Clinical Infectious Diseases Advance Access published December 8, 2016

Clinical Infectious Diseases

IDSA GUIDELINE

Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children

David M. Lewinsohn,[1,a] Michael K. Leonard,[2,a] Philip A. LoBue,[3,a] David L. Cohn,[4] Charles L. Daley,[5] Ed Desmond,[6] Joseph Keane,[7] Deborah A. Lewinsohn,[1] Ann M. Loeffler,[8] Gerald H. Mazurek,[3] Richard J. O'Brien,[9] Madhukar Pai,[10] Luca Richeldi,[11] Max Salfinger,[12] Thomas M. Shinnick,[3] Timothy R. Sterling,[13] David M. Warshauer,[14] and Gail L. Woods[15]

[1]Oregon Health & Science University, Portland, Oregon, [2]Emory University School of Medicine and [3]Centers for Disease Control and Prevention, Atlanta, Georgia, [4]Denver Public Health Department, Denver, Colorado, [5]National Jewish Health and the University of Colorado Denver, and [6]California Department of Public Health, Richmond; [7]St James's Hospital, Dublin, Ireland; [8]Francis J. Curry International TB Center, San Francisco, California; [9]Foundation for Innovative New Diagnostics, Geneva, Switzerland; [10]McGill University and McGill International TB Centre, Montreal, Canada; [11]University of Southampton, United Kingdom; [12]National Jewish Health, Denver, Colorado, [13]Vanderbilt University School of Medicine, Vanderbilt Institute for Global Health, Nashville, Tennessee, [14]Wisconsin State Laboratory of Hygiene, Madison, and [15]University of Arkansas for Medical Sciences, Little Rock

Background. Individuals infected with *Mycobacterium tuberculosis* (*Mtb*) may develop symptoms and signs of disease (tuberculosis disease) or may have no clinical evidence of disease (latent tuberculosis infection [LTBI]). Tuberculosis disease is a leading cause of infectious disease morbidity and mortality worldwide, yet many questions related to its diagnosis remain.

Methods. A task force supported by the American Thoracic Society, Centers for Disease Control and Prevention, and Infectious Diseases Society of America searched, selected, and synthesized relevant evidence. The evidence was then used as the basis for recommendations about the diagnosis of tuberculosis disease and LTBI in adults and children. The recommendations were formulated, written, and graded using the Grading, Recommendations, Assessment, Development and Evaluation (GRADE) approach.

Results. Twenty-three evidence-based recommendations about diagnostic testing for latent tuberculosis infection, pulmonary tuberculosis, and extrapulmonary tuberculosis are provided. Six of the recommendations are strong, whereas the remaining 17 are conditional.

Conclusions. These guidelines are not intended to impose a standard of care. They provide the basis for rational decisions in the diagnosis of tuberculosis in the context of the existing evidence. No guidelines can take into account all of the often compelling unique individual clinical circumstances.

EXECUTIVE SUMMARY

Individuals infected with *Mycobacterium tuberculosis* (*Mtb*) may develop symptoms and signs of disease (TB disease) or may have no clinical evidence of disease (latent tuberculosis infection [LTBI]). TB disease is a leading cause of infectious disease morbidity and mortality worldwide, with many diagnostic uncertainties. A task force supported by the supported by the American Thoracic Society, Centers for Disease Control and Prevention, and Infectious Diseases Society of America appraised the evidence and derived the following recommendations using the Grading, Recommendations, Assessment, Development, and Evaluation (GRADE) approach (Table 1):

Testing for LTBI

Our recommendations for diagnostic testing for LTBI are based upon the likelihood of infection with *Mtb* and the likelihood of progression to TB disease if infected, as illustrated in Figure 1.

- We recommend performing an interferon-γ release assay (IGRA) rather than a tuberculin skin test (TST) in individuals 5 years or older who meet the following criteria: (1) are likely to be infected with *Mtb*, (2) have a low or intermediate risk of disease progression, (3) it has been decided that testing for LTBI is warranted, and (4) either have a history of BCG vaccination or are unlikely to return to have their TST read (*strong recommendation, moderate-quality evidence*). Remarks: A TST is an acceptable alternative, especially in situations where an IGRA is not available, too costly, or too burdensome.
- We suggest performing an IGRA rather than a TST in all other individuals 5 years or older who are likely to be infected with *Mtb*, who have a low or intermediate risk of disease progression, and in whom it has been decided that testing for LTBI is warranted (*conditional recommendation, moderate-quality evidence*). Remarks: A TST is an

Received 4 October 2016; editorial decision 6 October 2016; accepted 14 October 2016.
These guidelines were endorsed by the European Respiratory Society on 20 June 2016.
[a]Authors are co-chairs of this guideline committee.
Correspondence: D. M. Lewinsohn, Pulmonary and Critical Care Medicine, Oregon Health & Science University, Portland, OR (lewinsod@ohsu.edu).
Clinical Infectious Diseases® 2016;00(0):1–33
© The Author 2016. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail journals.permissions@oup.com.
DOI: 10.1093/cid/ciw694

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

Table 1. Interpretation of Strong and Weak (Conditional) Recommendations

	Strong Recommendation	Weak (Conditional) Recommendation
Patients	Most individuals in this situation would want the recommended course of action, and only a small proportion would not.	The majority of individuals in this situation would want the suggested course of action, but many would not.
Clinicians	Most individuals should receive the intervention. Adherence to this recommendation according to the guideline could be used as a quality criterion or performance indicator. Formal decision aids are not likely to be needed to help individuals make decisions consistent with their values and preferences.	Recognize that different choices will be appropriate for individual patients and that you must help each patient arrive at a management decision consistent with his or her values and preferences. Decision aids may be useful in helping individuals to make decisions consistent with their values and preferences.
Policy makers	The recommendation can be adopted as policy in most situations.	Policymaking will require substantial debate and involvement of various stakeholders.

acceptable alternative, especially in situations where an IGRA is not available, too costly, or too burdensome.

- There are insufficient data to recommend a preference for either a TST or an IGRA as the first-line diagnostic test in individuals 5 years or older who are likely to be infected with *Mtb*, who have a high risk of progression to disease, and in whom it has been determined that diagnostic testing for LTBI is warranted.
- Guidelines recommend that persons at low risk for *Mtb* infection and disease progression NOT be tested for *Mtb* infection. We concur with this recommendation. However, we also recognize that such testing may be obliged by law or credentialing bodies. If diagnostic testing for LTBI is performed in individuals who are unlikely to be infected with *Mtb* despite guidelines to the contrary:
    - We suggest performing an IGRA instead of a TST (*conditional recommendation, low-quality evidence*). Remarks: A TST is an acceptable alternative in settings where an IGRA is unavailable, too costly, or too burdensome.
    - We suggest a second diagnostic test if the initial test is positive (*conditional recommendation, very low-quality evidence*). Remarks: The confirmatory test may be either an IGRA or a TST. When such testing is performed, the person is considered infected only if both tests are positive.
- We suggest performing a TST rather than an IGRA in healthy children <5 years of age for whom it has been decided that diagnostic testing for LTBI is warranted (*conditional recommendation, very low-quality evidence*).
- The preceding recommendations are summarized in Figure 2. While both IGRA and TST testing provide evidence for infection with Mtb, they cannot distinguish active from latent tuberculosis. Therefore, the diagnosis of active TB must be excludedprior to embarking on treatment for LTBI. This is typically done by determining whether or not symptoms suggestive of TB disease are present, performing a chest radiograph and, if radiographic signs of active tuberculosis (eg, airspace opacities, pleural effusions, cavities, or changes on serial radiographs) are seen, then sampling is performed and the patient managed accordingly.

