includes modular BSL-3 laboratories such as the one recently installed at the National TB and Leprosy Training Center in Zaria, Nigeria (Fig. 3).

Equipment and Equipment Maintenance

Harmonized equipment throughout the country. In order for a national laboratory system to minimize variations in test results, improve the operation and efficiency of laboratory networks, and reduce costs in equipment, reagents, and supplies, institutions throughout the country should harmonize TB testing equipment, methodology, and maintenance procedures for each tiered level of service (provincial, district, or health center) (146). As described in this review, a wide variety of technologies exist for TB testing. The following criteria for the selection of test methods and equipment should be considered:

- The TB diagnostic test method should be verified in each laboratory.
- The sensitivity and specificity of the assay should be well demonstrated.
- Assay throughput should fit the testing needs of the laboratory.

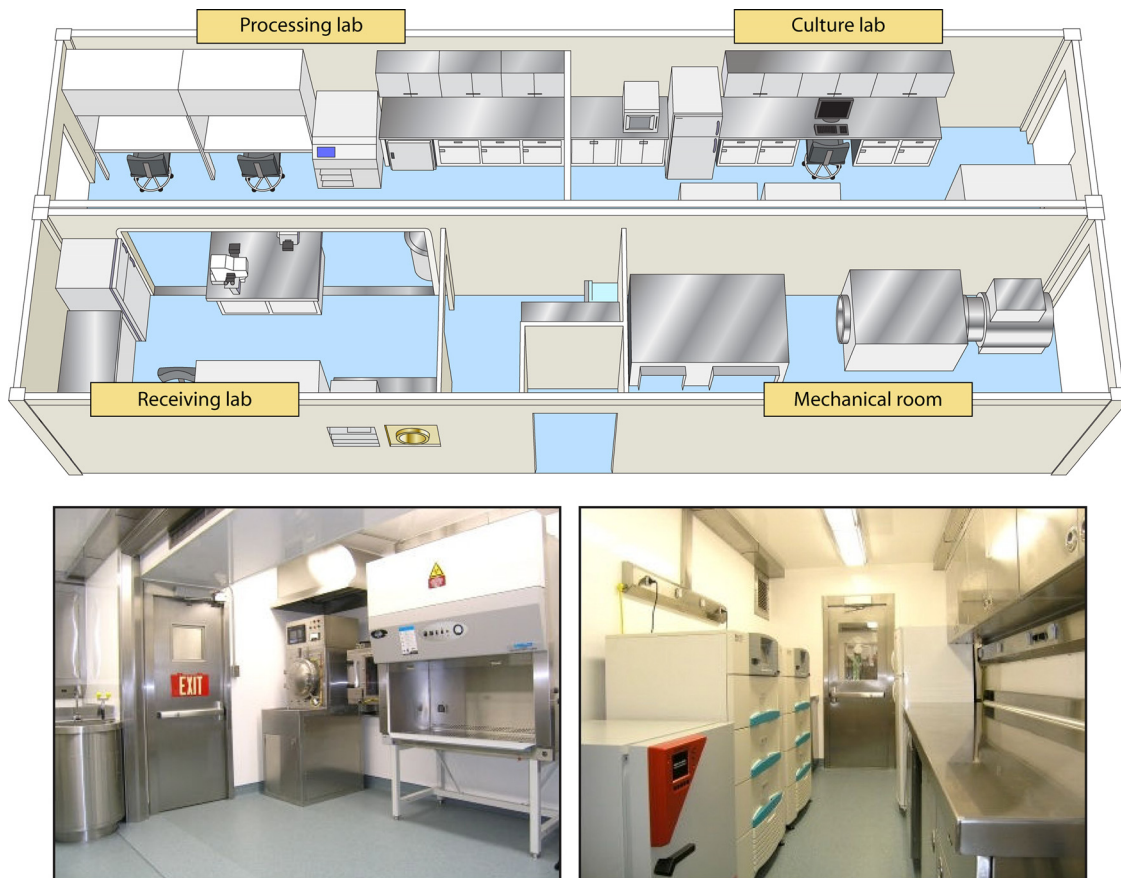


FIG. 3. Layout of a biosafety level 3 container laboratory.

- Availability, stability, and storage conditions for reagents should be addressed.
- Availability of instrument service and technical assistance should be addressed.

Once a national harmonized approach for the selection and purchase of equipment for a tiered laboratory system has been put into place, SOPs should be developed for instrument use and maintenance at each level of laboratory according to the manufacturer's protocol, with adaptations based on the specific situation in the testing facility. SOPs should be available, understandable, and followed by laboratory personnel to ensure the standardization of practices and to reduce instrument downtime, testing variations, and errors. The use of the same SOPs at a given level of laboratory throughout the country has the potential to increase reproducibility and the ability to more accurately compare patient results of tests performed in different laboratories. Each laboratory receiving new assays and instruments should perform a verification study to ensure that results obtained with the new technology are similar to those obtained during evaluation studies performed elsewhere. If the laboratory is already performing TB diagnostic testing with an established assay, the verification study should include comparisons of results obtained with the new technology with those from the original assay. If TB testing is new to the laboratory, sensitivity and specificity could be estimated by comparing results with those from a more experienced laboratory or with results from proficiency test panels.

Maintenance schedules. Each laboratory must have a plan to perform and implement scheduled and as-needed maintenance. The laboratory must be able to perform simple troubleshooting procedures and be informed of procedures to notify service providers for scheduled preventative maintenance and in the event of instrument or equipment failures.

The performance of daily maintenance procedures is essential to ensure that the instrument is working properly and any potential downtime is reduced. The performance of the required procedures should be documented on a form that is part of the maintenance SOP to ensure that all steps are performed with each use of the instrument. As with daily maintenance, the performance of scheduled and as-needed maintenance procedures is necessary to ensure that the instrument is working properly and downtime is reduced. A maintenance log for scheduled and as-needed maintenance should be developed to ensure that all maintenance procedures are performed and documented. In addition, a calendar should be developed to ensure that all scheduled maintenance is performed when required.

Troubleshooting. Testing failures or instrument malfunctions may occur during the routine use of automated TB culture instruments. Troubleshooting of these failures or malfunctions is necessary before TB growth detection testing is continued. A corrective action log sheet should be developed to record any problems and error messages that may occur. The corrective action taken to resolve the problem, including

advice or service calls from the manufacturer, should be documented. This log should be reviewed periodically to check for trends, and any technical errors that are identified should be immediately addressed. In addition, plans should be made to provide for manual testing during periods when a primary instrument is not functioning.

Service contracts. Service contracts for all major equipment should be acquired from the manufacturer or the manufacturer's representative. These contracts are necessary not only to maintain the proper functioning of the instrument but also to extend its life span. The contract should stipulate that the provider must be responsive for timely and appropriate service. Terms of a service contract must be very specific and include the following:

- The preventative maintenance procedures to be performed.
- The schedule for the maintenance procedures.
- Whether training will be provided for laboratory personnel or local technicians to perform routine maintenance.
- The phone number for and cost of an unscheduled service call.
- What parts are included or excluded.
- Availability and numbers of service representatives, including whether representatives are local or out of country.
- Availability of loaned instruments in case of instrument failure.
- Maximum time to response and maximum time to perform a service request.
- Whether troubleshooting help by phone or e-mail is included.
- Penalties incurred for faulty service, delays in service, or breaks in contract.

As described above, records of all maintenance, including preventative maintenance performed by a service representative, should be documented in specific log forms or books. Problems or issues that would require contacting the service representative should be clearly defined so that unnecessary service calls can be avoided. All direct and backup contact information such as phone numbers, fax numbers, and e-mail addresses should be readily available.

Ensuring Regular and Sufficient Supply of Consumables

Logistics management. Although supplies and equipment are standardized nationally, logistics management should be the responsibility of a unit of the MOH that works with national and international partners to integrate the management of laboratory supplies and reagents into the ordering and delivery system currently used for pharmaceuticals (22). With this integration, laboratory supplies and reagents can be managed by an administrator in charge of the stores for each health facility. Laboratory personnel should work closely with the supply manager to ensure that the needed laboratory materials are ordered along with essential medicines and other health commodities for the facility. The success of integrating the management of these commodities will depend on good communication and cooperation between the pharmacy, supply manager, and laboratory units of facilities. By integrating lab-

oratory supplies into the same system, the following benefits should be realized:

- Laboratory commodities would be ordered on a monthly basis and delivered directly to the facility.
- Stock levels of laboratory commodities would be monitored (logistics data) and maintained within established maximum and minimum stock levels to avoid stock-outs or overstocks.
- Stock cards (or computer-based bar coding, if available) would be maintained for all laboratory commodities so that logistics data for each facility are available.
- Logistics data on laboratory commodities should be reported monthly in a combined ordering and reporting system and would be available to a central level for forecasting, quantification, procurement, and program and logistics system performance monitoring.
- The integration of laboratory commodities should be planned through a review of the current system to determine what adjustments, if any, need to be made to the periodicity and flow of commodities, or additional pipeline parameters, for any special or highly sensitive laboratory commodities.

Forecasting and procurement. Logistics data in conjunction with the number of tests performed should be used for forecasting and quantification of laboratory supplies (22). Forecasting will be more accurate if current, appropriate data are collected through an integrated laboratory information management system (LIMS). Data should be collected and collated on a regular basis so that the information is available at the appropriate time for forecasting. While logistic data may be more difficult to obtain than service-related data, logistics data provide more accurate information, since the number of tests conducted does not always correlate with the supplies required. The central stores for supplies should establish contracts with vendors and suppliers to ensure that laboratory commodities are received on time and in the correct quantities to continuously maintain appropriate stock levels. This is particularly important for those laboratory commodities with short shelf lives or those which are more sensitive to changes in temperature of storage. In order to better coordinate commodity procurement, a laboratory supply management working group should be established to review supply needs, procurement progress, and other issues related to supply. The group should include all stakeholders and development partners who are involved in the use, procurement, or management of laboratory equipment and supplies. In addition, laboratory technical staff should be involved in assisting in the specification of orders from health facilities, in monitoring the quality of laboratory commodities received, and to assist with the management of laboratory supplies. Laboratory technical staff should also familiarize other staff involved in ordering with the characteristics and storage requirements of laboratory commodities and ensure that appropriate stock levels of laboratory commodities are held at each facility.

