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# The Diagnosis of Tuberculosis

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Tuberculosis is transmitted from person to person by respiratory droplets. Although some people develop active tuberculosis disease after infection, almost all tuberculosis infections are asymptomatic and remain latent. Latent tuberculosis infection (LTBI) itself progresses to active disease in approximately 5% to 10% of infected persons. The rate of progression is much greater in immunocompromised individuals. The estimated 2 billion people living with LTBI represent a vast reservoir of potential cases of tuberculosis around the world. This reservoir of LTBI is therefore a major barrier to the ultimate control and elimination of tuberculosis.

Strategies to combat tuberculosis in regions that are resource-rich aim, first, to identify and treat persons who have active disease; second, to find and treat contacts of cases of active disease who develop LTBI, and, third, to screen high-risk populations and treat LTBI [1]. Diagnosis and treatment of LTBI are crucial in this effort. In most of the world, however, resources are devoted exclusively to the highest priorities of tuberculosis control: identification and treatment of active disease [1]; for lack of resources, LTBI is neither diagnosed nor treated.

Diagnostic testing for both LTBI and active disease has changed little during the last century. Because of limitations in available tests, there has long been a clear need for better diagnostic tests. LTBI, until very recently, has been diagnosed exclusively by the tuberculin skin test (TST). The TST is fraught with problems including relatively poor sensitivity and specificity. Newer tests for LTBI offer the promise of greatly improved diagnostic accuracy.

Tools for the diagnosis of active disease include clinical suspicion, response to treatment, chest radiographs, staining for acid-fast bacilli (AFB), culture for mycobacteria, and, more recently, nucleic acid amplification (NAA) assays. AFB smears lack both sensitivity and specificity, and culture is very slow to produce results, limiting the ability to diagnose active disease effectively. NAA assays and several other experimental diagnostic tools can add significantly to the active disease diagnostic armamentarium. The suitability of newer diagnostic tests in a given population varies according to the resources available to pay for and implement those tests, however [2].

In resource-poor countries, where options are limited, current approaches, such as relying almost exclusively on the sputum smear for the diagnosis of active disease, leave a significant number of cases undetected [3–6]. This approach may be the only economically feasible strategy given the initial costs involved in the widespread use of other diagnostic modalities. Although smear-positive cases are the most infectious, neglecting smear-negative disease (approximately half of cases overall) increases the morbidity and mortality of the disease in those patients and does not account for the significant burden of transmission attributable to these smear-negative cases (17% of all transmission in one study using molecular epidemiology techniques) [6]. The increased likelihood of smear-negative tuberculosis in HIV patients, particularly those who have advanced immunosuppression [7], makes this diagnostic approach especially problematic, because the regions most afflicted by tuberculosis are similarly inundated with HIV infection.

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Meanwhile, in resource-rich countries, underdiagnosis is less an issue than overdiagnosis with its attendant costs (the production of specimens, the surveillance of cultures—most of which will ultimately be negative—use of isolation rooms, empiric therapy for tuberculosis, and expensive or invasive diagnostic testing) [8]. In part, the need is for more rapid diagnosis, allowing for earlier treatment of cases, decreased transmission of active disease, and decreased expenditure of resources. There is also a need for increased sensitivity of testing so that cases do not go unrecognized, and for increased specificity and negative predictive value to decrease the cost of having a high suspicion for this disease.

An ideal test for active tuberculosis would produce rapid results (available within 1 day), would have high sensitivity and specificity, low cost, and robustness (ability to provide reproducible results in a variety of settings), would be highly automated or easily performed without the need for excessive sample preparation or technical expertise, and would be able to provide drug-susceptibility data. Ideally, such a test would also be able to distinguish between LTBI and active disease. For LTBI, such a test would distinguish true infection from bacille Calmette-Guerin (BCG) vaccination and infection with nontuberculous mycobacteria (NTM). In cases of active disease, it would be valuable to be able to determine infectiousness, follow response to therapy, distinguish *Mycobacterium tuberculosis* from NTM in AFB-positive specimens and obtain drug-susceptibility information. No test performs all these functions at present, but several new tests are being used or are currently under study that incorporate many of these features and offer the possibility of improved diagnosis of LTBI and of active disease.

## Tests for latent tuberculosis infection

### *The tuberculin skin test*

Tuberculin, a broth culture filtrate of tubercle bacilli, was first described in detail by Robert Koch in 1891, a year after he introduced it as a potential cure for tuberculosis [9]. Although its purported curative properties proved unfounded, Koch observed that subcutaneous inoculation of tuberculin led to a characteristic febrile reaction in patients who had tuberculosis but not in those who did not have tuberculosis, giving rise to its use in the diagnosis of the disease. The technique was refined over the next 2 decades so that cutaneous or intradermal inoculation restricted the reaction to the skin. Subsequently, a standardized version of tuberculin, the purified protein derivative (PPD), was introduced in 1934 [9]. In 1939, the batch of PPD known as PPD-S was produced by Seibert and Glenn [3]. This batch remains the international standard for PPD to this day.

In the early years of the TST, the assumption that tuberculin reactions resulted solely from tuberculosis infections went virtually unchallenged [9]. By the mid-1930s, however, mounting evidence suggested that tuberculin reactions might not be restricted to such infections. In addition, investigators noted that if the dose of PPD were increased enough, almost everyone tested positive, including infants unlikely to have been exposed to tuberculosis [9]. These findings called into question the specificity of the TST, highlighting its limitations for the first time.

The current state of knowledge about the utility of tuberculin skin testing derives in large measure from a series of trials performed in epidemiologically well-defined populations of persons who have known

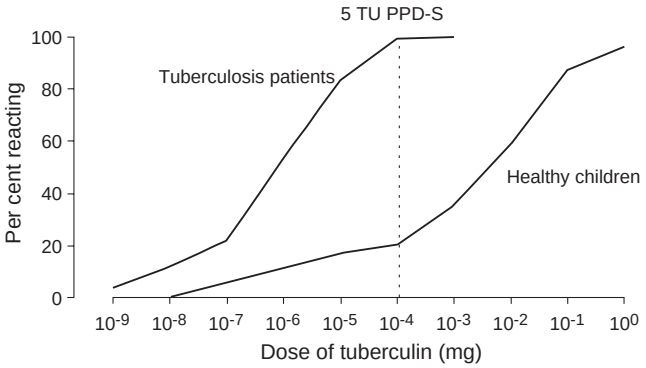


Fig. 1. Cumulative frequency of reactors responding to increasing doses of tuberculin among healthy children and patients with tuberculosis. TU, tuberculin units. (*From* Reider H. The epidemiologic basis of tuberculosis control. Paris: International Union Against Tuberculosis and Lung Disease; 1999. p. 32; with permission.)

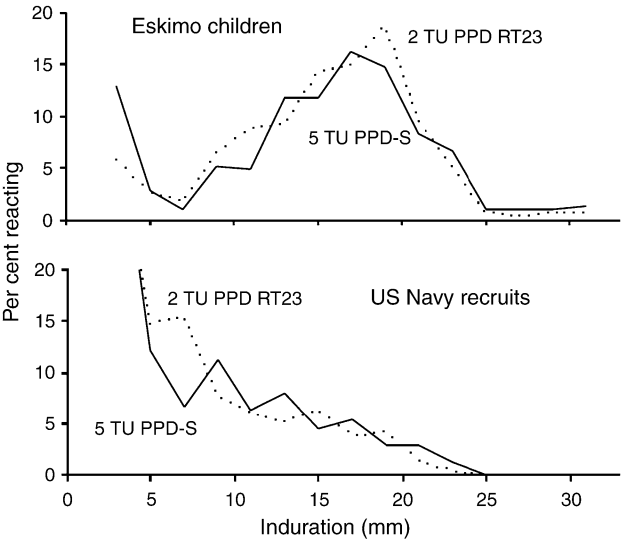


Fig. 2. Frequency distribution of skin test reaction with 5-TU PPD-S (*solid line*) and 2-TU PPD RT 23 (*dotted line*) among Eskimo children and United States Navy recruits. TU, tuberculin units. (*From* Reider H. The epidemiologic basis of tuberculosis control. Paris: International Union Against Tuberculosis and Lung Disease; 1999. p. 33; with permission.)

tuberculosis disease, those at extremely low likelihood of having latent infection, and those likely to be close contacts of persons who have active tuberculosis. Reider [10] has described these trials in detail.