Testing for TB Disease

- We recommend that acid-fast bacilli (AFB) smear microscopy be performed, rather than no AFB smear microscopy, in all patients suspected of having pulmonary TB (*strong recommendation, moderate-quality evidence*). Remarks: False-negative results are sufficiently common that a negative AFB smear result does not exclude pulmonary TB. Similarly, false-positive results are sufficiently common that a positive AFB smear result does not confirm pulmonary TB. Testing of 3 specimens is considered the normative practice in the United States and is strongly recommended by the Centers for Disease Control and Prevention and the National Tuberculosis Controllers Association in order to improve sensitivity given the pervasive issue of poor sample quality. Providers should request a sputum volume of at least 3 mL, but the optimal volume is 5–10 mL. Concentrated respiratory specimens and fluorescence microscopy are preferred.
- We suggest that both liquid and solid mycobacterial cultures be performed, rather than either culture method alone, for every specimen obtained from an individual with suspected TB disease (*conditional recommendation, low-quality evidence*). Remarks: The conditional qualifier applies to performance of both liquid and solid culture methods on all specimens. At least liquid culture should be done on all specimens as culture is the gold standard microbiologic test for the diagnosis of TB disease. The isolate recovered should be identified according to the Clinical and Laboratory Standards Institute guidelines and the American Society for Microbiology Manual of Clinical Microbiology.
- We suggest performing a diagnostic nucleic acid amplification test (NAAT), rather than not performing a NAAT, on the initial respiratory specimen from patients suspected of having pulmonary TB (*conditional recommendation, low-quality evidence*). Remarks: In AFB smear-positive patients, a negative NAAT makes TB disease unlikely. In AFB smear-negative patients with an intermediate to high level of suspicion for disease, a positive NAAT can be used as presumptive evidence of TB disease, but a

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

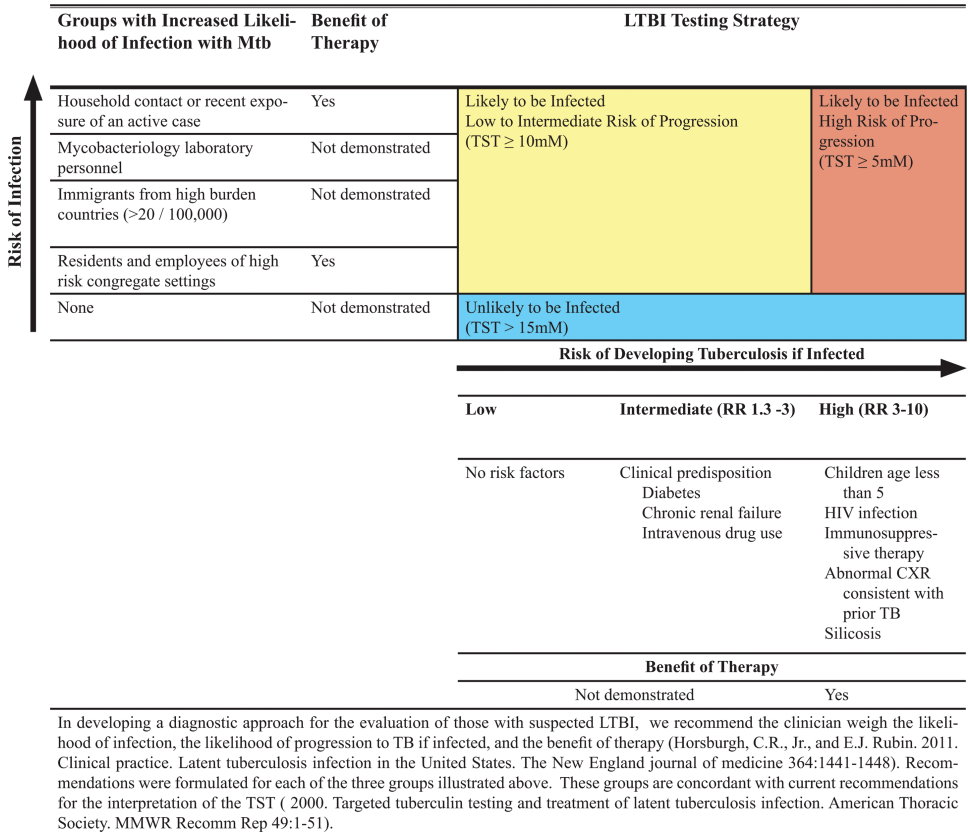

Figure 1. Paradigm for evaluation of those with latent tuberculosis infection (LTBI) based on risk of infection, risk of progression to tuberculosis, and benefit of therapy. In developing a diagnostic approach for the evaluation of those with suspected LTBI, we recommend the clinician weigh the likelihood of infection, the likelihood of progression to tuberculosis if infected, and the benefit of therapy (Horsburgh and Rubin, Clinical practice: latent tuberculosis infection in the United States. N Engl J Med 2011; 364:1441–8). Recommendations were formulated for each of the 3 groups illustrated above. These groups are concordant with current recommendations for the interpretation of the tuberculin skin test (American Thoracic Society, Targeted tuberculin testing and treatment of latent tuberculosis infection. MMWR Recomm Rep 2000; 49:1–51). Abbreviations: CXR, chest radiograph; HIV, human immunodeficiency virus; LTBI, latent tuberculosis infection; Mtb, Mycobacterium tuberculosis; RR, ; TB, tuberculosis; TST, tuberculin skin test.

negative NAAT cannot be used to exclude pulmonary TB. Appropriate NAAT include the Hologic Amplified *Mycobacteria Tuberculosis* Direct (MTD) test (San Diego, California) and the Cepheid Xpert MTB/Rif test (Sunnyvale, California).

- We recommend performing rapid molecular drug susceptibility testing for rifampin with or without isoniazid using the respiratory specimens of persons who are either AFB smear positive or Hologic Amplified MTD positive and who meet one of the following criteria: (1) have been treated for tuberculosis in the past, (2) were born in or have lived for at least 1 year in a foreign country with at least a moderate tuberculosis incidence (≥20 per 100 000) or a high primary multidrug-resistant tuberculosis prevalence (≥2%), (3) are contacts of patients with multidrug-resistant tuberculosis, or (4) are HIV infected (*strong recommendation, moderate-quality evidence*). Remarks: This recommendation specifically addresses patients who are Hologic Amplified MTD positive because the Hologic Amplified MTD NAAT only detects TB and not drug resistance; it is not applicable to patients who are positive for types of NAAT that detect drug resistance, including many line probe assays and Cepheid Xpert MTB/RIF.
- We recommend mycobacterial culture of respiratory specimens for all children suspected of having pulmonary TB (*strong recommendation, moderate-quality evidence*).
- We suggest sputum induction rather than flexible bronchoscopic sampling as the initial respiratory sampling method for individuals with suspected pulmonary TB who are either unable to expectorate sputum or whose expectorated sputum is AFB smear microscopy negative (*conditional recommendation, low-quality evidence*).
- We suggest flexible bronchoscopic sampling, rather than no bronchoscopic sampling, in individuals with suspected pulmonary TB from whom a respiratory sample cannot be obtained via induced sputum (*conditional recommendation, very low-quality evidence*). Remarks: In the committee members' clinical practices, bronchoalveolar lavage (BAL) plus brushings alone are performed for most

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

Group	Testing Strategy	Considerations
Likely to be Infected **High** Risk of Progression (TST ≥ 5mM)	**Adults** **Acceptable**: IGRA OR TST Consider dual testing where a positive result from either result would be considered **positive** **Children ≤ 5 years of age** **Preferred**: TST **Acceptable**: IGRA OR TST Consider dual testing where a positive result from either would be considered **positive**[1]	Prevalence of BCG vaccination Expertise of staff and/or laboratory Test availability Patient perceptions Staff perceptions Programmatic concerns
Likely to be Infected **Low** to **Intermediate** Risk of Progression (TST ≥ 10mM)	**Preferred**: IGRA where available **Acceptable**: IGRA or TST	
Unlikely to be Infected (TST > 15mM)	**Testing for LTBI is not recommended** **If necessary:** **Preferred:** IGRA where available. **Acceptable**: Either IGRA OR TST **For serial testing**: **Acceptable**: Either IGRA OR TST Consider repeat or dual testing where a negative result from either would be considered **negative**[2]	

1. Performing a second diagnostic test when the initial test is negative is a strategy to increase sensitivity. This may reduce specificity, but the panel decided that this is an acceptable tradeoff in situations in which the consequences of missing LTBI (i.e., not treating individuals who may benefit from therapy) exceed the consequences of inappropriate therapy (i.e., hepatotoxicity).
2. Performing a confirmatory test following an initial positive result is based upon both the evidence that false-positive results are common among individuals who are unlikely to be infected with Mtb and the committee's presumption that performing a second test on those whose initial test was positive will help identify initial false-positive results.