Human resources. Laboratories are becoming increasingly recognized globally as being integral components of national health systems. They provide testing for diagnosis as well as monitoring treatment and long-term surveillance. At the heart of clinical testing are people: the laboratory staff who carry out

their important work on a daily basis. However, most resource-poor countries carry the extra burden of having a severely low level of skilled laboratory workers. This factor, among others, has a huge negative impact on the ability of many countries to provide reliable, and clinically relevant, laboratory tests at all levels of the health care system in a timely manner, if at all. The underlying reasons are complex and many, but the following are often found in many resource-poor countries:

- Lack of strategy, planning, and concrete budget allotted to addressing laboratory human resource issues.
- Limited or inadequate preservice and continuing education to maintain a high level of testing proficiency.
- Lack of upwardly mobile career path and professional recognition and low compensation for laboratory workers, especially in government laboratories (this often leads to low retention rates, as many laboratory workers seek higher-paying jobs in the private sector).
- Weak or lack of quality management systems to optimize laboratory organization and use of personnel, i.e., clearly defining core laboratory functions and, in that context, the roles and responsibilities of laboratory staff.

To improve this human resource deficit, resource-poor countries must think beyond additional financial resources. Having new funds, specifically those directed at human resources, where funding is currently lagging far behind the funding devoted to laboratory commodities, will undoubtedly help. However, any human resource solution must be part of an integrated effort to strengthen laboratory quality management systems. Thus, there is a critical need for senior laboratory staff to be trained in laboratory management to develop skills in the following: management of human resources, organization of workload and workplace, supply forecasting and ordering, budgeting, and development and oversight of a quality assurance program.

Going forward, an accurate inventory of the national laboratory systems, their core functions, and the laboratory workforce obtained during the development of the national laboratory policy and strategic plan will be extremely useful. Furthermore, these documents should outline practical and meaningful ways to coordinate laboratory services among different ministries, institutions, and agencies, etc., across the entire government. It is critical that resources be pooled to (i) optimize the use of the existing pool of laboratory workers and (ii) develop creative solutions, i.e., incentives, to attract new and retain existing laboratory workers.

BEST PRACTICES FOR RAPID AND ACCURATE DIAGNOSIS OF TB IN RESOURCE-LIMITED COUNTRIES

Key Partners in TB Laboratory Strengthening

Overall, coordinated efforts of partners are essential for efficiently moving forward with laboratory strengthening. International as well as national and local partners, both public and private, must be engaged to increase productivity and maximize the results of laboratory capacity-building efforts.

Côte d'Ivoire. Addressing the need for the rapid and accurate diagnosis of TB and, as a result, TB laboratory strengthening may be overwhelming for ministry staff in resource-limited settings. An approach that has proven to be successful is the coordination of efforts by multiple partners. In Côte d'Ivoire, a broad coalition of partners (ASM-CDC-WHO-FIND-UNITAID) worked with the local office of the Elizabeth Glaser Pediatric AIDS Foundation (EGPAF) to coordinate efforts addressing TB-HIV coinfection, which affects almost one-half of the HIV-infected population. Specifically, the partners coordinated the procurement of essential commodities for three major laboratories and the rollout of essential training programs. The training provided TB laboratory staff with relevant skills, including the following: AFB smear microscopy optimization, TB liquid culture and DST training and implementation, and TB line probe assay training and implementation.

Haiti. Several partners selected by the Haitian MOH to support the scale-up of TB laboratory diagnostics met to begin the coordination of efforts. The key organizations included the NTRL for Haiti (Laboratoire National de Santé Public [LNSP]), the ASM, the Institut Pasteur-Guadeloupe (IPG), the Pan-American Health Organization (PAHO), and the CDC-Haiti. During the meeting, representatives laid out a coordinated plan for TB laboratory assistance in Haiti, with each organization slated with specific tasks, as outlined below.

The ASM will provide design plans for building a BSL-3 TB laboratory at the LNSP, present a 3-day TB workshop outlining the basics on mycobacteriology and the identification of MTBC isolates, assist with the establishment of training and EQA for AFB smear microscopy, and support six Haitians from the LNSP to attend 1- to 2-week TB culture and drug susceptibility testing training sessions at the IPG. The IPG will finalize lists of equipment and supply needs for a BSL-3 TB laboratory and provide TB culture and DST training both in Guadeloupe and through IPG staff visits to Haiti. The PAHO will provide guidelines for TB control and diagnostic algorithms and coordinate TB surveillance activities.

India. Another successful partnership is found in India, where recently the ASM and the Program for Appropriate Technology in Health (PATH), with funding from USAID, began providing technical assistance to the Intermediate Reference Laboratory (IRL) network. The partners work with the Indian Central TB Division to accelerate the process of accreditation of selected IRLs. The proficiency of these laboratories in TB culture and DST will be determined by an overseeing NRL. States (or territories) with accredited IRLs will then be able to conduct drug resistance surveys (DRSs) and implement the Directly Observed Treatment Short-Course Plus (DOTS-Plus) strategy to treat MDR-TB.

So far, this partnership has produced the following: customized laboratory assessment tools, detailed assessment reports for eight IRLs, and action plans for moving these IRLs forward toward accreditation. In addition, during this initial phase, the partners learned of the urgent need to improve communication between and among the NRLs and IRLs and allow for the standardization of SOPs and training. To this end, the partners coordinated joint NRL-IRL meetings.

In addition, FIND has been assisting the India Revised National TB Control Program (RNTCP) in upgrading the TB

laboratory services through funding from EXPANDx TB/UNITAID and GFATM round 9. Accordingly, from 2010 to 2014, FIND is charged with introducing LPA, liquid culture, and rapid identification of *M. tuberculosis* isolates by lateral flow assay in 43 state and local TB laboratories across the country. Previously, the FIND India office, established in New Delhi in 2007, performed demonstration studies on LED-based fluorescent microscopy in three supervisory sites and nine microscopy centers and on the Cepheid Xpert MTB/RIF assay in two sites. The data generated were submitted to the WHO STAG-TB in 2009 and 2010, respectively. Evidence was then collected for the possible scale-up of LPA in seven sites and liquid culture, DST, and rapid identification of isolates to the species level in three sites. Subsequently, the RNTCP approved the further expansion of the LPA and liquid culture testing. To facilitate QA, FIND introduced an LPA proficiency testing program that has been approved for implementation by the national laboratory committee and the RNTCP. The ongoing activities approved for funding under EXPANDx TB are (i) continuous assessment of TB laboratory networks; (ii) procurement of equipment for liquid culture, rapid identification of isolates to the species level, and LPA; (iii) national training in these rapid techniques and in laboratory management; and (iv) overall project management. Under GFATM round 9, TB laboratory activities will be further strengthened, and in addition, human resources for the identified 43 laboratories will be recruited. To facilitate the training of a large number of laboratory staff, the International Centre of Excellence in Laboratory Training (ICELT) was established at the National Tuberculosis Institute, Bangalore, India. By early 2011 at the ICELT, 15 participants from 10 sites had received three sessions of training on the LPA from both national and international trainers. Training on TB growth detection, identification, and DST; biosafety; and laboratory management will also be presented at this site. These efforts are expected to result in a marked decline in TB mortality in the populations served. Preliminary findings from a cohort of more than 200 drug-resistant TB patients diagnosed at the study sites since 2008 have shown a 15% reduction in mortality among these patients (B. Vollepore, FIND India, personal communication).

African Centre for Integrated Laboratory Training. The African Centre for Integrated Laboratory Training (ACILT) was established through PEPFAR funding in 2008 with the focus on the development and presentation of hands-on training courses of 1 to 3 weeks in duration for frontline laboratory staff. The ACILT is located on the campus of the South African National Institute for Communicable Diseases (NICD) and the National Health Laboratory Service (NHLS) in Johannesburg, South Africa. Prior to the establishment of the ACILT, needs assessments were performed in 10 resource-poor African countries to determine which topics would be most important for building capacity in their laboratory staffs. Courses were then developed by the ACILT, CDC, FIND, WHO, and other South African and international partners, thus ensuring that standardized materials were included. The first courses were offered at the ACILT in the fall of 2008, and by the end of 2010, more than 20 hands-on courses had been developed and presented to over 550 participants from 26 resource-poor countries on the following topics: TB culture

and identification, AFB smear microscopy, molecular line probe assay for MDR-TB, HIV DNA PCR for early infant diagnosis, HIV incidence assay, preparation of EQA panels for rapid HIV testing, national laboratory strategic planning, biosafety and biosecurity, and strengthening laboratory management toward accreditation. ACILT satellite courses have been offered at sites outside South Africa. One-week courses on molecular LPA testing for MDR-TB have been hosted by the new laboratory facility at the Ethiopian Health and Nutrition Research Institute (EHNRI) in Addis Ababa, Ethiopia; at the National Reference Laboratory for Mycobacteria in Maseru, Lesotho; and at the Institut Pasteur in Abidjan, Côte d'Ivoire. Also, the ACILT TB culture course was presented through partnerships with FIND, the CDC, Becton Dickinson, and the Thai NTRL in Bangkok. The course materials have recently been translated into French and Portuguese through a partnership with the ASM for the presentation of the courses in Francophone and Lusophone countries.