The dosing of tuberculin for use in skin testing was determined in studies such as one done in Ohio, in which skin testing was performed on tuberculosis patients and a group of children in orphanages who had little chance of tuberculosis exposure. At a dose of $10^{-4}$ mg of tuberculin, a clear distinction could be made between the two groups (Fig. 1). Refinements in dosing and criteria for positivity were achieved by using standardized preparations of PPD made by the Statenseruminstitut in Amsterdam and testing them in groups of Eskimo children (a group that has a high likelihood of latent infection acquired from close contact with active cases and very little exposure to environmental mycobacteria) and US Navy recruits who have little chance of contact with active tuberculosis but have frequent exposure to environmental mycobacteria (Fig. 2). Testing of 5440 tuberculosis patients revealed a normal distribution of extent of induration, with a mean of 16 to 17 mm (Fig. 3). Finally, a massive survey of more than 700,000 US military recruits, of whom 400,000 had no known

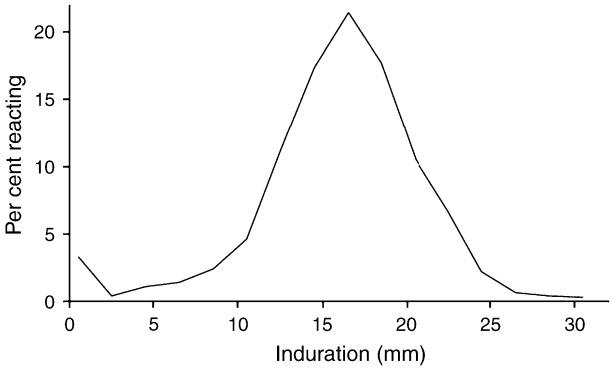


Fig. 3. Frequency distribution of tuberculin skin test results (5−tuberculin unit PPD S) among 5544 tuberculosis patients in the United States. (*From* Reider H. The epidemiologic basis of tuberculosis control. Paris: International Union Against Tuberculosis and Lung Disease; 1999. p. 35; with permission.)

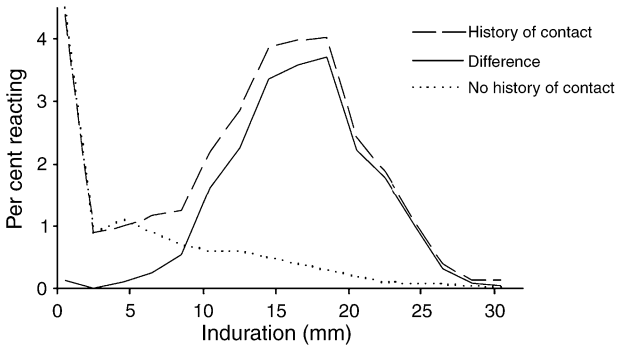


Fig. 4. Frequency distribution of tuberculin skin test results in United States Navy recruits with (*dashed line*) or without (*dotted line*) tuberculosis. The solid line shows the difference between the two groups. (*From* Reider H. The epidemiologic basis of tuberculosis control. Paris: International Union Against Tuberculosis and Lung Disease; 1999. p. 36; with permission.)

contact with tuberculosis patients and 10,000 had definite contacts, provided meaningful data on which to base recommendations for interpretation of skin tests that are still useful today (Fig. 4).

Although the TST has been in widespread use for a century and is the only universally accepted test for the diagnosis of LTBI, it suffers from significant inherent limitations. To understand these limitations, it is useful to review the mechanism of the TST. Infection with *M. tuberculosis* results in a cell-mediated immune response giving rise to sensitized T lymphocytes (both $CD4^+$ and $CD8^+$ [11]) targeted to *M. tuberculosis* antigens. Stimulation by *M. tuberculosis* antigens causes these T cells to release interferon-gamma (IFN-γ). The TST functions by eliciting this response in previously sensitized individuals. In such individuals, an intradermal injection of PPD evokes a delayed-type hypersensitivity response mediated by sensitized T cells and results in cutaneous induration. PPD, however, is a precipitate of *M. tuberculosis* culture supernatant which contains roughly 200 antigens, many of which are shared by other mycobacteria including many NTM and *M. bovis* BCG [12,13]. A response to PPD may signify infection with *M. tuberculosis* or, just as readily, infection with NTM [14–18] or vaccination with BCG [18–22]. This cross-reactivity seriously limits the specificity of the TST in many populations [23].

Given that one quarter to one half of the burden of tuberculosis in developed countries is found in foreign-born immigrants from high-prevalence countries, and this population is made up precisely of those who are likely to be BCG-vaccinated and to have been exposed to NTM, the TST is least reliable in those most in need of screening. Specificity is a major shortcoming of the TST. In addition, sensitivity of the TST may also be poorest in patients at high risk for developing tuberculosis. Anergy caused by an immunocompromised state (especially with HIV infection or medication-induced immunosuppression) may lead to false-negative results [24]. False-negative results also may occur up to about 10 weeks after infection with *M. tuberculosis* [25–27]. False negatives, particularly in the HIV population where the implications of active disease are most pressing [3,28,29], greatly limit the utility of this test.

The exact sensitivity and specificity of the TST for LTBI is impossible to know with certainty, given the lack of a reference standard for diagnosis. In that context, estimates of the global burden of LTBI are especially problematic. Estimates indicate that the problem is enormous, but these estimates are based on the performance of the TST, and such estimates, particularly in developing countries, are notoriously unreliable [30,31]. Studies of the prevalence of LTBI in India, for instance, have yielded prevalence rates ranging from 9% [32] to more than 80% in various populations [33]. A more accurate epidemiologic tool would greatly facilitate a better estimation of the true scope of the problem.

The TST is limited further by the subjectivity of its interpretation [34], in particular, by problems with interreader and intrareader reliability [25,35–37] and digit preference [38,39]. Also, the existence of the booster phenomenon [24,25,39–41], poor standardization of PPD preparations [31], and, logistically, the need for a return visit to have the test read make the TST a highly imperfect diagnostic tool. That it does not distinguish between LTBI and active disease also limits its usefulness.

Whether the extent of induration resulting from tuberculin skin testing can predict the development of tuberculosis in a linear (or at least dependent fashion) has also been the subject of considerable discussion

and investigation. Recently, Horsburgh [42] has provided a well-reasoned and -supported data set that gives guidance in this area.

Alternatives to the TST are lacking. Serologic tests for the diagnosis of *M. tuberculosis* have been disappointing [43,44]. Although an antibody response to *M. tuberculosis* antigens occurs, there is great individual variability in the number and type of serologically reactive antibodies [44], making this diagnostic tool too unreliable. Because no serologic tests for tuberculosis are remotely good enough to be used clinically at present, they are not discussed further in this article.

Despite its many limitations, the TST by necessity remains in widespread use. In 2000, the Centers for Disease Control and Prevention (CDC), the American Thoracic Society (ATS), and the Infectious Disease Society of America (IDSA) issued updated guidelines for the use of the TST in screening for LTBI [24]. These guidelines stress that in general one should not place a TST unless treatment would be offered in the event of a positive test. In addition, cut-off points of induration (5, 10, or 15 mm) for determining a positive test vary by the pretest risk category into which a patient falls. This approach may further decrease the specificity of the test, but it increases the sensitivity for capturing those at highest risk for developing active disease in the short term. Continued focus on this century-old test highlights its continued importance, but the need for a more accurate diagnostic tool is evident.

### *Beyond the tuberculin skin test*

Development of novel diagnostic tests for LTBI is hampered by the lack of a true reference standard for diagnosis. Without such a standard, the best approach might be to apply a new test to a population in a controlled study, observe all patients positive by the novel and reference tests, and determine which test more accurately predicts the development of active disease. This approach, however, is limited by ethical and practical considerations.

Demonstrating that any test is better than the TST is therefore difficult. Studies of the magnitude of those cited by Reider [10] for the development of tuberculin skin testing are unlikely to be repeated. What approach, then, might be taken? It is well documented that the greater the proximity to the source case of active disease and the greater the duration of exposure to that case, the more likely it is that a person will develop LTBI. Although the risk of LTBI cannot be precisely quantified for all degrees of contact, the risk of LTBI may be expressed as an increasing likelihood of infection with increasing exposure to the source case or increasing amounts of high-risk behavior. It is against this standard that the sensitivity of any new diagnostic test must be compared with the TST itself. A test that is superior to the TST in sensitivity would be more likely to be positive given a greater degree of contact with the source case. Specificity must similarly be gleaned from the expectation that only infection with *M. tuberculosis* and not with NTM or *M. bovis* BCG would give a positive result. A test would be superior in specificity if its results seemed to be independent of NTM exposure and BCG vaccination status.