Figure 2. Summary of recommendations for testing for latent tuberculosis infection (LTBI). [1]Performing a second diagnostic test when the initial test is negative is a strategy to increase sensitivity. This may reduce specificity, but the panel decided that this is an acceptable trade-off in situations in which the consequences of missing LTBI (ie, not treating individuals who may benefit from therapy) exceed the consequences of inappropriate therapy (ie, hepatotoxicity). [2]Performing a confirmatory test following an initial positive result is based upon both the evidence that false-positive results are common among individuals who are unlikely to be infected with Mycobacterium tuberculosis and the committee's presumption that performing a second test on those patients whose initial test was positive will help identify initial false-positive results. Abbreviations: IGRA, interferon-γ release assay; LTBI, latent tuberculosis infection; TST, tuberculin skin test.

patients; however, for patients in whom a rapid diagnosis is essential, transbronchial biopsy is also performed.

- We suggest that postbronchoscopy sputum specimens be collected from all individuals with suspected pulmonary TB who undergo bronchoscopy (*conditional recommendation, low-quality evidence*). Remarks: Postbronchoscopy sputum specimens are used to perform AFB smear microscopy and mycobacterial cultures.
- We suggest flexible bronchoscopic sampling, rather than no bronchoscopic sampling, in individuals with suspected miliary TB and no alternative lesions that are accessible for sampling whose induced sputum is AFB smear microscopy negative or from whom a respiratory sample cannot be obtained via induced sputum (*conditional recommendation, very low-quality evidence*). Remarks: Bronchoscopic sampling in patients with suspected miliary TB should include bronchial brushings and/or transbronchial biopsy, as the yield from washings is substantially less and the yield from BAL unknown. For patients in whom it is important to provide a rapid presumptive diagnosis of tuberculosis (ie, those who are too sick to wait for culture results), transbronchial biopsies are both necessary and appropriate.
- We suggest that cell counts and chemistries be performed on amenable fluid specimens collected from sites of suspected extrapulmonary TB (*conditional recommendation, very low-quality evidence*). Remarks: Specimens that are amenable to cell counts and chemistries include pleural, cerebrospinal, ascitic, and joint fluids.
- We suggest that adenosine deaminase levels be measured, rather than not measured, on fluid collected from patients with suspected pleural TB, TB meningitis, peritoneal TB, or pericardial TB (*conditional recommendation, low-quality evidence*).
- We suggest that free IFN-γ levels be measured, rather than not measured, on fluid collected from patients with suspected pleural TB or peritoneal TB (*conditional recommendation, low-quality evidence*).
- We suggest that AFB smear microscopy be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB (*conditional recommendation, very low-quality evidence*). Remarks: A positive result can be used as evidence of extrapulmonary TB and guide decision making because false-positive results are unlikely. However, a negative result may not be used to exclude TB because false-negative results are exceedingly common.
- We recommend that mycobacterial cultures be performed, rather than not performed, on specimens collected from

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

sites of suspected extrapulmonary TB (*strong recommendation, low-quality evidence*). Remarks: A positive result can be used as evidence of extrapulmonary TB and guide decision making because false-positive results are unlikely. However, a negative result may not be used to exclude TB because false-negative results are exceedingly common.

- We suggest that NAAT be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB (*conditional recommendation, very low-quality evidence*). Remarks: A positive NAAT result can be used as evidence of extrapulmonary TB and guide decision making because false-positive results are unlikely. However, a negative NAAT result may not be used to exclude TB because false-negative results are exceedingly common. At present, NAAT testing on specimens other than sputum is an off-label use of the test.
- We suggest that histological examination be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB (*conditional recommendation, very low-quality evidence*). Remarks: Both positive and negative results should be interpreted in the context of the clinical scenario because neither false-positive nor false-negative results are rare.

We recommend a culture isolate from each mycobacterial culture-positive patient be submitted to a regional genotyping laboratory for genotyping (*strong recommendation, very low-quality evidence*).

- We recommend one culture isolate from each mycobacterial culture-positive patient be submitted to a regional genotyping laboratory for genotyping (*strong recommendation, very low-quality evidence*).

Persons infected with *Mycobacterium tuberculosis (Mtb)* have a broad array of presentations, ranging from those with clinical, radiographic, and microbiological evidence of tuberculosis (TB disease) to those who are infected with *Mtb* but have no clinical evidence of TB disease (latent tuberculosis infection [LTBI]). Individuals with LTBI who have been recently exposed have an increased risk of developing TB, whereas those with remote exposure have less risk over time unless they develop a condition that impairs immunity. Operationally, recent exposure can be defined either epidemiologically (ie, as might occur in the setting of the household of an infectious case or occupational exposure) or immunologically (ie, conversion of a tuberculin skin test or interferon-γ release assay [IGRA] from negative to positive).

These clinical practice guidelines on the diagnosis and classification of tuberculosis in adults and children were prepared by a task force supported by the American Thoracic Society (ATS), the Centers for Disease Control and Prevention (CDC), and the Infectious Diseases Society of America (IDSA). Additionally, Fellows of the American Academy of Pediatrics participated in the development of these guidelines. The specific objectives of these guidelines are as follows:

- To define high- and low-risk patient populations based upon the results of epidemiological studies.
- To provide diagnostic recommendations that lead to beneficial treatments and favorable clinical outcomes.
- To describe a classification scheme for tuberculosis that is based on pathogenesis.

These guidelines target clinicians in high-resource countries with a low incidence of TB disease and LTBI, such as the United States. The recommendations may be less applicable to medium- and high-tuberculosis incidence countries. For such countries, guidance documents published by the World Health Organization (WHO) may be more suitable.

HOW TO USE THESE GUIDELINES

These guidelines are not intended to impose a standard of care. They provide the basis for rational decisions in the diagnostic evaluation of patients with possible LTBI or TB. Clinicians, patients, third-party payers, stakeholders, or the courts should never view the recommendations contained in these guidelines as dictates. Guidelines cannot take into account all of the often compelling unique individual clinical circumstances. Therefore, no one charged with evaluating clinicians' actions should attempt to apply the recommendations from these guidelines by rote or in a blanket fashion. Qualifying remarks accompanying each recommendation are its integral parts and serve to facilitate more accurate interpretation. They should never be omitted when quoting or translating recommendations from these guidelines.

METHODS

Committee Selection

The criteria for committee selection were an (1) established track record in the relevant clinical or research area; (2) involvement with the ATS Assembly on Microbiology, Tuberculosis and Pulmonary Infections, the IDSA Tuberculosis Committee, or employment by the United States CDC Division of Tuberculosis Elimination; and (3) absence of disqualifying conflicts of interest. Conflicts of interest were managed according to the policies and procedures agreed upon by the participating organizations [1].