WHO-AFRO Laboratory Accreditation Program. The outcome of two recent meetings on laboratory quality systems organized by the WHO, the CDC, and the WHO African Regional Office (AFRO) led to an agreement that countries with limited resources should be assisted in a stepwise approach for the accreditation of laboratories. The WHO-AFRO accreditation scheme developed after these meetings requires that laboratories implement and adhere to uniform standards using a simple and achievable stepwise approach. The accreditation scheme is not disease specific but focuses on all laboratories providing clinical testing services, including TB laboratories. The establishment of this stepwise laboratory accreditation scheme is in accordance with WHO's core functions of setting norms and standards and strengthening capacity in the laboratory systems of its member states. Currently, the WHO has developed laboratory accreditation standards for many diseases, including polio, measles, influenza, and HIV drug resistance, and this WHO-AFRO scheme is an important extension of the overall WHO laboratory accreditation effort.

The Laboratory Accreditation Program was launched by the WHO-AFRO, in partnership with the CDC, the American Society for Clinical Pathology (ASCP), and the Clinton HIV/AIDS Initiative (CHAI), in Kigali, Rwanda, July 2009 (130). The purpose of the launch was to (i) demonstrate to policy and decision makers a blueprint of the path toward accreditation, (ii) obtain key stakeholder support for accreditation, and (iii) showcase a task-based training program in support of laboratory improvement required for accreditation. Laboratories in the following 11 countries will be prioritized for the first round of the program: Botswana, Cameroon, Cote d'Ivoire, Ethiopia, Kenya, Malawi, Nigeria, Rwanda, Tanzania, Senegal, and Zambia. The launch event in Kigali also showcased Strengthening Lab Management Toward Accreditation (SLMTA), a training program that teaches laboratory managers critical management tasks and routines to foster the laboratory improvement necessary for accreditation.

External evaluation, which is the hallmark of accreditation, ensures customers that laboratory services meet acceptable quality and safety standards. Laboratory assessors recently trained in Nairobi, Kenya, are responsible for implementing the use of the WHO-AFRO checklists to perform laboratory

accreditation inspections. An African proficiency testing (PT) program will be established to provide specimens for the continent, collect and analyze the PT results, and provide these results to the WHO-AFRO accreditation governing board and technical working group. This PT program will provide measurable outcomes to document the continuous increase in the quality of testing resulting from the implementation of the WHO-AFRO accreditation scheme.

IMPACT OF INCREASED USE OF NOVEL DIAGNOSTIC METHODS

Cost-Effectiveness of the Introduction of New TB Diagnostics

The implementation of new diagnostics for the more accurate and rapid diagnosis of TB can be extremely costly for resource-poor countries. Not only must the country support the cost of the diagnostics themselves, it also must allocate significant funding for upgrading laboratory infrastructure, the procurement and distribution of equipment and supplies, and hiring and training sufficient laboratory technical staffs. In order to examine the cost-effectiveness of the new diagnostics for economic and epidemiologic impacts, an eight-step standardized methodology for costing was described (135). The steps involved include assessing laboratory needs and currently available new methods, gathering comparative pricing information, performing test evaluations, calculating unit costs, calculating costs based on current laboratory workflow volumes, and calculating performance/efficiency-related costs. The implementation of such a standardized methodology should eventually lead to the evidence-based adoption of new diagnostics with high sensitivity and specificity that are also affordable in resource-poor settings.

Impact of New Diagnostics on TB Control

Modeling studies have estimated that 400,000 lives could be saved each year with the introduction of an accurate, rapid, and widely available TB diagnostic system with a sensitivity greater than 85% for both smear-positive and smear-negative cases and 97% specificity (83). In addition, modeling has shown that expanding TB culture and drug susceptibility testing in South Africa could lead to reduced mortality, especially for those patients infected with MDR-TB, and there is significant potential that TB culture and new diagnostics could be effective and cost-effective for testing HIV-positive patients in resource-poor settings (41). Modeling studies are dependent on assumptions and cost estimates and therefore should be followed up with field studies, hopefully to demonstrate a positive impact on TB control and the lives of individual TB patients, many of whom suffer from dual infections with HIV. Field demonstrations and operational research should be a priority so that essential information necessary for government and donor advocacy, support, and funding will ensure the implementation of new diagnostics that improve patient care globally. A recent small study conducted in two of the five health districts of Lima, Peru, examined both the laboratory and programmatic factors influencing the overall turnaround time in diagnosing MDR-TB. The results highlighted that sig-

nificant delays were related not only to a lack of rapid diagnostic tests but rather to the low rate at which people acted on specimen management, specimen transfer for further testing, and results (215). A more recent study examined the impact and cost-effectiveness of a centralized growth detection program to serve HIV-positive patients in urban Brazil. The results suggest that in this setting, growth detection could avert an estimated 37 disability-adjusted life years (DALY) per 1,000 TB suspects and prevent 49% of all TB deaths occurring after initial presentation at a cost of 962 U. S. dollars (USD) per DALY averted if solid medium alone was used. The introduction of liquid medium base growth detection resulted in 21% more DALY at a cost of 2,751 USD per DALY. Those authors underlined that the primary drivers of cost-effectiveness were clinical practices such as empirical TB treatment, communications, and patient follow-up (e.g., translating culture results into treatment) (42).

CONCLUSIONS

Building capacity and enhancing universal access to rapid and accurate laboratory diagnostics are necessary to control TB and HIV-TB in resource-poor countries. This paper has described several new and established methods as well as some of the issues associated with implementing quality TB laboratory services in resource-limited countries, where there are challenges associated with retaining competent human resources and in establishing adequate infrastructure. Recently, several of these novel methods have been endorsed by the WHO and have been made available at discounted prices for procurement by the public health care sector of high-burden countries. These proven methods have already shown their potential to significantly improve case detection and management of patients, including drug-resistant TB cases, and enhance the identification of the disease in HIV-positive individuals. However, it is important to realize that at present, there is no stand-alone test for the rapid detection of tuberculosis in all patients. While some new techniques are simple, others have complex requirements, and therefore, it is vital to carefully determine how to link the new laboratory tests together and incorporate them within a country's national TB diagnostic algorithm, taking into account factors such as the capacity of different levels of the tiered health care system and the opportunity of decentralizing laboratory services that is possible through the recent improvements in integrated molecular testing. The effective functioning of a TB diagnostic network also relies on properly organized specimen collection, transportation, and communication of results. In order to implement and maintain the quality of the new diagnostic services, an adequate certification or quality assurance program needs to be inbuilt for all novel tests. Finally, laboratory results alone are not enough to dictate a particular strategy in the TB patient's care. Careful clinical correlation is necessary in determining the clinical significance of laboratory results and making the correct diagnosis or therapeutic decision. Health care providers and laboratory personnel need to communicate and cooperate to bridge any gap between them and thus optimize clinical outcomes. TB continues to be a complex disease to diagnose and manage due to the chronicity of the disease, the

nature of the host-pathogen relationship, and the resulting diversity in its clinical manifestations.