Recently, a new generation of tests for LTBI has been developed. They are the QuantiFERON-TB and QuantiFERON-TB Gold (QFN-Gold) tests (Cellestis Limited, St. Kilda, Australia) and the T SPOT-TB test (Oxford Immunotec, Oxford, UK) The basis of these tests is the detection in serum of either the release of IFN-$\gamma$ on stimulation of sensitized T cells by *M. tuberculosis* antigens in vitro (QuantiFERON) or detection of the T cells themselves (T SPOT-TB). In 1990, Wood and colleagues [45] developed a whole-blood assay for the detection of IFN-$\gamma$ in response to a specific antigen, PPD, intended for diagnosing bovine tuberculosis. Later that year, Rothel and colleagues [46] introduced a sandwich enzyme immunoassay for bovine IFN-$\gamma$ that streamlined the assay, making it more practical for widespread testing. This assay was shown to be both sensitive and specific in field comparisons with the intradermal tuberculin test [46–48] and later was accredited in Australia for use in the diagnosis of bovine tuberculosis [49]. In 1995, the test was successfully used in the diagnosis of *M. tuberculosis* and *M. avium* complex infection in humans [50,51].

Similar to the TST, the QuantiFERON-TB test detects cell-mediated immunity to tuberculin. In contrast to intradermal injection of PPD, however, whole blood is incubated overnight with PPD from *M. tuberculosis*, and the IFN-$\gamma$ that is released from sensitized lymphocytes is subsequently quantified by ELISA [38]. As discussed previously, PPD antigens are shared across mycobacterial species, including *M. bovis* BCG [13,52]. A positive response to the whole-blood IFN-$\gamma$ assay for PPD therefore, like the TST itself, lacks specificity for *M. tuberculosis* infection and may reflect infection with NTM or vaccination with BCG [18]. Early studies were nonetheless encouraging [38,49,53–56], demonstrating decreased false-positive results relative to the TST in BCG-vaccinated individuals [38] and those exposed to NTM [38], with equal or better apparent sensitivity

and specificity than the TST in multiple studies [38,49,53,56], including in populations of intravenous drug users and HIV-positive patients [54,55]. Few studies purported to demonstrate the superiority of the TST for LTBI [57,58].

The discovery of *M. tuberculosis*–specific antigens opened the way to improving the specificity of the assay. In 1986, Harboe and colleagues [59] reported the first *M. tuberculosis*–specific antigen, MPB-64 (later known as MPT-64). In 1995, Andersen and colleagues [60] reported the highly immunogenic antigen target of the cellular immune response to tuberculosis in mice, known as the early secreted antigenic target 6 (ESAT-6). Subsequently, in 1998, Berthet and colleagues [61] described culture filtrate protein (CFP-10) [61], another highly immunogenic antigen. MPT-64 has been studied extensively [62–66], but, because it is present in some strains of BCG and is a less potent target of the immune response, it has limited utility [62,63,67]. On the other hand, ESAT-6 [61–64,68–70] and CFP-10 [61,71] have demonstrated great potential.

In 1998, the complete genome sequence of *M. tuberculosis* was determined [72]. An earlier comparison of the *M. tuberculosis* genome with the genomic composition of *M. bovis* and *M. bovis* BCG in 1996 by subtractive genomic hybridization [68] and, subsequently, in 1999, by comparative hybridization experiments on a DNA microarray [73] led to the identification of a genomic region known as RD1. The gene products of RD1 are found only in *M. tuberculosis*, in pathogenic *M. bovis* strains [64,68,73], and in four NTM (*M. kansasii*, *M. szulgai*, *M. flavescens*, and *M. marinum*) [69,70]. Because, of these, only *M. kansasii* overlaps clinically with *M. tuberculosis*, and because *M. kansasii* infection is uncommon, the RD1 region encodes antigens that are essentially specific to *M. tuberculosis*. Among these antigens are, of course, ESAT-6 and CFP10, as well as MPT-64. ESAT-6 and CFP-10 are secreted by *M. tuberculosis* into the extracellular environment and are potent targets of the cell-mediated immune response [61–63,71,74]. ESAT-6, which has been shown to be highly immunogenic in animals [66,75–78], readily discriminated between bovine tuberculosis and cattle sensitized to environmental mycobacteria [75]. CFP-10 also has demonstrated utility in diagnosing bovine tuberculosis because of its significant specificity [78].

The melding of the ELISA-based QuantiFERON-TB test and the RD1 antigens, ESAT-6 and CFP-10, led to a more specific test, now known as the QFN-Gold. Similarly, the T SPOT-TB test employs the RD1 antigens but links them to an ELISPOT assay that identifies ESAT-6– or CFP-10–specific IFN-γ–secreting $CD4^+$ T cells.

Studies of the IFN-γ release assays employing ESAT-6 or CFP10 in humans have been promising [79–88]. ESAT-6 is a major target of the cellular immune response in humans [62,63]. Early on, assays employing ESAT-6 were shown to be more specific although less sensitive than PPD-based assays for active disease [79,82,89–91]. This improved specificity over PPD with loss of sensitivity was later shown by Arend and colleagues [80,81] to hold true in LTBI as well. More importantly, they also demonstrated improved specificity over the TST. They reported loss of sensitivity with respect to the TST, but, because the TST was used as the reference standard for LTBI, this result probably reflected the improved specificity rather than poorer sensitivity [80]. The improved specificity over the QuantiFERON-TB assay for PPD and over the TST was confirmed in a study by Johnson et al [79] of 60 Australian medical students who did not have BCG vaccination or known exposure to *M. tuberculosis* or NTM. The specificity of both the QuantiFERON-TB for PPD and the TST were reduced after BCG vaccination was administered to the students, but the QuantiFERON-TB for ESAT-6 was unaffected [79]. Brock and colleagues [85] also demonstrated improved specificity for both the ESAT-6 and CFP-10 over PPD in BCG-vaccinated versus nonvaccinated subjects. Recently, Brock and colleagues [88] published an outbreak study based on a case of active disease in a Danish high school student and the student's mostly non–BCG-vaccinated contacts. The TST was used as the reference standard for LTBI in this population, and there was excellent agreement between the TST and the QuantiFERON-TB ESAT-6/CFP-10 assay (94%) [88]. Superiority of the assay could not be established in this study, because the TST was itself the reference standard. Nonetheless, significant specificity with regard to BCG status was suggested by the findings in subjects who were BCG-vaccinated. Of these, 50% in the high-exposure group had a positive assay, compared with 53% of high-exposure subjects who did not have BCG vaccination. In the low-exposure group, 5% of BCG-vaccinated persons were assay positive, similar to the 6% of those who did not have BCG vaccination [88]. Finally, Mori and colleagues [87] studied a group of 216 Japanese student nurses who had no identified risk for *M. tuberculosis* exposure, all of whom had been vaccinated with BCG [87]. In this group 64.6% of the subjects had a TST response measuring 10 mm or more, yielding a specificity of 35.4% for the TST if it is assumed that none had true LTBI using exposure

history as the reference standard. The QuantiFERON-TB ESAT-6/CFP-10 assay, on the other hand, yielded a specificity of 98.1% in this group, far superior to the TST. The sensitivity of the assay, 89.0%, was determined in a separate group of patients who had culture-proven active disease. Extrapolating the sensitivity for LTBI from the sensitivity for active disease is problematic, however, because both IFN-γ activity and TST reactivity are reduced in active disease; extrapolation probably underestimates the sensitivity for LTBI [12,92,93].

Ajit Lalvani and colleagues [94] have adapted the ELISPOT technique, an ex vivo T-cell–based assay for the detection of cell-mediated immunity, for use in detecting *M. tuberculosis*. The technique detects and enumerates peripheral blood IFN-γ–secreting T cells that respond to ESAT-6 or CFP-10. In 2001, Lalvani and colleagues [94] established a sensitivity of 96% in active disease (100% in the subpopulation that had extrapulmonary tuberculosis) as compared with 69% sensitivity for the TST. In healthy BCG-vaccinated controls, the ELISPOT was not confounded by BCG. In TST-positive household contacts of a case of active disease (expected cases of LTBI), 85% were ELISPOT positive, suggesting a sensitivity for LTBI of 85% if the TST is taken to be the reference standard [94].