The committee was divided into subcommittees assigned to develop drafts for each of the following areas: (1) LTBI, (2) clinical and radiological aspects of TB diagnosis, (3) microbiological evaluation for TB diagnosis and detection of drug resistance, and (4) pediatric TB diagnosis. Meetings were held either in-person or via teleconference.

Evidence Synthesis

Each subcommittee identified key diagnostic questions and then performed a pragmatic evidence synthesis for each question, to identify and summarize the related evidence. The

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

subcommittees first sought studies comparing one diagnostic intervention with another and measuring clinical outcomes. Such evidence was unavailable, so the subcommittees next sought diagnostic accuracy studies. When published evidence was lacking, the collective clinical experience of the committee was used. The evidence syntheses were used to inform the recommendations. Though comprehensive, the evidence syntheses should not be considered systematic reviews of the evidence.

Developing and Grading Recommendations

Recommendations were formulated and the quality of evidence and strength of each recommendation were rated using the Grading, Recommendations, Assessment, Development and Evaluation (GRADE) approach [2, 3].

The quality of evidence is the extent to which one can be confident that the estimated effects are close to the actual effects and was rated as high, moderate, low, or very low. The quality of evidence rating derived from the quality of the accuracy studies that informed the panel's judgments, as randomized trials and controlled observational studies were lacking. Well-done accuracy studies that enrolled consecutive patients with legitimate diagnostic uncertainty and used appropriate reference standards represented high-quality evidence; lack of these characteristics constituted reasons to downgrade the quality evidence. Normally, the quality of evidence for first-line therapy would have been factored into such quality of evidence ratings but, in this case, the quality of evidence that treatment of TB disease and LTBI improve outcomes is high quality, so the overall quality of evidence rating was determined entirely by the accuracy study.

The decision to recommend for or against an intervention was based upon consideration of the balance of desirable consequences (ie, benefits) and undesirable consequences (ie, harms, burdens), quality of the evidence, patient values and preferences, cost, resource use, and feasibility. The subcommittees used open discussion to arrive at a consensus for each of the recommendations. An open voting procedure was reserved for situations when the subcommittee could not reach consensus through discussion, but this was not needed for any recommendation.

The strength of a recommendation indicates the committee's certainty that the desirable consequences of the recommended course of action outweigh the undesirable consequences. A strong recommendation is one for which the subcommittee is certain, whereas a conditional recommendation is one for which the subcommittee is uncertain. Uncertainty may exist if the quality of evidence is poor, there is a fine balance between desirable and undesirable consequences (ie, the benefits may not be worth the costs or burdens), the balance of desirable and undesirable consequences depends upon the clinical context, or there is variation about how individuals value the outcomes. A strong recommendation should be interpreted as the right thing to do for the vast majority of patients; a weak recommendation should be interpreted as being the right thing to do for the majority of patients, but maybe not for a sizeable minority of patients.

TUBERCULOSIS: EPIDEMIOLOGY, TRANSMISSION, AND PATHOGENESIS

A full discussion of these topics can be found in the Supplementary Materials. TB disease remains one of the major causes of morbidity and mortality in the world. The WHO estimates that 8.6 million new cases of tuberculosis occurred in 2014 and approximately 1.5 million persons died from the disease [4]. The emergence of drug-resistant tuberculosis has become apparent over the past 2 decades, and in particular, multidrug-resistant tuberculosis (MDR-TB; resistant to isoniazid and rifampin) and extensively drug-resistant tuberculosis (XDR-TB; resistant to isoniazid and rifampin, plus any fluoroquinolone and at least 1 of 3 injectable second-line drugs [ie, amikacin, kanamycin, or capreomycin]), which are more difficult to treat than drug-susceptible disease [5, 6]. The approximate number of cases of MDR-TB in the world is roughly 500 000 reported from at least 127 countries, and XDR-TB has been reported from 105 countries [4].

In the United States, 9412 cases of TB disease were reported in 2014, with a rate of 3.0 cases per 100 000 persons. Sixty-six percent of cases were in foreign-born persons; the rate of disease was 13.4 times higher in foreign-born persons than in US- born individuals (15.3 vs 1.1 per 100 000, respectively) [7]. An estimated 11 million persons are infected with *Mtb* [8]. Thus, although the case rate of TB in the United States has declined during the past several years, there remains a large reservoir of individuals who are infected with *Mtb*. Without the application of improved diagnosis and effective treatment for LTBI, new cases of TB will develop from within this group, which is therefore a major focus for the control and elimination of tuberculosis [9].

Mtb is transmitted from person to person via the airborne route [10]. Several factors determine the probability of *Mtb* transmission: (1) infectiousness of the source patient—a positive sputum smear for acid-fast bacilli (AFB) or a cavity on chest radiograph being strongly associated with infectiousness; (2) host susceptibility of the contact; (3) duration of exposure of the contact to the source patient; (4) the environment in which the exposure takes place (a small, poorly ventilated space providing the highest risk); and (5) infectiousness of the *Mtb* strain. In the United States, among contacts of patients with TB disease evaluated during a contact investigation, about 1% have TB disease themselves and 23% have a positive tuberculin skin test (TST) without evidence of tuberculosis disease and are considered to have LTBI [11]. Those who are household contacts and are exposed to patients who are smear positive have higher rates of both infection and disease [12]. Medical procedures that generate aerosols of respiratory secretions, such as sputum induction and bronchoscopy, entail significant risk for *Mtb* transmission unless proper precautions are taken [13].

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

Initial Infection: Acquisition of Latent *Mtb* Infection

After inhalation, the droplet nucleus is carried down the bronchial tree and implants in a respiratory bronchiole or alveolus. Whether or not inhaled tubercle bacilli establish an infection depends on both host and microbial factors [14]. It is hypothesized that, following infection, but before the development of cellular immunity, tubercle bacilli spread via the lymphatics to the hilar lymph nodes and then through the bloodstream to more distant anatomic sites [15]. The majority of pulmonary tuberculosis infections are clinically and radiographically unapparent [16]. A positive TST or IGRA result, most commonly, is the only indication that infection with *Mtb* has taken place.

Those who develop a positive TST are considered to have LTBI. It is estimated that, in the absence of treatment, approximately 4%–6% of individuals who acquire LTBI will develop active TB disease during their lifetime. The greatest risk of progression is during the first 2 years following exposure [11, 17]. The ability of the host to contain the organism is reduced in young (<4 years) children and by certain diseases such as silicosis, diabetes mellitus, and diseases associated with immunosuppression (eg, human immunodeficiency virus [HIV] infection), as well as by corticosteroids and other immunosuppressive drugs such as tumor necrosis factor alpha (TNF-α) inhibitors. In these circumstances, the likelihood of progression to TB disease is greater. For example, individuals who have a prior latent infection with *Mtb* (not treated) and then acquire HIV infection will develop TB disease at an approximate rate of 5%–10% per year (in the absence of effective HIV treatment) [18, 19].

DIAGNOSTIC TESTS FOR LTBI

The aim of testing for LTBI is to identify those who will benefit from prophylactic therapy. At present, the likelihood of completing LTBI treatment is relatively modest. In some reports, only 17%–37% of those eligible for LTBI therapy ultimately complete the treatment course, with higher rates of completion associated with shorter courses of therapy [20, 21]. Once therapy has been initiated, completion rates are more favorable [22]. It is hoped that better diagnostic tests, testing strategies, and treatment regimens will allow for resources to be focused on patients who are most deserving of evaluation and treatment of LTBI and, therefore, result in increased completion of therapy rates.

Tuberculin Skin Testing

The tuberculin skin test (TST) detects cell-mediated immunity to *Mtb* through a delayed-type hypersensitivity reaction using a protein precipitate of heat-inactivated tubercle bacilli (purified protein derivative [PPD]–tuberculin). The TST has been the standard method of diagnosing LTBI.