REFERENCES

- Ackah, A. N., et al. 1995. Response to treatment, mortality, and CD4 lymphocyte counts in HIV-infected persons with tuberculosis in Abidjan, Côte d'Ivoire. *Lancet* **345**:607–610.
- Albert, H., A. Trollip, T. Seaman, and R. J. Mole. 2004. Simple, phage-based (FASTPlaque) technology to determine rifampicin resistance of *Mycobacterium tuberculosis* directly from sputum. *Int. J. Tuberc. Lung Dis.* **8**:1114–1119.
- Andrews, R. H., and S. Radhakrishna. 1959. Comparison of two methods of sputum collection in the diagnosis of pulmonary tuberculosis. *Tubercle* **40**:155–162.
- Angeby, K. A., L. Klintz, and S. E. Hoffner. 2002. Rapid and inexpensive drug susceptibility testing of *Mycobacterium tuberculosis* with a nitrate reductase assay. *J. Clin. Microbiol.* **40**:553–555.
- Aranaz, A., et al. 1999. *Mycobacterium tuberculosis* subsp. *caprae* subsp. nov.: a taxonomic study of a new member of the *Mycobacterium tuberculosis* complex isolated from goats in Spain. *Int. J. Syst. Bacteriol.* **49**:1263–1273.
- Aziz, M., et al. 2002. External quality assessment for AFB smear microscopy. Association of Public Health Laboratories, Washington, DC. http://www.aplh.org/AboutAPHL/publications/Documents/External_Quality_Assessment_for_AFB_Smear_Microscopy.pdf.
- Barnard, M., H. Albert, G. Coetzee, R. O'Brien, and M. E. Bosman. 2008. Rapid molecular screening for multidrug-resistant tuberculosis in a high-volume public health laboratory in South Africa. *Am. J. Respir. Crit. Care Med.* **177**:787–792.
- Bártfai, Z., et al. 2001. Molecular characterization of rifampin-resistant isolates of *Mycobacterium tuberculosis* from Hungary by DNA sequencing and the line probe assay. *J. Clin. Microbiol.* **39**:3736–3739.
- Reference deleted.
- Blakemore, R., et al. 2010. Evaluation of the analytical performance of the Xpert MTB/RIF assay. *J. Clin. Microbiol.* **48**:2495–2501.
- Boehme, C. C., et al. 2007. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. *J. Clin. Microbiol.* **45**:1936–1940.
- Boehme, C. C., et al. 2010. Rapid molecular detection of tuberculosis and rifampin resistance. *N. Engl. J. Med.* **363**:1005–1015.
- Bogli-Stuber, K., et al. 2009. Evaluation of a novel line probe assay for the timely detection of extensively drug-resistant tuberculosis, abstr. P03, p. 79. Abstr. 5th Congr. Int. Union Tuberc. Lung Dis. Eur. Region, Dubrovnik, Croatia.
- Bonington, A., et al. 2000. TB PCR in the early diagnosis of tuberculous meningitis: evaluation of the Roche semi-automated COBAS AmpliCor MTB test with reference to the manual AmpliCor MTB PCR test. *Tuberc. Lung Dis.* **80**:191–196.
- Bonington, A., et al. 1998. Use of Roche AMPLICOR *Mycobacterium tuberculosis* PCR in early diagnosis of tuberculous meningitis. *J. Clin. Microbiol.* **36**:1251–1254.
- Bonnet, M., et al. 2007. Reducing the number of sputum specimens examined and thresholds for positivity: an opportunity to optimize smear microscopy. *Int. J. Tuberc. Lung Dis.* **11**:953–958.
- Braden, C. R., et al. 2001. Simultaneous infection with multiple strains of *Mycobacterium tuberculosis*. *Clin. Infect. Dis.* **33**:42–47.
- Breen, R. A., et al. 2007. Rapid diagnosis of smear-negative tuberculosis using immunology and microbiology with induced sputum in HIV-infected and uninfected individuals. *PLoS One* **2**:e1335.
- Brosch, R., et al. 2002. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proc. Natl. Acad. Sci. U. S. A.* **99**:3684–3689.
- Bruchfeld, J., et al. 2000. Sputum concentration improves diagnosis of tuberculosis in a setting with a high prevalence of HIV. *Trans. R. Soc. Trop. Med. Hyg.* **94**:677–680.
- Buszewski, B., M. Keszy, T. Ligor, and A. Amann. 2007. Human exhaled air analytics: biomarkers of diseases. *Biomed. Chromatogr.* **21**:553–566.
- Butao, D., B. Felling, and P. Msipa. 2009. Malawi: laboratory services and supply chain assessment. USAID Deliver Project, task order 1. USAID, Washington, DC.
- Butt, T., R. N. Ahmad, R. K. Afzal, A. Mahmood, and M. Anwar. 2004. Rapid detection of rifampicin susceptibility of *Mycobacterium tuberculosis* in sputum specimens by mycobacteriophage assay. *J. Pak. Med. Assoc.* **54**:379–382.
- Bwanga, F., S. Hoffner, M. Haile, and M. L. Joloba. 2009. Direct susceptibility testing for multi drug resistant tuberculosis: a meta-analysis. *BMC Infect. Dis.* **9**:67.
- Cain, K. P., et al. 2010. An algorithm for tuberculosis screening and diagnosis in people with HIV. *N. Engl. J. Med.* **362**:707–716.
- Cegielski, J. P., and D. N. McMurray. 2004. The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals. *Int. J. Tuberc. Lung Dis.* **8**:286–298.
- Centers for Disease Control and Prevention. 1994. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care facilities, 1994. *MMWR Recomm. Rep.* **43**(RR13):1–132.
- Centers for Disease Control and Prevention. 2009. Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. *MMWR Morb. Mortal. Wkly. Rep.* **58**:7–10.
- Chaisson, R. E., and G. J. Churchyard. 2010. Recurrent tuberculosis; relapse, reinfection, and HIV. *J. Infect. Dis.* **201**:653–655.
- Chedore, P., and F. B. Jamieson. 2002. Rapid molecular diagnosis of tuberculous meningitis using the Gen-Probe amplified *Mycobacterium tuberculosis* direct test in a large Canadian public health laboratory. *Int. J. Tuberc. Lung Dis.* **6**:913–919.
- Clinical and Laboratory Standards Institute. 2003. Susceptibility testing of mycobacteria, *Nocardiae*, and other aerobic *Actinomycetes*; approved standard. CLSI document M24-A. Clinical and Laboratory Standards Institute, Wayne, PA.
- Cockerill, F. R., III. 1999. Genetic methods for assessing antimicrobial resistance. *Antimicrob. Agents Chemother.* **43**:199–212.
- Conde, M., et al. 2009. Moxifloxacin versus ethambutol in the initial treatment of tuberculosis: a double-blind, randomised, controlled phase II trial. *Lancet* **373**:1183–1189.
- Cousins, D. V., et al. 2003. Tuberculosis in seals caused by a novel member of the *Mycobacterium tuberculosis* complex: *Mycobacterium pinnipedii* sp. nov. *Int. J. Syst. Evol. Microbiol.* **53**:1305–1314.
- Cristea-Fernström, M., M. Olofsson, E. Chrystanthou, J. Jonasson, and B. Petrini. 2007. Pyrosequencing of a short hypervariable 16S rDNA fragment for the identification of nontuberculous mycobacteria—a comparison with conventional 16S rDNA sequencing and phenotyping. *APMIS* **115**:1252–1259.
- Daley, P., et al. 2009. Blinded evaluation of commercial urinary lipoarabinomannan for active tuberculosis: a pilot study. *Int. J. Tuberc. Lung Dis.* **13**:989–995.
- De Beenhouwer, H., et al. 1995. Rapid detection of rifampicin resistance in sputum and biopsy specimens from tuberculosis patients by PCR and line probe assay. *Tuberc. Lung Dis.* **76**:425–430.
- De Viedma, D. G. 2003. Rapid detection of resistance in *Mycobacterium tuberculosis*: a review discussing molecular approaches. *Clin. Microbiol. Infect.* **9**:349–359.
- Diel, R., A. Nienhaus, C. Lange, and T. Schaberg. 2006. Cost-optimization of screening for latent tuberculosis in close contacts. *Eur. Respir. J.* **28**:35–44.
- Dorman, S. E., and R. E. Chaisson. 2007. From magic bullets back to the magic mountain: the rise of extensively drug-resistant tuberculosis. *Nat. Med.* **13**:295–298.
- Dowdy, D. W., R. E. Chaisson, G. Maartens, E. L. Corbett, and S. E. Dorman. 2006. Impact of enhanced tuberculosis diagnosis in South Africa: a mathematical model of expanded culture and drug susceptibility testing. *Proc. Natl. Acad. Sci. U. S. A.* **105**:11293–11298.
- Dowdy, D. W., et al. 2008. Impact and cost-effectiveness of culture for diagnosis of tuberculosis in HIV-infected Brazilian adults. *PLoS One* **3**:e4057.
- Dye, C., S. Scheele, P. Dolin, V. Pathania, and M. C. Raviglione. 1999. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *JAMA* **282**:677–686.
- Ecker, D. J., et al. 2009. Molecular genotyping of microbes by multilocus PCR and mass spectrometry: a new tool for hospital infection control and public health surveillance. *Methods Mol. Biol.* **551**:71–87.
- Ecker, D. J., et al. 2008. Ibis T5000: a universal biosensor approach for microbiology. *Nat. Rev. Microbiol.* **6**:553–558.
- Eshoo, M. W., et al. 2010. Detection and identification of *Ehrlichia* species in blood by use of PCR and electrospray ionization mass spectrometry. *J. Clin. Microbiol.* **48**:472–478.
- Evans, K. D., A. S. Nakasone, P. A. Sutherland, L. M. de la Maza, and E. M. Peterson. 1992. Identification of *Mycobacterium tuberculosis* and *Mycobacterium avium-M. intracellulare* directly from primary BACTEC cultures by using acridinium-ester-labeled DNA probes. *J. Clin. Microbiol.* **30**:2427–2431.
- Fluit, A. C., M. R. Visser, and F. J. Schmitz. 2001. Molecular detection of antimicrobial resistance. *Clin. Microbiol. Rev.* **14**:836–871.
- Fodor, T., and I. Szabo. 1980. Effect of chlorhexidine gluconate on the survival of acid fast bacteria. *Acta Microbiol. Acad. Sci. Hung.* **27**:343–344.
- Fox, W., G. A. Ellard, and D. A. Mitchison. 1999. Studies on the treatment of tuberculosis undertaken by the British Medical Research Council tuberculosis units, 1946–1986, with relevant subsequent publications. *Int. J. Tuberc. Lung Dis.* **10**:S231–S279.
- Franco-Alvarez de Luna, F., P. Ruiz, J. Gutierrez, and M. Casal. 2006. Evaluation of the GenoType Mycobacteria Direct assay for detection of *Mycobacterium tuberculosis* complex and four atypical mycobacterial species in clinical samples. *J. Clin. Microbiol.* **44**:3025–3027.
- Fraser, H. S. F., et al. 2007. Information systems for patient follow-up and chronic management of HIV and tuberculosis: a life-saving technology in resource-poor areas. *J. Med. Internet Res.* **9**:e29.