In 2001, Pathan and colleagues [92] also examined a low-exposure population of mostly BCG-vaccinated subjects. None of the 32 healthy controls were positive on ELISPOT. That year, Lalvani and colleagues [95] also reported on an outbreak study [95]. The odds ratio (OR) of a positive ELISPOT with increasing proximity and duration of exposure to the index case was 9.0, whereas the OR of a positive TST (by Heaf test, a less well-standardized approach to skin testing than the tuberculin test) was only 1.9. Another study published in 2001 by Lalvani and colleagues [12] looked at 40 healthy controls in the United Kingdom, 82% of whom were BCG-vaccinated and all of whom were ELISPOT negative. Another study in the United Kingdom similarly found that none of 40 healthy controls were ELISPOT positive [96].

In 2003, Ewer and colleagues [97] published a meticulously investigated outbreak study that evaluated the ESAT-6/CFP-10–based ELISPOT assay for LTBI. Two years earlier, in the United Kingdom, a secondary school student had been diagnosed with sputum smear–positive cavitary pulmonary tuberculosis. The health authority screened 1128 students at the school with the TST (HEAF test). Screening detected 69 cases of active disease and 254 cases of LTBI, 87% of whom had been vaccinated with BCG. Five hundred thirty-five representative students were enrolled in the study and underwent ELISPOT testing. The significance of the study derives from the detailed contact information available for each student by virtue of their mandatory, scheduled daily activities. Degree of exposure to the source case could be readily quantified and grouped as (1) same class, same year, with regularly shared lessons; (2) same year with only weekly shared events; and (3) other years. Using ORs, the authors provide an estimate of the increase in odds of a positive ELISPOT for each increase in level of exposure. The ELISPOT correlated significantly better than the TST with increasing exposure across each group. The relative risk (RR) of direct exposure to the index case if one was both TST and ELISPOT positive was 17.6. If one was ELISPOT positive but TST negative, it was 11.7. If one was ELISPOT negative and TST positive, the RR was only 2.97. Also, TST positivity was significantly associated with BCG vaccination status and with birth in a region of high prevalence for NTM, whereas no significant association was found for the ELISPOT. The superior correlation with degree of exposure strongly suggests improved sensitivity with the ELISPOT. The lack of confounding by BCG or NTM suggests improved specificity with the ELISPOT.

The role of IFN-γ– or T-cell–based assays in the diagnosis of LTBI is being defined. These tests show promise as replacements for the TST in diagnosing LTBI among persons at risk for infection in the developed world. Both the QFN-Gold and T SPOT-TB tests are approved for diagnostic use throughout the European Union. In addition, the QFN-GOLD was approved by the United States Food and Drug Administration (FDA) in December 2004. Clinical experience with these tests should accumulate rapidly. The less accurate original QuantiFERON-TB is approved for use in the United States by the FDA, but guidelines for its use are confusing, and the test has not been widely adopted in clinical settings.

The improved sensitivity of these tests over the TST would capture a cohort of patients who otherwise would go without treatment of LTBI. Within that cohort, those that would have progressed to active disease would be spared the attendant morbidity and mortality. The future contacts of those destined to progress to active disease would likewise be spared. This cohort would be overrepresented by those most likely to have false-negative TST results, namely immunosuppressed individuals. This population is precisely the one in which it is most important to identify LTBI because of their increased risk for developing active disease [3]. Unanswered is whether

there is something different about this cohort that gives positive results on these assays and negative results on the TST and whether their risk for developing active disease is less than that of those who are also TST positive. The TST has predictive value for the subsequent development of active disease in both HIV-negative [9,25] and HIV-positive patients [98]: a stronger skin test response indicates an increased risk of developing active disease [99,100]. Longitudinal studies linking positive assays with risk for development of active disease are ongoing and are crucial to demonstrating the true role of these tests. If they demonstrate a high degree of accuracy, treatment of LTBI might, under the right conditions, become a viable strategic component of tuberculosis control efforts in high- and low-prevalence countries [97]. One small study of 24 healthy household contacts of persons who had smear-positive pulmonary tuberculosis in Ethiopia looked at QuantiFERON-TB ESAT-6 responses at the initial visit and approximately 2 years later. The subjects were not treated for LTBI. Seven of 24 patients went on to develop pulmonary active disease. The subjects who responded to ESAT-6 at study entry were significantly more likely to develop active disease than those who were not responsive [101].

The improved specificity would decrease unnecessary treatment in those who are not truly infected, thereby avoiding the costs to the health care system for medication, follow-up, and management of complications. It would also spare the individual patient these same costs.

Costs would also likely be reduced by the increased capture of cases of LTBI, because their identification would eliminate the future—much greater—costs of treating an outbreak of active disease. Costs also might be reduced by the decreased number of clinic visits required, because the TST requires a follow-up visit for reading the TST and may require a second skin test to overcome the booster phenomenon.

At present, LTBI is neither diagnosed nor treated in most high-burden, resource-poor countries, except in certain situations, such as young children who are close contacts of active cases. The expense of a novel diagnostic test must be justified in terms of the cost savings realized from treating LTBI. Such savings could accrue by reducing the number of cases of active disease that develop from the vast reservoir of LTBI that exists in the developing world.

Overall, the potential advantages of the IFN-$\gamma$–release and T-cell–based assays over the TST in diagnosing LTBI seem to include improved specificity (lack of confounding by BCG and NTM) and improved sensitivity. Operator bias and inter- and intrareader variability are significantly reduced. Only a single patient visit is required. There is no booster effect. The results are obtained rapidly, within 24 hours. There may well be cost savings to the health care system. The assays have proven to be robust in different populations and in different settings, in both the developed and developing worlds. The technology for running the assays has improved so that requirements for equipment and technical expertise are reasonable.

Further study is needed. Longitudinal data, as mentioned previously, are critical. To date there are no large-scale trials of these assays, and few studies have employed the Mantoux test while using current ATS/CDC/IDSA criteria for a positive TST. Study of the effect of treatment on assay results in LTBI may provide the data necessary to monitor therapy for LTBI, allowing the clinician, for example, to distinguish response to therapy from lack of response caused by noncompliance or isoniazid resistance. Another area of interest would be the ability to distinguish past exposure to *M. tuberculosis* without ongoing infection from true ongoing LTBI. It may be that detection of T cells specific for ESAT-6 or CFP-10 suggests that tubercle bacilli continue to secrete these antigens [12]. Identification of an antigen that is expressed either during LTBI or during active disease, but not during both, and that could distinguish these two states would greatly enhance the role of the assays in active disease and would increase the specificity for LTBI. Finally, the point after infection at which the RD1 antigens become detectable has yet to be defined precisely and has significant implications for testing in contact investigations and for reducing false-negative results soon after infection with *M. tuberculosis*.

## Tests for tuberculosis disease

The reference standard for diagnosing active disease remains largely clinical: documented response to appropriate therapy. Of course, establishing a microbiologic diagnosis is preferable. AFB smear, mycobacterial culture, and NAA assays may all be used in confirming a diagnosis of active disease (both pulmonary and extrapulmonary). In the case of pulmonary tuberculosis, the method of obtaining a sample greatly affects the sensitivity of testing. Extrapulmonary tuberculosis frequently poses a diagnostic challenge because specimens may be difficult to obtain. After identifying *M. tuberculosis*, the most pressing issue is drug-susceptibility testing, in which

traditional culture techniques are giving way to more advanced technologies that produce rapid results.

## Mode of diagnosis of pulmonary tuberculosis

### *Is there a role for the tuberculin skin test in the diagnosis of active disease?*

The TST was originally a test for active disease [9]. It is unsuited for that purpose because its specificity is limited by cross reactions with NTM and *M. bovis* BCG, and, more importantly, by its detection of LTBI itself, and by alterations in general immune responsiveness that may occur in cases of active tuberculosis. The sensitivity of the TST for active disease varies considerably, from 65% to 94% [31,34,58,87,89,94]. A study of 3600 hospitalized patients done by the World Health Organization in the 1950s found a sensitivity of 93% for reactions of 10 mm or more and a sensitivity of 78% when a cut-off of 14 mm or more was used [31]. The sensitivity is decreased in certain populations (eg, to less than 50% in critically ill patients who have disseminated tuberculosis) [25]. Lacking both specificity and sensitivity for active disease, the TST is not particularly useful in this setting.