The TST is administered by the intradermal injection of 0.1 mL of PPD (5 TU) into the volar surface of the forearm (Mantoux method) to produce a transient wheal. The test is interpreted at 48–72 hours by measuring the transverse diameter of the palpable induration. TST interpretation is risk-stratified [23]. A reaction of 5 mm or greater is considered positive for close contacts of tuberculosis cases; immunosuppressed persons, in particular persons with HIV infection; individuals with clinical or radiographic evidence of current or prior TB; and persons receiving TNF blocking agents. A reaction of ≥10 mm is considered positive for other persons at increased risk of LTBI (eg, persons born in high TB incidence countries and those with at risk of occupational exposure to TB) and for persons with medical risk factors that increase the probability of progression from LTBI to TB (Figure 1). A reaction of 15 mm or greater is considered positive for all other persons. Serious adverse reactions to PPD-tuberculin are rare. However, strong reactions with vesiculation and ulceration may occur.

The sensitivity of the TST, as measured in clinically well persons with previously treated tuberculosis, is high (95%–98%). False-negative reactions occur more frequently in infants and young children, early (<6–8 weeks) after infection, in persons having recently received viral vaccination, in persons with clinical conditions associated with immunosuppression (eg, HIV infection) or overwhelming illness (including extensive or disseminated tuberculosis), after recent viral and bacterial infections, and in association with treatment with immunosuppressive drugs (eg, high-dose corticosteroids, TNF inhibitors).

Test specificity of the TST is decreased among persons with prior BCG vaccination, especially those vaccinated postinfancy and those with repeat vaccination. Similarly, persons living in areas where nontuberculous mycobacteria are common are at increased risk of having false-positive TST reactions. Repeated administration of TSTs cannot induce reactivity; however, a repeat TST can restore reactivity in persons whose TST reactivity has waned over time. Because of this "boosting phenomenon," initial repeat testing is recommended for persons with a negative TST who are to undergo periodic TST screening and who have not been tested with tuberculin recently (eg, 1 year)." This "2-step" testing, with a repeat TST within 1–3 weeks after an initial negative TST, is intended to avoid misclassification of subsequent positive TSTs as a TST conversion, indicating recent infection, when they are actually a result of boosting.

Benefits and Limitations of the TST

The benefits of the TST include its simplicity to perform (it does not require a laboratory or equipment and can be done by a trained healthcare worker in remote locations), its low cost, no need for phlebotomy, the observation that it reflects a polycellular immune response, and the foundation of well-controlled studies that support the use of the TST to detect LTBI and guide the use of prophylactic therapy [24]. In addition, there are well-established definitions of TST conversion, which

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

are particularly helpful when using the TST in the setting of serial testing.

Limitations include the need for trained personnel to both administer the intradermal injection and interpret the test, inter- and intrareader variability in interpretation, the need for a return visit to have the test read, false-positive results due to the cross-reactivity of the antigens within the PPD to both BCG and nontuberculous mycobacteria, false-negative results due to infections and other factors, rare adverse effects, and complicated interpretation due to boosting, conversions, and reversions [24].

Interferon-Gamma Release Assays

Overview of IGRAs

Until recently, the TST has been the only method to test for latent infection with *Mtb*. Ideally, an improved diagnostic test would specifically identify those with *Mtb* infection and would delineate those at risk for disease progression. In this regard, the TST has well-known strengths and limitations [23, 25, 26]. The IGRAs are newer tests to diagnose infection with *Mtb*. IGRAs are in vitro, T cell–based assays that measure interferon gamma (IFN-γ) release by sensitized T cells in response to highly specific *Mtb* antigens.

Immune Basis of IGRAs

Like the TST, the IGRA is a reflection of the cellular immune response. The discovery of antigens that have elicited robust immune responses and are relatively specific for infection with *Mtb* has enabled the development of IGRA assays, which are more specific for *Mtb* infection than the TST [27], particularly in the setting of BCG vaccination. Of particular interest has been the RD-1 gene segment, a 9.5-kb DNA segment absent from all strains of *Mycobacterium bovis* BCG but present in wild-type *M. bovis* and *Mtb* [28]. This region, containing 11 open reading frames, is responsible for the transcription and translation of a variety of antigenic proteins, including early secretory antigen (ESAT-6) [29–33] and culture filtrate protein (CFP-10) [34–37]. Both antigens are absent from all attenuated strains of *M. bovis* (BCG strains) and most nontuberculous mycobacteria with the important exceptions of *Mycobacterium kansasii*, *Mycobacterium szulgai*, *Mycobacterium marinum* [32, 38], and *Mycobacterium leprae* [39, 40].

IGRA assays are primarily a reflection of a $CD4^+$ T-cell immune response to these antigens. Immunologic memory is characterized by the clonal expansion of antigen-specific T cells following exposure to an antigen. Effector memory T cells are defined by their capacity to respond rapidly to subsequent antigenic exposure. This response is characterized by the release of cytokines, as well as further expansion of these cells. Responses measured in current short-term IGRA assays reflect the presence of these cells. Although it has been postulated measurement of these short-term effectors might reflect recent infection and/or ongoing bacterial replication, current evidence does not support this hypothesis [41–43].

Commercially Available IGRAs

Currently, there are 2 commercially available IGRA platforms that measure interferon-γ release in response to *Mtb*-specific antigens: the QuantiFERON TB Gold In Tube (QFT-GIT; Cellestis Limited, Carnegie, Victoria, Australia) and T-SPOT. TB test (T-SPOT, manufactured by Oxford Immunotec Ltd, Abingdon, United Kingdom). The QFT-GIT measures IFN-γ plasma concentration using an enzyme-linked immunosorbent assay (ELISA), while the T-SPOT assay enumerates T cells releasing IFN-γ using an enzyme-linked immunospot (ELISPOT) assay.

QuantiFERON Assays

The QFT-GIT method has been approved by the US Food and Drug Administration (FDA) and has replaced the QuantiFERON-TB Gold (QFT-G) test. Whole blood (minimum 3 mL) is drawn directly into heparinized tubes coated with lyophilized antigen and agitated. In this case, peptides from ESAT-6, CFP-10, and TB7.7 are found within the same tube. Two additional tubes are drawn as controls (mitogen control and nil control). The mitogen control (phytohemagglutinin [PHA]) stimulates T-cell proliferation and ensures that viable cells are present. After incubation for 16–24 hours at 37°C, plasma is collected from each tube and the concentration of IFN-γ is determined for each by ELISA. The in-tube methodology requires no additional sample handling. Perhaps because of the nearly immediate exposure of T cells to antigen, as well as the addition of the TB7.7 peptide, the QFT-GIT may be more sensitive than the QFT-G test. Studies reporting the sensitivity and specificity of the QFT-GIT test are provided in Supplementary Tables 1 and 2, respectively. The next generation of QFT (QFTPlus) has been introduced in Europe and is pending approval in the United States. QFTPlus contains a tube of short peptides derived from CFP-10, which are designed to elicit an enhanced CD8 T-cell response. There is no TB7.7 peptide. No published information is available to evaluate the performance of this test.

The QFT-GIT assay is considered positive if the difference between the IFN-γ concentration in response to the *Mtb* antigens and the IFN-γ response to the nil control is ≥0.35 IU. In addition, to control for high background in the nil control, the IFN-γ response to antigen must be 25% greater than the IFN-γ concentration in the NIL control. An indeterminate response defined as either a lack of response in the PHA control well (IFN-γ concentration ≤0.5 IU) or a nil control that has a very high background (IFN-γ concentration >8 IU).