53. **Gandhi, N. R., et al.** 2006. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet* **368**:1575–1580.
54. **Ganoza, C. A., et al.** 2008. Novel hypertonic saline-sodium hydroxide (HS-SH) method for decontamination and concentration of sputum samples for *Mycobacterium tuberculosis* microscopy and culture. *J. Med. Microbiol.* **57**: 1094–1098.
55. **Gegia, M., et al.** 2008. Prevalence of and molecular basis for tuberculosis drug resistance in the Republic of Georgia: validation of a QIAplex system for detection of drug resistance-related mutations. *Antimicrob. Agents Chemother.* **52**:725–729.
56. **Getahun, H., M. Harrington, R. O'Brien, and P. Nunn.** 2007. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. *Lancet* **369**:2042–2049.
57. **Gingeras, T. R., et al.** 1998. Simultaneous genotyping and species identification using hybridization pattern recognition analysis of generic *Mycobacterium* DNA arrays. *Genome Res.* **8**:435–448.
58. **Grant, R. J., et al.** 2010. Application of the Ibis-T5000 pan-orthopoxvirus assay to quantitatively detect monkeypox viral loads in clinical specimens from macaques experimentally infected with aerosolized monkeypox virus. *Am. J. Trop. Med. Hyg.* **82**:318–323.
59. **Gryadunov, D., et al.** 2005. Evaluation of hybridisation on oligonucleotide microarrays for analysis of drug-resistant *Mycobacterium tuberculosis*. *Clin. Microbiol. Infect.* **11**:531–539.
60. **Guio, H., et al.** 2006. Method for efficient storage and transportation of sputum specimens for molecular testing of tuberculosis. *Int. J. Tuberc. Lung Dis.* **10**:906–910.
61. **Gutierrez, M. C., et al.** 2005. Ancient origin and gene mosaicism of the progenitor of *Mycobacterium tuberculosis*. *PLoS Pathog.* **1**:55–61.
62. **Halse, T. A., et al.** 2010. Combined real-time PCR and *rpoB* gene pyrosequencing for rapid identification of *Mycobacterium tuberculosis* and determination of rifampin resistance directly in clinical specimens. *J. Clin. Microbiol.* **48**:1182–1188.
63. **Heep, M., et al.** 2001. Frequency of *rpoB* mutations inside and outside the cluster I region in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates. *J. Clin. Microbiol.* **39**:107–110.
64. **Helb, D., et al.** 2010. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J. Clin. Microbiol.* **48**:229–237.
65. **Heller, L. C., M. Jones, and R. H. Widen.** 2008. Comparison of DNA pyrosequencing with alternative methods for identification of mycobacteria. *J. Clin. Microbiol.* **46**:2092–2094.
66. **Heym, B., et al.** 1994. Implications of multidrug resistance for the future of short-course chemotherapy of tuberculosis: a molecular study. *Lancet* **344**: 293–298.
67. **Hillemann, D., S. Rüscher-Gerdes, and E. Richter.** 2005. Application of the Capilia TB assay for culture confirmation of *Mycobacterium tuberculosis* complex isolates. *Int. J. Tuberc. Lung Dis.* **9**:1409–1411.
68. **Hillemann, D., S. Rüscher-Gerdes, and E. Richter.** 2007. Evaluation of the GenoType MTBDRplus assay for rifampin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and clinical specimens. *J. Clin. Microbiol.* **45**:2635–2640.
69. **Hillemann, D., S. Rüscher-Gerdes, and E. Richter.** 2009. Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of *Mycobacterium tuberculosis* strains and clinical specimens. *J. Clin. Microbiol.* **47**:1767–1772.
70. **Hillemann, D., M. Weizenegger, T. Kubica, E. Richter, and S. Niemann.** 2005. Use of the genotype MTBDR assay for rapid detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* complex isolates. *J. Clin. Microbiol.* **43**:3699–3703.
71. **Ho, Y. P., and P. M. Reddy.** 2010. Identification of pathogens by mass spectrometry. *Clin. Chem.* **56**:525–536. [Epub ahead of print.]
72. **Huard, R. C., et al.** 2006. Novel genetic polymorphisms that further delineate the phylogeny of the *Mycobacterium tuberculosis* complex. *J. Bacteriol.* **188**:4271–4287.
73. **Huebner, R. E., M. F. Schein, and J. B. Bass, Jr.** 1993. The tuberculin skin test. *Clin. Infect. Dis.* **17**:968–975.
74. **Inangolet, F. O., B. Demelash, J. Oloya, J. Opuda-Asibo, and E. Skjerve.** 2008. A cross-sectional study of bovine tuberculosis in the trans-human and agro-pastoral cattle herds in the border areas of Katakwi and Moroto districts, Uganda. *Trop. Anim. Health Prod.* **40**:501–508.
75. **Iwamoto, T., T. Sonobe, and K. Hayashi.** 2003. Loop-mediated isothermal amplification for direct detection of *Mycobacterium tuberculosis* complex, *M. avium*, and *M. intracellulare* in sputum samples. *J. Clin. Microbiol.* **41**:2616–2622.
76. **Janosy, G.** 2008. The changing pattern of “smart” flow cytometry (S-FC) to assist the cost-effective diagnosis of HIV, tuberculosis, and leukemias in resource-restricted conditions. *Biotechnol. J.* **3**:32–42.
77. **Janosy, G., et al.** 2008. The role of flow cytometry in the interferon-gamma-based diagnosis of active tuberculosis and its coinfection with HIV-1—a technically oriented review. *Cytometry B Clin. Cytom.* **74**:S141–S151.
78. **Janosy, G., and H. Shapiro.** 2008. Simplified cytometry for routine monitoring of infectious diseases. *Cytometry B Clin. Cytom.* **74**:S6–S10.
79. **Johansen, I. S., et al.** 2004. Improved sensitivity of nucleic acid amplification for rapid diagnosis of tuberculous meningitis. *J. Clin. Microbiol.* **42**: 3036–3040.
80. **Kalantri, S., M. Pai, L. Pascopella, L. Riley, and A. Reingold.** 2005. Bacteriophage-based tests for the detection of *Mycobacterium tuberculosis* in clinical specimens: a systematic review and meta-analysis. *BMC Infect. Dis.* **5**:59.
81. **Karp, C. L., and P. G. Auwaerter.** 2007. Coinfection with HIV and tropical infectious diseases. I. Protozoal pathogens. *Clin. Infect. Dis.* **45**:1208–1213.
82. **Kasai, H., T. Ezaki, and S. Harayama.** 2000. Differentiation of phylogenetically related slowly growing mycobacteria by their *gyrB* sequences. *J. Clin. Microbiol.* **38**:301–308.
83. **Keeler, E., et al.** 2006. Reducing the global burden of tuberculosis: the contribution of improved diagnostics. *Nature* **444**(Suppl. 1):49–57.
84. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology: a guide for the level III laboratory. Centers for Disease Control, Atlanta, GA.
85. **Kim, B. J., et al.** 1999. Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (*rpoB*). *J. Clin. Microbiol.* **37**:1714–1720.
86. **Kirschner, P., and E. C. Bottger.** 1998. Species identification of mycobacteria using rDNA sequencing. *Methods Mol. Biol.* **101**:349–361.
87. **Kirschner, P., et al.** 1996. Diagnosis of mycobacterial infections by nucleic acid amplification: 18-month prospective study. *J. Clin. Microbiol.* **34**:304–312.
88. **Kirschner, P., et al.** 1993. Genotypic identification of mycobacteria by nucleic acid sequence determination: report of a 2-year experience in a clinical laboratory. *J. Clin. Microbiol.* **31**:2882–2889.
89. **Kiwanuka, N., et al.** 2008. Effect of human immunodeficiency virus type 1 (HIV-1) subtype on disease progression in persons from Rakai, Uganda, with incident HIV-1 infection. *J. Infect. Dis.* **197**:707–713.
90. **Kluge, C., M. C. Gutierrez, K. Delabre, and G. Marchal.** 2008. Improving detection of mycobacteria by immunocapture-qPCR in water samples: a rapid and sensitive method for routine testing, abstr. OP-24. 29th Annu. Congr. Eur. Soc. Mycobacteriol., Plovdiv, Bulgaria.
91. **Kolmer, J. A., and F. Boerner.** 1945. Approved laboratory technic, 4th ed. Appellon Century, New York, NY.
92. **Korbel, D. S., B. E. Schneider, and U. E. Schaible.** 2008. Innate immunity in tuberculosis: myths and truth. *Microbes Infect.* **10**:995–1004.
93. **Kudoh, S., and T. Kudoh.** 1974. A simple technique to culturing tubercle bacilli. *Bull. World Health Organ.* **51**:71–82.
94. **Lalvani, A., L. Richeldi, and H. Kunst.** 2005. Interferon gamma assays for tuberculosis. *Lancet Infect. Dis.* **5**:322–324.
95. **Lama, J., and V. Planelles.** 2007. Host factors influencing susceptibility to HIV infection and AIDS progression. *Retrovirology* **4**:57.
96. **Lang, A. M., et al.** 1998. Clinical evaluation of the Gen-Probe Amplified Direct Test for detection of *Mycobacterium tuberculosis* complex organisms in cerebrospinal fluid. *J. Clin. Microbiol.* **36**:2191–2194.
97. **Laszlo, A., M. Rahman, M. Raviglione, F. Bustreo, and the WHO/IUATLD Network of Supranational Reference Laboratories.** 1997. Quality assurance programme for drug susceptibility testing of *Mycobacterium tuberculosis* in the WHO/IUATLD Supranational Laboratory Network: first round of proficiency testing. *Int. J. Tuberc. Lung Dis.* **1**:231–238.
98. **Lawn, S. D., et al.** 2009. Urine lipoarabinomannan assay for tuberculosis screening before antiretroviral therapy: diagnostic yield and association with immune reconstitution disease. *AIDS* **23**:1875–1880.
99. **Lee, H., T. J. Yoon, and R. Weissleder.** 2009. Ultrasensitive detection of bacteria using core-shell nano-particles and an NMR-filter system. *Angew. Chem. Int. Ed.* **48**:5657–5660.
100. **Lin, S. Y., E. Desmond, D. Bonato, W. Gross, and S. Siddiqi.** 2009. Multi-center evaluation of Bactec MGIT 960 system for second-line drug susceptibility testing of *Mycobacterium tuberculosis* complex. *J. Clin. Microbiol.* **47**:3630–3634.
101. **Ling, D. I., L. L. Flores, L. W. Riley, and M. Pai.** 2008. Commercial nucleic-acid amplification tests for diagnosis of pulmonary tuberculosis in respiratory specimens: meta-analysis and meta-regression. *PLoS One* **3**:e1536.
102. **Ling, D. I., A. A. Zwerling, and M. Pai.** 2008. Rapid diagnosis of drug-resistant TB using line probe assays: from evidence to policy. *Exp. Rev. Respir. Med.* **2**:583–588.
103. **Ling, D. I., A. A. Zwerling, and M. Pai.** 2008. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. *Eur. Respir. J.* **32**:1165–1174.
104. **Lumb, R., et al.** 2006. An alternative method for sputum storage and transport for *Mycobacterium tuberculosis* drug resistance surveys. *Int. J. Tuberc. Lung Dis.* **10**:172–177.
105. **Mahairas, G. G., P. J. Sabo, M. J. Hickey, D. C. Singh, and C. K. Stover.** 1996. Molecular analysis of genetic differences between *Mycobacterium bovis* BCG and virulent *M. bovis*. *J. Bacteriol.* **178**:1274–1282.
106. **Mase, S. R., et al.** 2007. Yield of serial sputum specimen examinations in