### *Sputum-based diagnosis*

To establish a diagnosis of pulmonary tuberculosis, respiratory samples are submitted to the laboratory for microscopy (AFB smear) and for mycobacterial culture. NAA assays may also be used in the diagnostic algorithm, as discussed later.

The technique used to obtain the respiratory sample strongly influences the ability to detect pulmonary tuberculosis. Expectorated sputum is generally the starting point. Three samples are collected on three separate days and stained for AFB [102,103]. Although, the utility of collecting three samples has been questioned [104], the overall yield for smear and culture is superior to collecting fewer specimens [105,106]. Samples are generally sent simultaneously for smear and culture, because culture data are essential for confirmation of the diagnosis. In resource-poor countries, the cost of culture is often too great, resulting in reliance solely on AFB smears.

The sensitivity of sputum AFB smears for detecting pulmonary tuberculosis is limited by the need for 5000 to 10,000 bacilli per milliliter to be present in a specimen to allow detection [3]. The sensitivity of expectorated sputum ranges from 34% to 80% [3–5,104,107–116]; the sensitivity tends to be highest in patients who have cavitary disease and lowest in patients who have weak cough or less advanced disease. In no way does a negative sputum smear eliminate the diagnosis of active tuberculosis, particularly if the clinical suspicion is high. Instituting therapy in such cases often is warranted while awaiting culture results. If a patient is suspected of having pulmonary tuberculosis but is smear negative on expectorated sputum or is unable to produce sputum for testing (30% of patients in one series [117]), further diagnostic testing may be warranted. The options include sputum induction (SI), fiberoptic bronchoscopy (FOB), and perhaps gastric washings (GW). The following discussion refers specifically to patients who are expectorated sputum smear negative or who cannot produce an expectorated sputum sample.

### *Sputum induction*

SI in the diagnosis of active disease was first described in 1961 by Hensler and colleagues [117]. They adapted an earlier technique used to obtain sputum for cytology in diagnosing lung cancer. Early studies compared SI with the well-established method of gastric aspiration [117–119]. In patients unable to expectorate or who had smear-negative sputum samples, SI was superior to GW in obtaining a suitable sample for culture, although the two techniques were noted to be complementary [119]. GW probably adds to overall diagnosis, and, according to one author, its value has been underestimated in recent years [120]. The role of GW in adults is probably quite limited, however. SI, on the other hand, has proven effective in patients clinically suspected of having pulmonary tuberculosis who are either unable to produce sputum or are sputum smear negative.

SI has performed well in resource-poor countries with little added cost [121–123]. In South Africa, SI performed on 51 patients yielded a suitable sample in 36 [123]. Fifteen of the 36 patients (42%) were smear positive, 12 of whom were ultimately culture positive as well. In Malawi, Parry and colleagues [122] were able to obtain SI specimens in 73 of 82 patients. Eighteen of the 73 (25%) were smear positive, and 30 of 73 (42%) were culture positive. Similarly, of 1648 patients in China, 558 (34%) were smear positive on SI samples. The direct cost per SI in that study was 37 cents [121]. In these studies, SI provided appropriate samples for diagnosis and increased the early diagnostic yield significantly. SI also seems to be cost-effective in the resource-poor setting.

Conversely, in a retrospective review of 114 patients who had culture-positive *M. tuberculosis* infection at an urban hospital in New York, SI added little to overall diagnosis and was deemed costly by the investigators [124]. In 1 year, they performed 1566 SIs yielding only 16 positive smears in 10 patients. At a cost of $28.65 per SI, the annual cost of $45,000 would indeed be difficult to justify [124]. A study in the United Kingdom confirmed a low yield but suggested there might be a role for SI [125].

Is there a role for SI in resource-rich countries? A large, prospective study from Montreal, Canada, assessed 500 patients who were either smear negative (5%) or could not produce sputum (95%) with repeated SI [126]. An adequate sample was obtained in 99.8% of patients. The cumulative yield of SI for smear-positive samples with successive attempts was 64%, 81%, 91%, and, after four inductions, 98%. The culture yield also increased with each attempt from 70% to 91% to 99% to 100%. This study suggests that the use of repeated SI has a high yield in this setting and that repeated SI should be considered seriously in this subset of patients [126].

### *Sputum induction versus fiberoptic bronchoscopy*

How does SI compare with FOB in the diagnosis of pulmonary tuberculosis in expectorated-sputum smear-negative patients or patients unable to produce sputum? A study by McWilliams and colleagues [127] from New Zealand compared repeated SI with FOB, which was performed if at least two SIs were smear negative. They prospectively studied 129 patients who underwent both procedures. Each successive SI, up to three in total, increased the yield for culture-positive samples significantly. SI was performed without difficulty in 96% of patients and had an overall yield of 96.3% after three tests, confirming the utility of repeated SIs. By contrast, the yield of FOB was only 51.9%, making SI significantly more sensitive in this population. The authors also noted that the overall cost of FOB was three times that of doing three SIs. They offered several strategies for diagnosis: FOB alone was too insensitive, whereas SI alone was sensitive (missed only one case) and cost effective. Although the combination of SI and FOB would have captured all culture-confirmed cases of pulmonary tuberculosis, it would have done so at four times the cost. The preferred strategy, according to the authors, would employ SI followed by FOB only in patients who were negative on SI but had features of pulmonary tuberculosis on chest radiograph. This strategy missed no cases and was only 2.5 times the cost of SI alone [127]. This strategy may be worthwhile in resource-rich settings but may be less applicable in resource-poor settings where repeated SI alone would diagnose most of the cases at a substantially reduced cost.

Anderson [128] prospectively compared SI and FOB with bronchoalveolar lavage (BAL) in 101 patients who had suspected pulmonary tuberculosis in Montreal. SI yielded a positive smear in 19% of cases; the yield of FOB smear was 12%. The yield was much higher in obtaining culture-positive samples: 87% with SI, as compared with 73% for FOB. Overall, SI performed better than FOB, and direct costs of FOB were more than eight times those of SI [128].

A Brazilian study compared SI with FOB in HIV-positive and HIV-negative patients [129]. One hundred forty-three patients were diagnosed with pulmonary tuberculosis, 17% of whom were HIV positive. The sensitivity of SI smear was 33.8%, and that of FOB was 38.1% in HIV-negative patients. In HIV-positive patients, the sensitivities were similar: 36% for SI smear and 40% for FOB smear. SI produced an adequate sample in 97% of patients in this study [129].

SI performs well in both resource-poor and resource-rich countries, is useful in HIV-positive and -negative patients and compares favorably with FOB in diagnostic yield and cost. Some authors argue that neither SI nor FOB should be performed unless absolutely necessary, given the risk of exposure of health care workers and other patients to the aerosol-generating procedures [130]. This warning, however, applies mostly to environments where proper respiratory protective equipment and exhaust ventilation devices or appropriate isolation rooms are in short supply [130].

### *The role of fiberoptic bronchoscopy*

FOB encompasses BAL, bronchial washings (BW), bronchial brushings (BB), transbronchial biopsy (TBB), and postbronchoscopy sputum collection (PBS). FOB has been studied by several investigators (although usually in relatively small studies) in pulmonary tuberculosis suspects who are smear negative or unable to produce a sputum sample. The utility of FOB (or SI) in this setting is twofold. First, generating a sample in patients who do not have spontaneous sputum creates the potential for making a diagnosis. Second, rapid diagnosis (by positive smear or histopathology) in either subset of patients provides the potential for earlier intervention and treatment while awaiting culture results.

In 1988, Chawla and colleagues [131] at the University of Delhi in India prospectively studied 50 pulmonary tuberculosis suspects who were smear negative or unable to produce sputum. Overall, cultures of *M. tuberculosis* from FOB were positive in 90%. More significantly, a rapid diagnosis was made in fully 72% of cases. Smear-positive samples were obtained in 28% of PBS specimens, 24% of BW specimens, and 56% of BB specimens. In the case of BB specimens, 10 patients (20% of those studied) were rapidly diagnosed exclusively by this means. PBS and BW each provided the exclusive diagnosis for 6% of patients. TBB was performed in 30 patients, and histopathology was positive in 9 (3 were exclusively diagnosed on biopsy). The authors comment that the high yield from the BB smears was a result of brushing caseous material in the bronchi when visible [131].