T-SPOT.TB Assays

The T-SPOT.TB assay is currently available in Europe, Canada, and has been approved for use in the United States with revised

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

criteria for test interpretation. For the T-SPOT.TB assay, blood (minimum 2 mL) is drawn into either a heparin or CPT Ficoll tube, and must be processed within 8 hours. More recently, this time has been extended to 32 hours if the "T-cell Xtend" additive is used and the blood kept between 10°C and 25°C. Peripheral blood mononuclear cells (PBMCs) are separated using density gradient centrifugation, enumerated, and then added to microtiter wells at 2.5×10^5 viable PBMCs per well that have been coated with monoclonal antibodies to IFN-γ (ELISPOT assay). Peptides derived from ESAT-6 and CFP-10 antigens are then added and the plate is developed following overnight (16–20 hours) incubation at 37°C. Cells are then washed away and "captured" IFN-γ is then detected via a sandwich capture technique by conjugation with secondary antibodies hence revealing a "spot." These spots are then enumerated as "footprints" [44] of effector T cells [44, 45].

For the T-SPOT.TB assay, a positive response is based on spot-forming units (SFU). Outside of the United States, if the negative control well contains ≤5 SFU and there are >6 SFU above the media nil control in either of the antigen wells, then this is considered positive. If the negative control well has ≥6 SFU, then the antigen wells must be at least 2 times the negative control well for a response to be considered positive. An invalid response is defined as high background in the negative control well (≥10 SFU) or if the positive control well is not responsive to mitogen (PHA, <20 SFU). The FDA has published revised criteria for T-SPOT.TB interpretation in the United States, in which a test is considered negative if there are ≤4 spots. Eight spots or greater is considered positive. Five, 6, and 7 spots are considered "borderline" and would be interpreted in conjunction with the subject's pretest probability of infection with *Mtb*. Studies reporting the sensitivity and specificity of the T-SPOT test are provided in Supplementary Tables 3 and 4, respectively.

Indeterminate/Invalid IGRA Responses

Unlike the TST, in which the results are interpreted categorically based on the size of the reaction [46], the IGRAs currently have a trichotomous outcome yielding a positive, negative, or indeterminate result (T-SPOT may also yield a borderline result as described above). As described above, an indeterminate/invalid IGRA can result from either a high background (nil) response or from a poor response to positive control mitogen. Indeterminate IGRA results are associated with immunosuppression [47–49], although they may occur in healthy individuals (studies reporting the test characteristics of IGRAs in individuals with immunosuppression are provided in Supplementary Tables 5 and 6). With regard to those with a poor response to the positive control mitogen, there are at least 2 possibilities. First, the test may not have been correctly performed. For example, errors in specimen collection, long delays in specimen processing, incubator malfunction, or technical errors might result in a poor mitogen response. Here, it is reasonable to simply repeat the assay. Second, a persistently diminished response to mitogen may be a reflection of anergy. Thus, the reproducibility and details regarding the reason for an indeterminate result may provide clinically useful information.

Reproducibility of IGRAs

Because IGRAs are predicated on in vitro release of cytokines from stimulated cells, there is likely to be more variability in these tests than those based on the measurement of a circulating substance such as sodium. There are at least 4 sources of variability which are inherent in the IGRA: (1) the type of measurement itself (ie, ELISA or ELISPOT), (2) reproducibility of a complex biological reaction, (3) the natural variability of immune responses, and (4) variability introduced during the course of test performance or manufacturing variances.

Reproducibility has been evaluated for both the QFT and T-SPOT assays. Although published information regarding currently available tests is limited [50, 51] the QFT-IT result was reported to have an 11% variance (http://www.accessdata.fda.gov/cdrh_docs/pdf/P010033). Studies on within subject variability of the QFT-IT are limited and most were performed in areas of the world where *Mtb* is endemic and variability over time due to reinfection would be expected [50, 51]. Recently, intrasubject variability of QFT-IT was assessed using available plasma, and a discordance rate of 8% between the first and second tests was observed. While the variations were quantitatively modest, results at or near the cutoff resulted in differing test results [52]. This variability might spuriously change the test result (positive to negative or negative to positive). Consequently, values at or near the test cutoff should be interpreted with caution. Variability of the T-SPOT was dependent on the strength of the response, and varied from 4% in those with robust responses, to 22% in those whose responses were close to the cutoff (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cftopic/pma/pma.cfm?num=p070006).

Boosting of IGRAs

Initial studies found that repeat TST testing did not alter the IGRA response [53, 54]. However, more recent evidence [50, 51] suggests that the prior placement of a TST can boost an IGRA, particularly in those individuals who were already IGRA positive to begin with (ie, previously sensitized to *Mtb* or possibly other mycobacteria). Additionally, it was found that this could be observed in as little as 3 days post-TST administration, and that the boosting effect may wane after several months [50, 51]. While these data do not detract from the excellent overall agreement that has been reported, they suggest when dual testing is to be considered that the IGRA be collected either concurrently or prior to TST placement.

Special Considerations

Because IGRAs rely on a functional assessment of viable lymphocytes, these tests require special attention to the technical

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

aspects of the test. This includes proper filling of the blood collection tube, proper mixing, timely transport to the laboratory, and timely processing of the specimen. Additionally, for the laboratory, performance of cellular assays may pose unique challenges with regard to reagent storage and preparation as well as the separation of viable cells. Finally, manufacturing problems such as endotoxin contamination can confound assays that depend on cellular activation.

Benefits and Limitations of IGRAs

The benefits of IGRAs include the use of antigens that are largely specific for *Mtb* (ie, no cross-reactivity with BCG and minimal cross-reactivity with nontuberculous mycobacteria), the test can be performed in a single visit, and both the performance and reporting of results in a laboratory setting fall under the auspices of regulatory certification [24].

Limitations include cost, the need for phlebotomy (which may be particularly challenging in children), complicated interpretation due to frequent conversions and reversions and lack of consensus on thresholds, and inconsistent test reproducibility [24]. The reproducibility of results is particularly problematic in the setting of serial testing. While some of this can be attributed to results that fall near the cutoff, this is not always the case, and current data does not provide specific guidance. Data on the effect of IGRA-guided therapy on prevention of TB disease is limited, although one study demonstrated a roughly 84% reduction in TB disease among household contacts who received IGRA-based preventive therapy [55]. Finally, several studies have reported an increased rate of indeterminate IGRA results in children <5 years of age [47, 56–60] and one study described an increased rate of indeterminate IGRA results among individuals with HIV infection and a CD4 count ≤200 cells/μL [61]

DIAGNOSTIC APPROACH: TESTING FOR SUSPECTED LTBI

This section addresses how to test for LTBI. A complementary ATS/CDC/IDSA guideline that addresses who to screen for LTBI and how to treat LTBI is in development and forthcoming.

No definitive diagnostic test for LTBI exists. Our recommendations for diagnostic testing for LTBI are based upon the likelihood of infection with *Mtb* and the likelihood of progression to TB disease if infected, as illustrated in Figure 1. The recommendations are summarized in Figure 2. As our literature searches failed to identify randomized trials or observational studies that directly compared different diagnostic approaches and measured clinical outcomes, our recommendations are based upon evidence about the accuracy of various tests combined with evidence that treatment of LTBI improves clinical outcomes.

There are 2 major benefits of treating LTBI: Treating LTBI prevents progression to active TB disease with its attendant morbidities [62] and has public health benefits, as each new case is likely to infect others. The consequences of failing to prevent progression to active TB disease may be especially severe in the young or immunocompromised host, in whom the disease is more likely to be disseminated and elude discovery, and has a higher mortality rate. Failure to rapidly diagnose TB disease also poses a risk of widespread transmission in hospitals, homeless shelters, and prisons.