- the diagnosis of pulmonary tuberculosis: a systematic review. *Int. J. Tuberc. Lung Dis.* **11**:485–495.
107. Mawak, J., N. Gomwalk, C. Bello, and Y. Kandakai-Olukemi. 2006. Human pulmonary infections with bovine and environment (atypical) mycobacteria in Jos, Nigeria. *Ghana Med. J.* **40**:132–136.
 108. Mazurek, G. H., et al. 2005. Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. *MMWR Recommend. Rep.* **54**(RR15):49–55.
 109. Mazurek, G. H., M. E. Villarino, and the Centers for Disease Control and Prevention. 2003. Guidelines for using the QuantiFERON-TB test for diagnosing latent *Mycobacterium tuberculosis* infection. *MMWR Recommend. Rep.* **52**(RR2):15–18.
 110. McCarthy, K. D., et al. 2008. Monitoring the performance of mycobacteriology laboratories: a proposal for standardized indicators. *Int. J. Tuberc. Lung Dis.* **12**:1–6.
 111. Metchock, B. G., F. S. Nolte, and R. J. Wallace. 1999. *Mycobacterium*, p. 399–437. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, DC.
 112. Mikhailovich, V., et al. 2001. Identification of rifampin-resistant *Mycobacterium tuberculosis* strains by hybridization, PCR, and ligase detection reaction on oligonucleotide microchips. *J. Clin. Microbiol.* **39**:2531–2540.
 113. Minion, J., H. Sohn, and M. Pai. 2009. Light-emitting diode technologies for TB diagnosis: what is on the market? *Expert Rev. Med. Devices* **6**:341–345.
 114. Miotto, P., F. Piana, D. M. Cirillo, and G. B. Migliori. 2008. Genotype MTBDRplus: a further step toward rapid identification of drug-resistant *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* **46**:393–394.
 115. Miotto, P., F. Piana, G. B. Migliori, and D. M. Cirillo. 2008. Evaluation of the GenoCard as a tool for transport and storage of samples for tuberculosis molecular drug susceptibility testing. *New Microbiol.* **31**:147–150.
 116. Mitchison, D. A. 2000. Role of individual drugs in the chemotherapy of tuberculosis. *Int. J. Tuberc. Lung Dis.* **4**:796–806.
 117. Reference deleted.
 118. Mohamed, A. M., et al. 2005. Computational approach involving use of the internal transcribed spacer 1 region for identification of *Mycobacterium* species. *J. Clin. Microbiol.* **43**:3811–3817.
 119. Monkongdee, P., et al. 2009. Yield of acid-fast smear and mycobacterial culture for tuberculosis diagnosis in people with human immunodeficiency virus. *Am. J. Respir. Crit. Care Med.* **180**:903–908.
 120. Moore, D. A., et al. 2006. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. *N. Engl. J. Med.* **355**:1539–1550.
 121. Moore, D. F., J. A. Guzman, and L. T. Mikhail. 2005. Reduction in turnaround time for laboratory diagnosis of pulmonary tuberculosis by routine use of a nucleic acid amplification test. *Diagn. Microbiol. Infect. Dis.* **52**:247–254.
 122. Mostowy, S., D. Cousins, J. Brinkman, A. Aranaz, and M. A. Behr. 2002. Genomic deletions suggest a phylogeny for the *Mycobacterium tuberculosis* complex. *J. Infect. Dis.* **186**:74–80.
 123. Mukadi, Y. D., D. Maher, and A. Harries. 2001. Tuberculosis case fatality rates in high HIV prevalence populations in sub-Saharan Africa. *AIDS* **15**:143–152.
 124. Müller, B., et al. 2009. African 1, an epidemiologically important clonal complex of *Mycobacterium bovis* dominant in Mali, Nigeria, Cameroon, and Chad. *J. Bacteriol.* **191**:1951–1960.
 125. Mutetwa, R., et al. 2009. Diagnostic accuracy of commercial urinary lipoarabinomannan detection in African tuberculosis suspects and patients. *Int. J. Tuberc. Lung Dis.* **13**:1253–1259.
 126. Nadol, P., et al. 2008. Electronic tuberculosis surveillance systems: a tool for managing today's TB programs. *Int. J. Tuberc. Lung Dis.* **12**(Suppl. 1):8–16.
 127. Ngamert, K., et al. 2009. Diagnostic performance and costs of Capilia TB for *Mycobacterium tuberculosis* complex identification from broth-based culture in Bangkok, Thailand. *Trop. Med. Int. Health* **14**:748–753.
 128. Nkengasong, J. N., et al. 2009. Critical role of developing national strategic plans as a guide to strengthen laboratory health systems in resource-poor settings. *Am. J. Clin. Pathol.* **131**:852–857.
 129. Noordhoek, G. T., S. Mulder, P. Wallace, and A. M. van Loon. 2004. Multicentre quality control study for detection of *Mycobacterium tuberculosis* in clinical samples by nucleic amplification methods. *Clin. Microbiol. Infect.* **10**:295–301.
 130. Nordling, L. 27 July 2009, posting date. African disease labs to get health check. *Nature News* <http://www.nature.com/news/2009/090727/full/news.2009.735.html>.
 131. Noussair, L., F. Bert, V. Leflon-Guibout, N. Gayet, and M. H. Nicolas-Chanoine. 2009. Early diagnosis of extrapulmonary tuberculosis by a new procedure combining broth culture and PCR. *J. Clin. Microbiol.* **47**:1452–1457.
 132. Padilla, E., et al. 2004. Comparative evaluation of the new version of the INNO-LiPA *Mycobacteria* and genotype *Mycobacterium* assays for identification of *Mycobacterium* species from MB/BacT liquid cultures artificially inoculated with mycobacterial strains. *J. Clin. Microbiol.* **42**:3083–3088.
 133. Padilla, E., et al. 2005. Comparison of the sodium hydroxide specimen processing method with the C18-carboxypropylbetaine specimen processing method using independent specimens with auramine smear, the MB/BacT liquid culture system, and the COBAS AMPLICOR MTB test. *J. Clin. Microbiol.* **43**:6091–6097.
 134. Pai, M., S. Kalantri, L. Pascopella, L. W. Riley, and A. L. Reingold. 2005. Bacteriophage-based assays for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: a meta-analysis. *J. Infect. Dis.* **51**:175–187.
 135. Pai, M., J. Minion, H. Sohn, A. Zwerling, and M. D. Perkins. 2009. Novel and improved technologies for tuberculosis diagnosis: progress and challenges. *Clin. Chest Med.* **30**:701–716.
 136. Pai, M., L. W. Riley, and J. M. Colford, Jr. 2004. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect. Dis.* **4**:761–776.
 137. Park, M. Y., et al. 2009. Evaluation of an immunochromatographic assay kit for rapid identification of *Mycobacterium tuberculosis* complex in clinical isolates. *J. Clin. Microbiol.* **47**:481–484.
 138. Parsons, L. M., et al. 2002. Rapid and simple approach for identification of *Mycobacterium tuberculosis* complex isolates by PCR-based genomic deletion analysis. *J. Clin. Microbiol.* **40**:2339–2345.
 139. Parsons, L. M., J. R. Driscoll, H. W. Taber, and M. Salfinger. 1997. Drug resistance in tuberculosis. *Infect. Dis. Clin. North Am.* **11**:905–928.
 140. Parsons, L. M., et al. 1999. Rifampin-resistant *Mycobacterium tuberculosis* isolates with rpoB mutation, L533P, appear to be rifampin susceptible in the BACTEC system, abstr. U4. Abstr. 99th Gen. Meet. Am. Soc. Microbiol., Chicago, IL.
 141. Parsons, L. M., A. Somoskovi, R. Urbanczik, and M. Salfinger. 2004. Laboratory diagnostic aspects of drug resistant tuberculosis. *Front. Biosci.* **9**:2086–2105.
 142. Patel, J. B., et al. 2000. Sequence-based identification of *Mycobacterium* species using the MicroSeq 500 16S rDNA bacterial identification system. *J. Clin. Microbiol.* **38**:246–251.
 143. Pavlou, A. K., et al. 2004. Detection of *Mycobacterium tuberculosis* (TB) in vitro and in situ using an electronic nose in combination with a neural network system. *Biosens. Bioelectron.* **20**:538–544.
 144. Pavlou, A. K., and A. P. Turner. 2000. Sniffing out the truth: clinical diagnosis using the electronic nose. *Clin. Chem. Lab. Med.* **38**:99–112.
 145. Persidis, A. 1998. Biochips. *Nat. Biotechnol.* **16**:981–983.
 146. Peter, T. F., et al. 2009. The need for standardization in laboratory networks. *Am. J. Clin. Pathol.* **131**:867–874.
 147. Pfyffer, G. 2007. *Mycobacterium*: general characteristics, laboratory detection, and staining procedures, p. 543–572. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller (ed.), *Manual of clinical microbiology*, 9th ed. ASM Press, Washington, DC.
 148. Pfyffer, G. E., et al. 1996. Diagnostic performance of amplified *Mycobacterium tuberculosis* direct test with cerebrospinal fluid, other nonrespiratory, and respiratory specimens. *J. Clin. Microbiol.* **34**:834–841.
 149. Pfyffer, G. E., P. Kissling, R. Wirth, and R. Weber. 1994. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by a target-amplified test system. *J. Clin. Microbiol.* **32**:918–923.
 150. Phillips, M., et al. 2007. Volatile biomarkers of pulmonary tuberculosis in the breath. *Tuberculosis* **87**:44–52.
 151. Piatek, A. S., et al. 2000. Genotypic analysis of *Mycobacterium tuberculosis* in two distinct populations using molecular beacons: implications for rapid susceptibility testing. *Antimicrob. Agents Chemother.* **44**:103–110.
 152. Piatek, A. S., et al. 1998. Molecular beacon sequence analysis for detecting drug resistance in *Mycobacterium tuberculosis*. *Nat. Biotechnol.* **16**:359–363.
 153. Piersimoni, C., and C. Scarparo. 2003. Relevance of commercial amplification methods for direct detection of *Mycobacterium tuberculosis* complex in clinical samples. *J. Clin. Microbiol.* **41**:5355–5365.
 154. Pounder, J. I., et al. 2010. *Mycobacterium tuberculosis* complex differentiation by genomic deletion patterns with multiplex polymerase chain reaction and melting analysis. *Diagn. Microb. Infect. Dis.* **67**:101–105.
 155. Pym, A. S., and S. T. Cole. 2002. Drug resistance and tuberculosis chemotherapy—from concept to genomics, p. 355–403. In K. Lewis, A. A. Salyers, H. W. Taber, and R. G. Wax (ed.), *Bacterial resistance to antimicrobials*. Marcel Dekker Inc., New York, NY.
 156. Raizada, N., et al. 2009. Establishing laboratory proficiency to conduct line probe assay for MDR-TB diagnosis. *Int. J. Tuberc. Lung Dis.* **13**(S1):309.
 157. Ramsay, A., et al. 15 March 2009, posting date. Front-loading sputum microscopy services: an opportunity to optimize smear-based case detection of tuberculosis in high prevalence countries. *J. Trop. Med.* doi:10.1155/2009/398767.
 158. Reither, K., et al. 2009. Low sensitivity of a urine LAM-ELISA in the diagnosis of pulmonary tuberculosis. *BMC Infect. Dis.* **9**:141.
 159. Richardson, M., et al. 2002. Multiple *Mycobacterium tuberculosis* strains in early cultures from patients in a high-incidence community setting. *J. Clin. Microbiol.* **40**:2750–2754.
 160. Richeldi, L. 2006. An update on the diagnosis of tuberculosis infection. *Am. J. Respir. Crit. Care Med.* **174**:736–742.
 161. Richter, E., M. Weizenegger, A. M. Fahr, and S. Rusch-Gerdes. 2004. Usefulness of the GenoType MTBC assay for differentiating species of the