In a study from Hong Kong in 1982, So and colleagues [132] also prospectively examined the capability of FOB for rapid diagnosis. They performed FOB in 65 pulmonary tuberculosis suspects. Overall, rapid diagnosis was achieved in 42 of 65 (65%). TBB gave a rapid diagnosis in 33 of the 57 patients in whom it was performed (58%) and was the exclusive means of rapid diagnosis in 12% [132].

Willcox and colleagues [133] conducted a study in Cape Town, South Africa, in 1982 that looked at 275 pulmonary tuberculosis suspects. Seventy-nine were diagnosed with pulmonary tuberculosis. FOB made the culture diagnosis in 60 of 79 (76%). BB gave a rapid diagnosis in 33%, and TBB did so in 43%. Similarly, Sarkar and colleagues [134] prospectively performed FOB in 30 pulmonary tuberculosis suspects in Rajasthan, India. Rapid diagnosis was made in 22 of 30 persons (73%).

In a retrospective review of 41 patients who had culture-proven pulmonary tuberculosis and underwent FOB, a rapid diagnosis was obtained in 34% of patients [135]. Finally, Mehta and colleagues [112] looked retrospectively at 30 patients who had culture-positive pulmonary tuberculosis and a negative sputum smear or no sample. FOB (BW and BB) made a rapid diagnosis in 18 of 30 patients (60%).

The potential utility of BAL and TBB for rapid diagnosis in HIV-positive and HIV-negative patients was demonstrated in a study by Kennedy and colleagues [136]. They retrospectively reviewed 67 HIV-positive and 45 HIV-negative patients who had culture-proven pulmonary tuberculosis. Of those who had smear-negative sputum, BAL provided a rapid diagnosis in 24% of HIV-positive and 8% of HIV-negative patients. BAL was the exclusive means of diagnosis in seven HIV-positive patients and in one HIV-negative patient. TBB yielded a rapid diagnosis in 16% of HIV-positive and 42% of HIV-negative patients. Overall, TBB provided the exclusive early diagnosis in 10% of patients [136].

Although not all studies report such high yields from FOB [137–142], it definitely has utility [103]. The ability to achieve rapid diagnosis—a crucial step in the management of pulmonary tuberculosis—with FOB generally ranges from around 30% to 70%, and the overall yield of culture from FOB specimens is much higher [112,113,131,132,134–136,143–147]. Although the yield of the different techniques varied significantly among studies, each one clearly contributed to the overall yield of FOB.

The most productive use of FOB is in pulmonary tuberculosis suspects who produce no sputum or who are smear negative and in patients in whom there is considerable diagnostic uncertainty, where lung biopsy may produce an alternative diagnosis. These benefits must always be weighed against the costs of the procedure, concerns regarding infection control, and the risk of TBB in any given patient.

### *Cultures*

Cultures of mycobacteria require only 10 to 100 organisms to detect *M. tuberculosis*. As a result, the sensitivity of culture is excellent, ranging from 80% to 93% [3,107]. Moreover, the specificity is quite high, at 98% [3]. Cultures increase the sensitivity for diagnosis of *M. tuberculosis* and allow speciation, drug-susceptibility testing, and, if needed, genotyping for epidemiologic purposes [3]. Therefore, all specimens should be cultured.

There are three types of culture media: solid media, which includes egg-based media (Lowenstein-Jensen) and agar-based media (Middlebrook 7H10 and 7H11), and liquid media (Middlebrook 7H12 and other broths). Solid media, long the standard for culturing mycobacteria, are slower than liquid media, which now are widely used alongside solid media to increase sensitivity and decrease recovery time [148,149]. In fact, Lowenstein-Jensen 7H10 and 7H11 media may detect mycobacteria in less than 4 weeks [148,150,151], but they require incubation for as long as 6 to 8 weeks before they can be classified as negative. In contrast, broth media combined with DNA probes for rapid species identification typically provide results in less than 2 weeks with smear-positive samples and somewhat longer with smear-negative samples [148,151,152]. Broth media formulations include both manual and automated systems using radiometric or colorimetric methods for detection of mycobacteria. Examples of broth media

include the BACTEC 460TB and BACTEC MB9000 radiometric methods, the Mycobacterial Growth Indicator Tube or MGIT nonradiometric method, and the manual Septi-Chek AFB system (all from Becton Dickinson Microbiology Systems, Franklin Lakes, NJ), the MB/Bac T (Biomerieux, Durham, NC), Extra Sensing Power (ESP) and Myco-ESPculture System II (Trek Diagnostic Systems, Cleveland, OH), and BacT/ALERT MB Susceptibility Kit (Organon Teknika, Durham, NC).

Broth media also may allow more rapid determination of drug susceptibilities, particularly if direct susceptibility testing is used. Direct susceptibility testing may be done with smear-positive samples that are simultaneously inoculated into bottles lacking and containing antibiotics. With this technique, drug susceptibilities can be known at the same time as culture results.

Newer culture technologies are in development. One such product is TK Medium (Salubris, Inc., Cambridge, MA). TK Medium uses multiple-color dye indicators to identify *M. tuberculosis* rapidly. It can also be used for drug-susceptibility testing and can differentiate a contaminated specimen. Information is available at www.salubrisinc.com.

### *Nucleic acid amplification assays*

NAA assays amplify *M. tuberculosis*–specific nucleic acid sequences using a nucleic acid probe. NAA assays enable direct detection of *M. tuberculosis* in clinical specimens. Such assays complement the conventional laboratory approach to the diagnosis of active disease. Whereas AFB smears are rapid but lack sensitivity and specificity, and culture is both sensitive and specific but may take from 2 to 8 weeks to produce results, NAA assays allow rapid, sensitive, and specific detection of *M. tuberculosis*. The sensitivity of the NAA assays currently in commercial use is at least 80% in most studies, and these assays require as few as 10 bacilli from a given sample under research conditions [3]. Although the sensitivity of these assays in AFB smear-negative samples is lower than for smear-positive samples, newer assays perform much better in this regard than earlier versions, increasing the sensitivity for smear-negative specimens as well as overall sensitivity [4,108]. NAA assays are also quite specific for *M. tuberculosis*, with specificities in the range of 98% to 99%.

At present two FDA-approved NAA assays are widely available for commercial use: the AMPLICOR M. tuberculosis (Roche Diagnostic Systems, Inc., Branchburg, NJ), and the Amplified Mycobaterium Tuberculosis Direct (MTD) Test (Gen-Probe, Inc., San Diego, CA).

The AMPLICOR assay uses DNA polymerase chain reaction (PCR) to amplify nucleic acid targets. The FDA approved its use in smear-positive respiratory specimens in December 1996. The COBAS AMPLICOR is an automated version of the AMPLICOR MTB. The MTD assay is an isothermal strategy for detection of *M. tuberculosis* rRNA. The FDA approved its use for use with smear-positive respiratory specimens in December 1995. A reformulated MTD (AMTDII or E-MTD, for enhanced MTD) was approved by the FDA in May 1998 for smear-positive specimens and in September 1999 for detection of *M. tuberculosis* in both smear-positive and smear-negative respiratory specimens.

In clinical and laboratory studies, the original MTD assay ranged in sensitivity from 83% to 98% for smear-positive respiratory samples [107,153–160] and from 70% to 81% for smear-negative respiratory samples. In a recent study in Zambia (one of relatively few studies in a resource-poor country), the sensitivity was only 64% [116]. The specificity in these studies was 98% to 99%. The AMPLICOR assay performed similarly. The sensitivity was 74% to 92% for smear-positive respiratory samples [5,107,109,157,161–166] and 40% to 73% for smear-negative samples [5,107,161,164–166]. Specificity ranged from 93% to 99%. In Switzerland, Laifer and colleagues [167] recently tested the AMPLICOR assay in 3119 war refugees from Kosovo and found a sensitivity of only 64% for pulmonary tuberculosis [167]. They noted, however, that the negative predictive value of three consecutive PCRs (in two sputa and one BAL) was 100%. In studies where MTD and AMPLICOR have been compared directly, MTD has consistently had a small advantage [107,157,159].