Patients with LTBI have a 4%–6% lifetime risk of developing TB disease, with approximately half of these cases occurring following recent exposure [11, 17]. Multiple placebo-controlled trials in adults and children with LTBI have shown that isoniazid reduces the subsequent development of TB disease in patients at high risk of progression. As an example, in a trial of 28 000 individuals with LTBI and radiographic evidence of healed tuberculosis, isoniazid taken for 52 weeks reduced the subsequent development of TB disease from 14.3% to 3.6% [62]. Other groups in which the treatment of LTBI has been demonstrated to reduce the incidence of TB disease include household contacts of active TB patients [55, 63], native Alaskan communities [64], residents of mental health facilities [65], persons with HIV infection [66–69], and individuals treated with TNF inhibitors [70, 71]. These data can be extrapolated to populations at low risk for progression (ie, no risk factors) and intermediate risk for progression (ie, diabetes, chronic renal disease, intravenous drug abuse); while the relative benefit of treatment is probably similar in these lower risk populations, the absolute benefit is almost certainly smaller due to the lower baseline risk of progression to TB disease.

These studies provide high-quality evidence that treatment of LTBI reduces the incidence of TB disease in populations at high risk for progression. However, they provide only moderate-quality evidence that treatment of LTBI reduces the incidence of TB disease in populations at low or intermediate risk for progression because the data are from high-risk populations.

Our recommendations for the diagnosis of LTBI reflect both the likelihood of infection (either likely or unlikely, based upon studies that used the TST to detect LTBI) and the risk of progression if infected (low; intermediate, RR 1.3–3; and high, RR 3–10). This paradigm is summarized in Figure 1.

Question 1: Should an IGRA or a TST be performed in individuals 5 years or older who are likely to be infected with *Mtb*, who have a low or intermediate risk of disease progression, and in whom it has been decided that testing for LTBI is warranted?

Evidence

In individuals who are likely to be infected with *Mtb* but at low or intermediate risk of disease progression, the sensitivity of IGRAs in the detection of *Mtb* infection has been consistently reported as either equal (QFT; 81%–86%) or superior (T-SPOT; 90%–95%) to the sensitivity of the TST (71%–82%) [47, 72–94]

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

when a final diagnosis of either microbiologically confirmed or clinical TB is used as the reference standard. Individuals who are likely to be infected with *Mtb* include household contacts (studies reporting the test characteristics of IGRAs in contacts are provided in Supplementary Table 7), recent exposures of an active case, mycobacteriology laboratory personnel, immigrants from high-burden countries, and residents or employees of high-risk congregate settings. Individuals at low risk of progression to TB include those with no risk factors, while those at intermediate risk of progression to TB include those with diabetes, chronic renal failure, or intravenous drug abuse.

In patients who are known to have received vaccination with BCG, the specificity of IGRAs has also been consistently superior to TST testing, presumably because IGRAs rely on responses to antigens absent in BCG and many nontuberculous mycobacteria. In contrast, among patients who have not received vaccination with BCG, the specificity of IGRAs and TST appears similar. Meta-analyses estimate that the specificity of QFT–IT to be >95%, whereas the specificity for TST is roughly 97% in those with no prior exposure to BCG. The specificity is reduced to roughly 60% in those with a history of BCG vaccination [24]. Data for the commercially available T-SPOT are more limited. In German healthcare workers, specificity (using a cutoff of 6 spots) was reported at 97%, whereas in Korean adolescents the specificity was 85% [49]. In Navy recruits, specificity was 99% using the 8-spot cutoff [95].

Our confidence in the estimated test characteristics was moderate because many of the studies did not report whether the subjects were consecutively enrolled.

Recommendation 1a: We recommend performing an IGRA rather than a TST in individuals 5 years or older who meet the following criteria: (1) are likely to be infected with *Mtb*, (2) have a low or intermediate risk of disease progression, (3) it has been decided that testing for LTBI is warranted, and (4) either have a history of BCG vaccination or are unlikely to return to have their TST read (*strong recommendation, moderate-quality evidence*). Remarks: A TST is an acceptable alternative, especially in situations where an IGRA is not available, too costly, or too burdensome.

Recommendation 1b: We suggest performing an IGRA rather than a TST in all other individuals 5 years or older who are likely to be infected with *Mtb*, who have a low or intermediate risk of disease progression, and in whom it has been decided that testing for LTBI is warranted (*conditional recommendation, moderate-quality evidence*). Remarks: A TST is an acceptable alternative, especially in situations where an IGRA is not available, too costly, or too burdensome.

Rationale

Accuracy studies indicate that IGRAs are more specific and equally or more sensitive than TST in individuals who have received the BCG vaccination; therefore, false-positive results are less likely with IGRAs than TST. This is important because false-positive results may lead to unnecessary treatment and its accompanying risks (ie, hepatotoxicity) [96–98]. To minimize these risks, the guideline development panel chose to recommend IGRA testing for individuals who received the BCG vaccination.

In contrast, the accuracy of TST and IGRAs appears similar in those without a history of BCG vaccination. Despite the similar test characteristics, the guideline development committee chose to suggest IGRA testing over TST testing in such patients because it was concerned about the reliability of a history of having received or not received the BCG vaccination. Because many of the individuals who fall into the likely to be infected with *Mtb* category are from regions of the world in which the BCG vaccination is routinely administered, the committee concluded that individuals who are likely to be infected with *Mtb* and provide history of not having received the BCG vaccination should be treated the same as those who provide a history of having received the BCG vaccination, unless there is a reason to choose an alternate approach such as IGRA testing not being available, being too costly, or being too burdensome.

The recommendation to perform IGRA testing rather than TST testing is strong for those who have received the BCG vaccination or who are not likely to return for TST read, reflecting the guideline development committee's certainty that avoiding the serious consequences of false-positive results and obtaining a result to guide therapy outweigh the additional cost and need to perform phlebotomy for IGRA testing. In contrast, the suggestion to perform IGRA testing rather than TST testing on all other patients who are likely to be infected with *Mtb* and have a low or moderate risk of progressing to TB disease is conditional, reflecting the committee's recognition that the choice should depend upon the clinical context as the test characteristics are similar. While the committee concluded that IGRA testing is preferable in most patients, it recognized that TST testing may be more appropriate in a sizeable minority due to availability, feasibility, cost, or burden.

Justification for Extending the Recommendation Down to 5 Years of Age

Young children are at increased risk of developing TB following infection and more likely to develop severe disease than older children and adults [99, 100]. This risk is highest in the youngest infants, diminishes with increasing age, and becomes equivalent with older children and adults at approximately 5 years of age. Thus, children ≥5 years old have a similar risk of TB as adults and display a similar disease spectrum. With respect to *Mtb* infection, children aged ≥5 years possess a functional immune response equivalent to that of adults. In addition, the results of existing studies of IGRA performance in children ≥5 years of age, albeit limited, are consistent with results of studies of IGRA performance in adults. The sensitivity of IGRAs in

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

children with TB [56, 59, 101–105] and in older children who are household [106, 107] or school [108] contacts and the specificity of IGRAs in children [102, 108] are comparable to those of adults. For these reasons, it seems reasonable to extrapolate the results of studies of IGRA performance in healthy adults to children aged ≥5 years.

Cautions and Limitations

While both IGRA and TST testing provide evidence for infection with *Mtb*, they cannot distinguish active from latent tuberculosis. Therefore, the diagnosis of active TB must be excluded prior to embarking on treatment for LTBI. This is typically done by determining whether or not symptoms suggestive of TB disease are present, performing a chest radiograph and, if radiographic signs of active tuberculosis (eg, airspace opacities, pleural effusions, cavities, or changes on serial radiographs) are seen, then sampling is performed and the patient managed accordingly.