- Mycobacterium tuberculosis* complex in cultures obtained from clinical specimens. J. Clin. Microbiol. **42**:4303–4306.
162. Richter, E., M. Weizenegger, S. Rusch-Gerdes, and S. Niemann. 2003. Evaluation of Genotype MTBC assay for differentiation of clinical *Mycobacterium tuberculosis* complex isolates. J. Clin. Microbiol. **41**:2672–2675.
 163. Ringuet, H., et al. 1999. hsp65 sequencing for identification of rapidly growing mycobacteria. J. Clin. Microbiol. **37**:852–857.
 164. Robledo, J., G. I. Mejía, L. Paniagua, A. Martín, and A. Guzmán. 2008. Rapid detection of rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* by the direct thin-layer agar method. Int. J. Tuberc. Lung Dis. **12**:1482–1484.
 165. Roth, A., et al. 1998. Differentiation of phylogenetically related slowly growing mycobacteria based on 16S-23S rRNA gene internal transcribed spacer sequences. J. Clin. Microbiol. **36**:139–147.
 166. Sarkola, A., et al. 2004. Prospective evaluation of the GenoType assay for routine identification of mycobacteria. Eur. J. Clin. Microbiol. Infect. Dis. **23**:642–645.
 167. Service, R. F. 1998. Microchip arrays put DNA on the spot. Science **282**:396–399.
 168. Siddiqi, S. H., and S. Rüscher-Gerdes. 2006. MGIT procedure manual. Foundation for Innovative New Diagnostics, Geneva, Switzerland.
 169. Soini, H., E. C. Bottger, and M. K. Viljanen. 1994. Identification of mycobacteria by PCR-based sequence determination of the 32-kilodalton protein gene. J. Clin. Microbiol. **32**:2944–2947.
 170. Somoskovi, A., J. Dormandy, D. Mitsani, J. Rivenburg, and M. Salfinger. 2006. Use of smear-positive samples to assess the PCR-based Genotype MTBDR assay for rapid, direct detection of the *Mycobacterium tuberculosis* complex as well as its resistance to isoniazid and rifampin. J. Clin. Microbiol. **44**:4459–4463.
 171. Somoskovi, A., et al. 2008. Direct comparison of the Genotype MTBC and genomic deletion assays in terms of ability to distinguish between members of the *Mycobacterium tuberculosis* complex in clinical isolates and in clinical specimens. J. Clin. Microbiol. **46**:1854–1857.
 172. Somoskovi, A., L. M. Parsons, and M. Salfinger. 2001. The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in *Mycobacterium tuberculosis*. Respir. Res. **2**:164–168.
 173. Somoskovi, A., et al. 2003. Use of molecular methods to identify the *Mycobacterium tuberculosis* complex (MTBC) and other mycobacterial species and to detect rifampin resistance in MTBC isolates following growth detection with the BACTEC MGIT 960 system. J. Clin. Microbiol. **41**:2822–2826.
 174. Song, S. H., et al. 2009. Electrospray ionization-tandem mass spectrometry analysis of the mycolic acid profiles for the identification of common clinical isolates of mycobacterial species. J. Microbiol. Methods **77**:165–177.
 175. Steingart, K. R., et al. 2007. Commercial serological antibody detection tests for the diagnosis of pulmonary tuberculosis: a systematic review. PLoS Med. **4**:e202.
 176. Steingart, K. R., et al. 2006. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect. Dis. **6**:570–581.
 177. Steingart, K. R., A. Ramsay, and M. Pai. 2007. Optimizing sputum smear microscopy for the diagnosis of pulmonary tuberculosis. Expert Rev. Anti Infect. Ther. **5**:327–331.
 178. Streitz, M., et al. 2007. Loss of receptor on tuberculin-reactive T-cells marks active pulmonary tuberculosis. PLoS One **2**:e735.
 179. Stürenburg, E., and R. Junker. 2009. Point-of-care testing in microbiology: the advantages and disadvantages of immunochromatographic test strips. Dtsch. Arztebl. Int. **106**:48–54.
 180. Tang, X., S. L. Morris, J. J. Langone, and L. E. Bockstahler. 2005. Microarray and allele specific PCR detection of point mutations in *Mycobacterium tuberculosis* genes associated with drug resistance. J. Microbiol. Methods **63**:318–330.
 181. Torres, M. J., A. Criado, J. C. Palomares, and J. Aznar. 2000. Use of real-time PCR and fluorimetry for rapid detection of rifampin and isoniazid resistance-associated mutations in *Mycobacterium tuberculosis*. J. Clin. Microbiol. **38**:3194–3199.
 182. Tortoli, E., et al. 2001. Performance assessment of new multiplex probe assay for identification of mycobacteria. J. Clin. Microbiol. **39**:1079–1084.
 183. Troesch, A., et al. 1999. *Mycobacterium* species identification and rifampin resistance testing with high-density DNA probe arrays. J. Clin. Microbiol. **37**:49–55.
 184. Tuohy, M. J., G. S. Hall, M. Sholtis, and G. W. Procop. 2005. Pyrosequencing as a tool for the identification of common isolates of *Mycobacterium* sp. Diagn. Microbiol. Infect. Dis. **51**:245–250.
 185. Turenne, C. Y., L. Tschetter, J. Wolfe, and A. Kabani. 2001. Necessity of quality-controlled 16S rRNA gene sequence databases: identifying nontuberculous *Mycobacterium* species. J. Clin. Microbiol. **39**:3637–3648.
 186. UNAIDS. 2009. AIDS epidemic update. UNAIDS, Geneva, Switzerland. <http://www.unaids.org/en/dataanalysis/epidemiology/2009aidsupdate/>.
 187. United Nations Foundation. 2011. mHealth for development. United Nations Foundation, Washington, DC. <http://www.unfoundation.org/global-issues/technology/mhealth-report.html>.
 188. Van Deun, A., et al. 2009. *Mycobacterium tuberculosis* strains with highly discordant rifampin susceptibility test results. J. Clin. Microbiol. **47**:3501–3506.
 189. van Soolingen, D., et al. 1997. A novel pathogenic taxon of the *Mycobacterium tuberculosis* complex, Canetti: characterization of an exceptional isolate from Africa. Int. J. Syst. Bacteriol. **47**:1236–1245.
 190. van Soolingen, D., et al. 1998. Diagnosis of *Mycobacterium microti* infections among humans by using novel genetic markers. J. Clin. Microbiol. **36**:1840–1845.
 191. Vernet, G., C. Jay, M. Rodrigue, and A. Troesch. 2004. Species differentiation and antibiotic susceptibility testing with DNA microarrays. J. Appl. Microbiol. **96**:59–68.
 192. Victor, T. C., et al. 2002. Molecular detection of early appearance of drug resistance during *Mycobacterium tuberculosis* infection. Clin. Chem. Lab. Med. **40**:876–881.
 193. Walusimbi, M., et al. 2010. Randomized trial of a 6-month punctuated course of ART in Ugandan HIV+ adults with pulmonary TB and CD4 >350, abstr. 105. Abstr. 17th Conf. Retrovir. Oppor. Infect., San Francisco, CA.
 194. Warren, R. M., et al. 2006. Differentiation of *Mycobacterium tuberculosis* complex by PCR amplification of genomic regions of difference. Int. J. Tuberc. Lung Dis. **10**:818–822.
 195. Warren, R. M., et al. 2009. The clinical relevance of mycobacterial pharmacogenetics. Tuberculosis **89**:199–202.
 196. Warren, R. M., et al. 2004. Patients with active tuberculosis often have different strains in the same sputum specimen. Am. J. Respir. Crit. Care Med. **169**:610–614.
 197. Weizenegger, M., M. Hengstler, K. Feldmann, and A. M. Fahr. 2004. Direct detection of *Mycobacterium tuberculosis* complex and four nontuberculous mycobacteria: preliminary data of an evaluation in a hospital and a private laboratory, abstr. 128. Abstr. 25th Annu. Meet. Eur. Soc. Mycobacteriol., Alghero, Italy.
 198. Woods, G., N. G. Warren, and C. B. Inderlied. 2007. Susceptibility test methods: mycobacteria, norcardia, and other actinomycetes, p. 1223–1247. In P. R. Murray, E. J. Baron, J. H. Tenover, and M. A. Tenover (ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, DC.
 199. Reference deleted.
 200. World Health Organization. 2006. Patient monitoring guidelines for HIV care and antiretroviral therapy. World Health Organization, Geneva, Switzerland. <http://www.who.int/hiv/pub/imai/patientguide/en/index.html>.
 201. World Health Organization. 2006. TB e-Recording and Reporting Portal. World Health Organization, Geneva, Switzerland. http://www.who.int/tb/country/recording_reporting/en/index.html.
 202. World Health Organization. 3 February 2011, accession date. Indicator. World Health Organization, Geneva, Switzerland. http://www.stop.tb.org/wg/tb_hiv/assets/documents/cgd1/IRUM%20Draft%20WHO-PEPFAR%20Indicator%2004%2018%2008.doc.
 203. Reference deleted.
 204. World Health Organization. 2007. Reduction of number of smears for the diagnosis of pulmonary TB. World Health Organization, Geneva, Switzerland. <http://www.who.int/tb/dots/laboratory/policy/en/index2.html>.
 205. World Health Organization. 2007. The use of liquid medium for culture and DST. World Health Organization, Geneva, Switzerland. <http://www.who.int/tb/dots/laboratory/policy/en/index3.html>.
 206. World Health Organization. 2008. Anti-tuberculosis drug resistance in the world. Report no. 4. WHO/HTM/TB/2008.394. World Health Organization, Geneva, Switzerland.
 207. World Health Organization. 2008. Interim policy guidance on drug susceptibility testing (DST) of second-line anti-tuberculosis drugs. WHO/HTM/TB/2008.392. World Health Organization, Geneva, Switzerland.
 208. World Health Organization. 2008. New laboratory diagnostic tools for tuberculosis control. Stop TB Partnership: Retooling Task Force and the New Diagnostics Working Group. World Health Organization, Geneva, Switzerland.
 209. World Health Organization. 2008. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). World Health Organization, Geneva, Switzerland. http://www.who.int/tb/features_archive/policy_statement.pdf.
 210. World Health Organization. 2008. Laboratory-based evaluation of 19 commercially available rapid diagnostic tests for tuberculosis. Special Program for Research and Training in Tropical Diseases. Diagnostics evaluation series, 2. World Health Organization, Geneva, Switzerland.
 211. World Health Organization. 2009. Global tuberculosis control: a short update to the 2009 report. WHO/HTM/TB/2009.426. World Health Organization, Geneva, Switzerland.
 212. World Health Organization. 2009. Report of the 9th meeting of STAG-TB, Geneva, Switzerland. World Health Organization, Geneva, Switzerland. http://www.who.int/tb/advisory_bodies/stag_tb_report_2009.pdf.
 213. World Health Organization. 2010. Roadmap for rolling out Xpert MTB/RIF