The E-MTD brings with it an improved sensitivity [4,108,153,168], especially in smear-negative specimens [4,108]. Bergmann and colleagues [4] investigated the E-MTD in a 1999 study of Texas prison inmates [4]. One thousand four respiratory specimens from 489 inmates tested with E-MTD were compared with culture, smear, and clinical course. Twenty-two inmates were diagnosed with pulmonary tuberculosis (10 smear-positive and 12 smear-negative.) Overall, the E-MTD had a sensitivity of 95.2% and a specificity of 99.1%. In smear-positive patients, the sensitivity and specificity were both 100%. In smear-negative patients, the sensitivity was 90.2%, and the specificity was 99.1% [4]. A 1999 study from the Central Public Health Laboratory in Etobicoke,

Ontario, looked at 823 specimens (616 respiratory) over a 1-year period [108]. Using clinical diagnosis as the reference standard, the specificity approximated 100%, and the sensitivity for either smear-positive or smear-negative respiratory samples was 100%, an exceptionally high value, especially for the smear-negative specimens. Specimens that were smear negative were preselected for testing with the E-MTD based on a clinical determination that the patients were at high risk for tuberculosis. Preselection no doubt contributed to the high sensitivity and specificity in this study, but results indicate there is great utility in selecting appropriate patients for testing [108].

An investigation of the E-MTD with particular clinical relevance was undertaken by Catanzaro and colleagues [169] who evaluated the performance of the E-MTD in a multicenter, prospective trial. In this study, the E-MTD was evaluated against the backdrop of a patient's clinical suspicion for pulmonary tuberculosis, which was stratified into low, intermediate, or high risk as determined by physicians who had expertise in evaluating patients for tuberculosis. Clinical investigators determined the risk for 338 patients. The specificity of the E-MTD was high in all groups. The sensitivities were 83%, 75%, and 87% respectively. The positive predictive value, however, was low in the low-risk group (59%, as compared with 100% in the other two groups). The negative predictive value was especially high in the low-risk group (99%) and remained high (91%) in the intermediate- and high-risk groups. These results compared favorably with the AFB smear, which had positive predictive values of 36% (low), 30% (intermediate), and 94% (high), respectively, and negative predictive values of 96% (low), 71% (intermediate), and 37% (high), respectively. This study demonstrates the clear utility of the E-MTD test and suggests that it may be particularly helpful for confirming disease in intermediate- and high-risk patients and for excluding cases in low-risk patients [169].

Other NAA assays have been tested, such as a ligase chain-reaction–based test (LCx test; Abbott Diagnostics Division, Abbott Park, IL), and the strand displacement amplification (SDA) test known as the BDProbeTec ET Mycobacterium tuberculosis Complex Direct Detection Assay (DTB) (Becton Dickinson Biosciences, Sparks, MD). DTB is a 1-hour assay that couples SDA to a fluorescent energy-transfer detection system. DTB performs similarly to the E-MTD [170,171]. A variety of less standardized PCR assays have been developed and tested [172–175]. Real-time PCR assays have compared favorably with AMPLICOR [174,175] and E-MTD [173]. None of these tests has been approved for use in the United States.

In 2000, the CDC updated its recommendations for use of NAA tests for the diagnosis of active disease [176]. The CDC now recommends that AFB smear and NAA be performed on the first sputum smear collected. If smear and NAA are both positive, pulmonary tuberculosis is diagnosed with near certainty. If the smear is positive and the NAA is negative, the statement recommends testing the sputum for inhibitors by spiking the sputum sample with an aliquot of lysed *M. tuberculosis* and repeating the assay. If inhibitors are not detected, the process is repeated on additional specimens. If the sputum remains smear positive without inhibitors and NAA negative, the patient can be assumed to have NTM. If a sputum sample is smear negative but E-MTD positive (only the E-MTD is approved for smear-negative specimens), the CDC recommends testing additional samples. If further samples are E-MTD positive, the patient can be assumed to have pulmonary tuberculosis. If both the smear and E-MTD are negative, an additional specimen should be tested by E-MTD. If negative, the patient can be assumed not to have infectious pulmonary tuberculosis. The recommendations conclude by noting that clinicians must always rely on clinical judgment and that, ultimately, definitive diagnosis rests on response to therapy and culture results [176]. Although they have a certain logic, these recommendations are expensive and based on few published data.

Overall, a reasonable use of NAA assays for rapid diagnosis of pulmonary tuberculosis is as follows: NAA assays should be used to confirm that a positive AFB smear does indeed represent *M. tuberculosis*. If both smear and NAA are positive, pulmonary tuberculosis is diagnosed with near certainty. If the smear is positive and the NAA is negative, testing the sputum for inhibitors and repeating the assay is warranted [177]. If inhibitors are not detected, and the process is repeated on additional specimens and is negative, the patient can be presumed to have NTM. If smears are negative, but clinical suspicion is intermediate or high (based on the impression of experienced observers [169,178,179]), NAA should be performed on a sputum sample, and a presumptive diagnosis of tuberculosis is made if the test is positive. NAA should not be performed on sputum samples from cases in which the AFB smear is negative and the clinical index of suspicion is low [169, 179,180]. Testing should also be limited to those who have not been treated recently for active disease [177].

Cost is the main consideration limiting the use of the NAA assays, particularly in the developing world. A study in Nairobi, Kenya, compared the cost-effectiveness of AMPLICOR and that of an AFB smear [181]. The AFB smear was 1.8 times as cost-effective. The authors, however, concluded that AMPLICOR could be cost-effective if "the largest contributing component, the costs of the PCR-kit, can be reduced substantially." A cost-effectiveness analysis conducted in Finland in 2004 showed that the addition of COBAS AMPLICOR PCR to smear and culture was not cost-effective unless limited to smear-positive specimens [182]. Extending this assay to smear-negative specimens may be possible when g the E-MTD is used, however, because of its superior sensitivity in smear-negative patients who have pulmonary tuberculosis. Furthermore, centralized laboratories offer the ability to invest in technology, conduct batch testing, develop expertise, and benefit from economies of scale. In such settings, regular NAA testing may be economically feasible [108,183].

A major limitation of NAA tests is that they give no drug-susceptibility information. In addition, they are able to detect nucleic acids from both living and dead organisms and may be falsely positive for active disease in patients who have a recent history of infection and have been adequately treated [156, 184–186]. In contrast to NAAs that employ DNA or rRNA, the use of an assay to detect *M. tuberculosis* mRNA, with a half-life of only minutes, offers an indicator of the viability of *M. tuberculosis*. Assays that detect mRNA remain positive only while viable mycobacteria persist and therefore are useful as sensitive indicators of adequate treatment and for rapid determination of drug susceptibility [187]. This technology is under study.

## Extrapulmonary tuberculosis

Diagnosing extrapulmonary tuberculosis presents the clinician with many challenges. In most cases, the samples are paucibacillary, decreasing the sensitivity of diagnostic tests. Testing for extrapulmonary tuberculosis follows the same principles as for pulmonary tuberculosis, but, because accuracy of diagnosis is attenuated in extrapulmonary tuberculosis, clinicians must rely more heavily on clinical judgment and response to treatment to diagnose extrapulmonary tuberculosis. Meanwhile, the increased incidence of extrapulmonary tuberculosis in HIV patients makes it all the more urgent to improve diagnostic strategies for this entity.

AFB smear and culture are used but generally are less sensitive in nonrespiratory samples. Respiratory samples are sometimes of benefit in extrapulmonary tuberculosis. In the case of pleural tuberculosis, the finding of *M. tuberculosis* in the sputum is diagnostic of tuberculosis in patients who have an effusion. Such patients may not easily give expectorated sputum samples, however. In this setting, IS has been shown to have a sensitivity of 52% for *M. tuberculosis* [188], compared with the 60% to 80% sensitivity of the more invasive pleural biopsy [189].

In the case of miliary tuberculosis, sputum smears are warranted, but if smears are negative, FOB may play a significant role. FOB was performed in 41 patients who had miliary tuberculosis and smear-negative sputum [190]. Diagnosis was obtained in 34 patients (83%). BB captured 57% of cases, and TBB was diagnostic in 73% of cases. A rapid diagnosis was made in 27 of 34 patients [190]. In a separate study, 22 patients who had smear-negative miliary tuberculosis underwent FOB with brushings, aspirate, and TBB. Tuberculosis was diagnosed in 16 of the 22 patients (73%). A rapid diagnosis was made in 14 of 16, from brush smears alone in 3 patients, aspirate alone in 1, and biopsy alone in 7 [191]. Sampling multiple sites may also be of benefit in miliary tuberculosis.