- for rapid diagnosis of TB and MDR-TB. World Health Organization, Geneva, Switzerland. http://www.who.int/entity/tb/laboratory/roadmap_xpert_mtb_rif_rev23dec2010.pdf.
214. **Wrighton-Smith, P., and J. P. Zellweger.** 2006. Direct costs of three models for the screening of latent tuberculosis infection. *Eur. Respir. J.* **28**:45–50.
215. **Yagui, M., et al.** 2006. Timely diagnosis of MDR-TB under program conditions: is rapid drug susceptibility testing sufficient? *Int. J. Tuberc. Lung Dis.* **10**:838–843.
216. **Zelazny, A. M., L. B. Calhoun, L. Li, Y. R. Shea, and S. H. Fischer.** 2005. Identification of *Mycobacterium* species by *secA1* sequences. *J. Clin. Microbiol.* **43**:1051–1058.
217. **Zhang, Y., B. Heym, B. Allen, D. Young, and S. Cole.** 1992. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature* **358**:591–593.
218. **Zhang, Y., and A. Telenti.** 2000. Genetics of drug resistance in *Mycobacterium tuberculosis*, p. 235–254. In G. F. Hatfull and W. R. Jacobs, Jr. (ed.), *Molecular genetics of mycobacteria*. ASM Press, Washington, DC.
219. **Zheng, X., M. Pang, H. D. Engler, S. Tanaka, and T. Reppun.** 2001. Rapid detection of *Mycobacterium tuberculosis* in contaminated BACTEC 12B broth cultures by testing with Amplified Mycobacterium Tuberculosis Direct Test. *J. Clin. Microbiol.* **39**:3718–3720.

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Evan Lee is currently Senior Policy Officer at FIND. He has several years of experience practicing internal medicine in the United States; international public health experience with leading organizations, including the Médecins Sans Frontières and Management Sciences for Health; as well as an M.B.A. from the MIT Sloan School of Management. At FIND, his multifaceted role includes leadership of policy-related aspects of the organization's projects to implement new diagnostics in resource-limited countries. His accomplishments have included a leading role in the UNITAID-funded EXPAND-TB project to implement new TB diagnostics in up to 27 countries; leadership and guidance in FIND's support, along with other partners, for the Ministry of Health of Uganda to develop a national laboratory policy; and mobilization of resources for FIND's work across a number of diseases. He is also a focal point for working on access-related activities with drug and vaccine product development partnerships.



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C. N. Paramasivan joined FIND in 2006 from his position as Head of the Bacteriology Division of the Tuberculosis Research Centre of the Indian Council of Medical Research in Chennai, India. He earned his Ph.D. in Medical Microbiology as well as a D.Sc. from Madras University. Dr. Paramasivan has made several important contributions to tuberculosis research, including studies with newer antitubercular drugs and standardizing the quality-assured laboratory diagnosis of tuberculosis. He took active interest in training laboratory personnel and establishing TB laboratory services in several countries. He also played a major role in the drug resistance surveillance studies in India and other countries. Since 1990, Dr. Paramasivan has served as Head of the WHO Supranational Reference Laboratory in Chennai, and in 2001, he became a member of the WHO Expert Advisory Panel on Tuberculosis. In 2004, the IUATLD elected him to the Chair of the Bacteriology and Immunology Section, and in 2007, the IUATLD elected him as the Secretary of the TB Section for their Global Meetings.



Alash'le Abimiku's international focus in Nigeria has been pivotal to the establishment of the Institute of Human Virology-Nigeria (IHV-N) and the PEPFAR program and the growing portfolio of grants linked to the program. Dr. Abimiku first demonstrated the unique nature of the HIV strain prevalent in Nigeria in 1993 as subtype G and provided the first reliable HIV research laboratory in central Nigeria to support her current research focuses on the role of subtypes in disease pathogenesis using the mother-to-child model to investigate the transmitting subtype(s) and by studying effects of coinfections with TB on HIV pathogenesis. Dr. Abimiku has trained a cadre of pre- and postdoctoral Nigerian students who are engaged in advanced research through the UM-IHV NIH Fogarty AIDS International Training and Research Program Grant. She is internationally recognized for her leadership in HIV research and her role as the cochair of the African AIDS vaccine program.



Steven Spector received a B.A. in Biology (1969) and a Ph.D. in Microbiology and Immunology (1975) from Temple University, Philadelphia, PA. He was a postdoctoral fellow at Albert Einstein Medical Center, Philadelphia, PA, in Experimental Immunology; Director of Clinical Virology at the same institution (1976 to 1979); and assistant Professor of Medical Microbiology and Immunology (1979), Professor (1991), and Associate Dean for Pre-Clinical Education from 1998 to 2001, University of South Florida College of Medicine (USF COM), Tampa, FL, and is currently Associate Dean for Student Affairs at the USF COM. He has written more than 175 publications and has edited a dozen books. He is Chairman of the Clinical Virology Symposium (1985 to present). Dr. Spector has received 2 outstanding teaching awards (1991 and 1996), the Theodore and Vanette Askonas-Ashford Distinguished Scholar Award by the USF in 1997, the Professorial Excellence Program award (1998), and the PASCV Diagnostic Virology Award (2004). He is the chair of the American Society for Microbiology Laboratory Capacity Building Committee.



Giorgio Roscigno has 20 years of experience in the pharmaceutical industry in the medical and clinical research of anti-infectives. He joined the industry after 5 years of field medical practice in Ethiopia, Sudan, and the Democratic Republic of Congo. He was closely associated with the clinical work and the subsequent registration (including the FDA) of all the TB fixed-dose combinations containing rifampin, and he also contributed to the clinical development of rifapentine, a long-acting rifamycin. Dr. Roscigno has led the clinical research work on efloornithine for the treatment of sleeping sickness and the coordination of the African multicenter clinical trial in collaboration with the WHO Special Programme for Research and Training in Tropical Diseases. In 2000, Dr. Roscigno was among the founding members of the Global Alliance for TB Drug Development (GATB). He left the industry and joined the GATB, initially as Acting Chief Executive Officer and later as Strategic Development Director. In May 2003, he joined FIND as Chief Executive Officer.



John Nkengasong is currently Chief of the Global AIDS Program's International Laboratory Branch at the CDC in Atlanta, GA, and has been the Acting Associate Director for Laboratory Science at the Center for Global Health at the CDC since January 2010. In addition to his CDC post, Dr. Nkengasong is also cochair of the US\$49-billion U.S. President's Emergency Plan for AIDS Relief's (PEPFAR) Laboratory Technical Working Group. He received a master's degree in Tropical Biomedical Science at the Institute of Tropical Medicine in Antwerp, Belgium; a master's degree in Medical and Pharmaceutical Sciences at the University of Brussels School of Medicine, Brussels, Belgium; and a doctorate in Medical Sciences from the University of Brussels. He joined the CDC in 1995 as Chief of the Virology Laboratory, CDC Abidjan, Côte d'Ivoire, and became Chief of the International Laboratory Branch, Global AIDS Program, in 2005.