There is clearly a role for NAA assays in the diagnosis of extrapulmonary tuberculosis, although this role needs to be better defined. The overall sensitivity in nonrespiratory specimens for the MTD or E-MTD ranges from 67% to 100% [108,153–155, 160,168,170,192]. In smear-negative samples, the sensitivity was 52% in one study [160] and 100% in another [108]. The AMPLICOR had a similar sensitivity [162,193], and the specificity of both assays remains high in nonrespiratory samples. The assays do not perform equally well in all sample types; for example, they are much more sensitive in cerebrospinal fluid [192,194] than in pleural fluid [154]. The sensitivities vary significantly among studies, as shown in recent meta-analyses of the use of NAA tests in tuberculous meningitis [195] and tuberculous pleuritis [196]. In one study, the combination of AFB smear and MTD in cerebrospinal fluid had a sensitivity of 64%, which increased to 83% by the third sample tested [197]. The DTB system delivers sensitivity similar to the E-MTD in nonrespiratory samples [170,198,199].

The use of adenosine deaminase (ADA) levels, especially in pleural fluid samples, to diagnose extrapulmonary tuberculosis has shown great promise. A recent meta-analysis of 40 studies investigating ADA for the diagnosis of tuberculous pleuritis yielded the

summary measure of test characteristics derived from the receiver operator characteristic curve where sensitivity equaled specificity at 92.2% [200]. Similarly, a meta-analysis of 31 studies on ADA in pleural tuberculosis yielded a joint sensitivity and specificity of 93% [201]. The performance of ADA in diagnosing pleural tuberculosis is inconsistent across studies, however. In one study, the sensitivity and specificity were both 55% [202]; in another, they were 88% and 85.7%, respectively [203]. Some authors report the need to combine ADA determination with PCR analysis, yielding a combined sensitivity of 87.5% [204], but others argue that ADA alone is superior to ADA combined with PCR [205].

ADA use outside the pleural space has been explored as well. ADA may be of limited value in diagnosing tuberculous meningitis [206] but was sensitive for tuberculous pericarditis in one study [207].

Another test that has received some attention for the diagnosis of pleural and pericardial tuberculosis is pleural or pericardial fluid IFN-$\gamma$, which has proven comparable to or even better than ADA in some studies [201,203,207]. Finally, there may be a role for the serum IFN-$\gamma$ assays, discussed earlier, in the diagnosis of extrapulmonary tuberculosis [81,208].

## Rapid detection of drug resistance

Multidrug-resistant (MDR) tuberculosis poses a major public health problem in many parts of the world. Traditional methods of drug-susceptibility testing rely on cultures of *M. tuberculosis* inoculated with antibiotics and can take weeks for results to be known. The ability to detect drug resistance rapidly would be vitally important to tuberculosis-control efforts, enabling expeditious administration of appropriate treatment and a decrease in transmission of the MDR strain. The detection of rifampin resistance may be used as a surrogate for uncovering multidrug resistance, because most rifampin-resistant isolates are also isoniazid-resistant [209,210]. Rifampin resistance signals the need for treatment with second-line drugs. It is currently feasible to detect rifampin resistance rapidly. One approach takes advantage of genotypic abnormalities by identifying mutations primarily in the region of the *M. tuberculosis rpoB* gene associated with most rifampin-resistant strains of *M. tuberculosis*. Coupling a variety of assays that identify genetic mutations (line probe assays and molecular beacons, for instance) to PCR or related technologies allows rapid detection of the drug-resistant mutations from smear-positive respiratory specimens or from culture specimens [209,211–215]. Another approach detects actual phenotypic resistance seen as persistence of the organism in a rifamycin-containing medium (eg, luciferase reporter phage assays.)

### *Line probe assays*

Line probe assays use PCR and reverse hybridization with specific oligonucleotide probes fixed to nitrocellulose strips in parallel lines. These assays may be used for the detection and identification of mycobacterial species or for rapid identification of mutations in the *rpoB* gene. The INNO-LiPA MYCOBACTERIA v2 (Innogenetics, Ghent, Belgium) and GenoType Mycobacterium (Hain Diagnostika, Nehren, Germany) are line probe assays for the simultaneous detection and identification of mycobacteria; both are very sensitive [216]. The INNO-LiPA Rif.TB assay detects *M. tuberculosis* and is very sensitive for detecting rifampin resistance [213–215,217,218].

### *Molecular beacons*

Molecular beacons are nucleic acid hybridization probes. They are designed to bind to target DNA sequences in regions, such as the *rpoB*, where resistance mutations are known to occur. Molecular beacons fluoresce only when bound to their targets, so that a mutation—even a single-nucleotide substitution—prevents fluorescence. A PCR assay using molecular beacons can identify drug resistance in sputum samples in less than 3 hours and is both sensitive and specific [219]. Lin and colleagues [211] designed a set of molecular beacons for the detection of isoniazid- and rifampin-resistant mutations in *M. tuberculosis* organisms from both culture- and smear-positive respiratory specimens [211]. The sensitivity and specificity for detection of isoniazid resistance were 82.7% and 100%, respectively, and for rifampin resistance were 97.5% and 100%, respectively. Piatek et al [220] previously reported similar findings.

### *Phage amplification*

Phage amplification uses a bacteriophage to detect *M. tuberculosis* in a given sample within 48 hours. FASTPlaqueTB (Biotec, Ipswich, Suffolk, UK) uses phage amplification technology to detect viable *M. tuberculosis* in sputum samples and has had mixed results with excellent specificity (96%–99%) but lesser overall sensitivity (70%–87%) [116,221–223].

It detected 48.8% of smear-negative cases in one study [223]. The FASTPlaqueTB-MDRi or FASTPlaqueTB-RIF uses the phage amplification technology to determine rifampin resistance in culture or sputum specimens. Albert and colleagues [224] demonstrated a sensitivity of 100% and a specificity of 97% for identifying rifampicin-resistant strains in solid culture media and in a separate study demonstrated similar results using a liquid culture system [210]. A more recent study by Albert and colleagues [225] showed a 100% sensitivity and specificity for determining rifampin resistance directly from smear-positive sputum, with results also available within 48 hours.

*Luciferase reporter phages*

Firefly luciferase catalyzes the reaction of luciferin with ATP to generate photons efficiently and thereby emit light. Mycobacteriophages expressing the firefly luciferase gene may be introduced into viable mycobacteria [226]. The presence of cellular ATP in viable mycobacteria causes visible light to be emitted when exogenous luciferin is added. The emitted light is measured by a luminometer or on film (eg, with the Bronx box [227,228]). In the presence of adequate antimycobacterial therapy, mycobacteria are rendered nonviable, and the light is extinguished. Drug-resistant strains of *M. tuberculosis* continue to produce light in the presence of antimycobacterial therapy, revealing their resistance. This method can determine drug susceptibility in 1 to 4 days, and it is also a sensitive and specific means for identifying *M. tuberculosis* [228–232].

Cost and the need for advanced technology and laboratory skills limit the applicability of most of these technologies. Efforts to reduce costs and simplify the technology may make these tests practical for widespread use in the near future in resource-rich and, perhaps, even in resource-poor countries.

## Summary

Diagnostic testing for tuberculosis remained unchanged for nearly a century, but newer technologies hold the promise of a true revolution in tuberculosis diagnostics. The IFN-$\gamma$ release and T-cell–based assays may well supplant the TST in diagnosing LTBI in much of the world. NAA assays are proving their worth in more rapidly diagnosing both pulmonary and extrapulmonary tuberculosis with great sensitivity and specificity. The role of line probe assays, molecular beacons, phage amplification, and luciferase reporter phages in diagnosing tuberculosis and rapidly detecting drug resistance is still being defined. These tests are likely to play an ever-increasing role in the coming years. Ultimately, the appropriate and affordable use of any of these tests depends on the setting (low or high prevalence of active disease, low or high clinical suspicion in a given patient, available resources, and laboratory capabilities) in which they are employed.

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