Quantitative aspects of the tests are poorly understood. With respect to the TST, the result is categorized as positive or negative and quantitative data are of limited utility, with the exception of recognition that a large (>15 mm) skin test reaction is more likely to reflect infection with *Mtb* [109, 110]. The dichotomous characterization of the result, coupled with the fact that repeat testing is not recommended in the setting of a prior positive test result, has resulted in a paucity of information about the variability of the TST result over time. With respect to IGRAs, measurement of IFN-γ over time may reflect inherent variability in the test result (the FDA accepts a variance of 11%) or true immunological variation due to alterations in the abundance of *Mtb* antigens, exposure to other antigens, and/or the health and nutritional status of the host. As an example, it is possible that a rise in IFN-γ might reflect ongoing exposure and/or growth of the bacteria. Alternatively, a rise in IFN- γ may reflect variability of the test. At present, there are insufficient data upon which to base any recommendations for quantitative interpretation of IGRAs beyond those cut-points recommended by the FDA. However, it is important to recognize that the optimal cut-points are controversial and results near the cut-point are less reliable than results far above or below the cut-point. The results of IFN-γ testing should be reported quantitatively such that these immune correlates of the natural history of TB can be prospectively discerned and ultimately applied to clinical practice.

Discordance between TST and IGRA testing is common. Not surprisingly, TST-positive/IGRA-negative discordance is often seen in persons with prior exposure to BCG. However, TST-positive/IGRA-negative discordant results where the TST is well over 15 mm have also been reported. The reasons for this discordance are not understood. It could relate to the possibility that discordance may reflect immune responses that have occurred in the remote past (and where the antigen is currently not available to drive an ongoing response that can be measured by IGRA), may reflect immune differences inherent in a delayed-type hypersensitivity versus blood assay, or may reflect exposures to nontuberculous mycobacteria. In low-risk populations, discordant tests are likely to be false positives [61, 111]. Clearly, more information is desirable regarding which test best reflects productive infection and, therefore, best reflects the likelihood of disease progression.

The benefit of targeted testing for LTBI resides not in the test employed, but in its programmatic use. We acknowledge that programmatic considerations such as cost, test availability, prevalence of BCG exposure in the target population, ability to reevaluate the patient 2–3 days after testing, and the training and expertise of program staff might all affect the decision to use IGRA- or TST-based evaluations.

Question 2: Should an IGRA or a TST be performed in individuals 5 years or older who are likely to be infected with *Mtb*, who have a high risk of progression to disease, and in whom it has been decided that testing for LTBI is warranted?

Evidence

Individuals at high risk of progression to TB include those with HIV infection, an abnormal chest radiograph consistent with prior TB, or silicosis. It also includes those who are receiving immunosuppressive therapy. Most data about the accuracy of the TST and IGRA are from patients who are immunocompromised.

Studies have compared TST and IGRAs in the setting of immunocompromise. Both diagnostic tests have diminished sensitivity in this setting. The sensitivity of IGRAs (QFT-IT and T-SPOT) for detecting LTBI in individuals with HIV infection has been estimated to be from 65% to 100% [112–114], while the sensitivity of TST is only 43% (25,85) when a final diagnosis of either microbiologically confirmed or clinical TB is used as the reference standard. These limited data suggest that IGRAs are at least as sensitive as TST in the setting of HIV infection. Studies have also compared IGRAs with TST in populations that were heterogeneous with respect to both the type of underlying immunocompromise and the reasons for testing. These studies demonstrated significant discord between TST and IGRA results, but the source of the discordance has not been elucidated [61]. The panel's confidence in the estimated test characteristics of IGRA and TST testing was moderate because it was not reported whether patients were consecutively enrolled or whether there was true diagnostic uncertainty.

Recommendation 2: There are insufficient data to recommend a preference for either a TST or an IGRA as the first-line diagnostic test in individuals 5 years or older who are likely to be infected with *Mtb*, who have a high risk of progression to disease, and in whom it has been determined that diagnostic testing for LTBI is warranted.

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

Rationale

The committee judged the body of evidence insufficient to render a recommendation for either IGRA or TST testing in patients likely to be infected with *Mtb* who are at high risk for progression to disease because the estimated test characteristics were widely variable and derived from only a small subgroup of such patients (ie, immunocompromised patients).

As part of the discussion about which diagnostic test to perform in patients likely to be infected who are at high risk for progression to disease, many committee members acknowledged that they perform a second test in their clinical practices when such patients test negative; specifically, they perform a TST if an initial IGRA is negative or an IGRA if an initial TST is negative. If the second test is positive, they consider this evidence for infection with *Mtb*. Their practice is not based upon empirical evidence, but rather, the following clinical rationale. A sensitive diagnostic test is important for individuals who are likely to be infected with *Mtb* and at high risk of progression, so that such individuals are less likely to receive false-negative results that will result in delayed diagnosis and treatment. Performing a second diagnostic test when the initial test is negative is one strategy to increase sensitivity. While this strategy to increase sensitivity may reduce the specificity of diagnostic testing, this may be an acceptable tradeoff in situations in which it is determined that the consequences of missing LTBI (ie, not treating individuals who may benefit from therapy) exceed the consequences of inappropriate therapy (ie, hepatotoxicity).

Cautions and Limitations

While both QFT and T-SPOT rely on the release of IFN-γ in response to RD-1 antigens, limited data have suggested that indeterminate results are more common for QFT-IT when the CD4 count is <200 cells/μL than T-SPOT [115]. This may be the result of T-SPOT using a defined number of PBMCs, which may better, but not completely, normalize for the lack of $CD4^+$ T cells. By incorporating a measure of anergy into the test (Mitogen control), IGRAs may more accurately allow the clinician to discriminate a test that is negative from one that is indeterminate (anergic by virtue of inadequate responses to mitogen).

Question 3: Should an IGRA or a TST be performed in individuals 5 years or older who are unlikely to be infected with *Mtb*, but in whom it has been decided that testing for LTBI is warranted?

Evidence

There is a lack of direct evidence regarding the relative test characteristics of IGRA and TST testing in individuals who are unlikely to be infected with *Mtb*. Indirect evidence from individuals likely to be infected with *Mtb* indicates that IGRA testing is more specific than TST testing and equally or more sensitive than TST testing. We have no reason to suspect that these relative test characteristics will be different among individuals who are unlikely to be infected with *Mtb*. However, it is likely that false-positive results are more common for both IGRAs and TST in populations with a lower prevalence of LTBI. This is supported by a study of longitudinal testing of healthcare workers residing in areas of low TB prevalence, which found that most conversions were false-positive results as evidenced by a negative result on repeat testing [116].

The evidence provides low confidence in the estimated test characteristics in our population of interest because many of the estimates are based upon evidence from patients who are likely to be infected with a high risk for progression rather than patients who are unlikely to be infected, and many of the studies did not report whether subjects were consecutively enrolled.

Guidelines recommend that persons at low risk for *Mtb* infection and disease progression NOT be tested for *Mtb* infection. We concur with this recommendation. However, we also recognize that such testing may be obliged by law or credentialing bodies. If diagnostic testing for LTBI is performed in individuals who are unlikely to be infected with *Mtb* despite guidelines to the contrary.

Recommendation 3a: We suggest performing an IGRA instead of a TST (*conditional recommendation, low-quality evidence*). Remarks: A TST is an acceptable alternative in settings where an IGRA is unavailable, too costly, or too burdensome.

Recommendation 3b: We suggest a second diagnostic test if the initial test is positive (*conditional recommendation, very low-quality evidence*). Remarks: The confirmatory test may be either an IGRA or a TST. When such testing is performed, the person is considered infected only if both tests are positive.

Rationale

Current ATS/CDC and American Academy of Pediatrics guidelines recommend that testing for LTBI not be performed in individuals at low risk for infection with *Mtb* because the risk of isoniazid chemoprophylaxis may outweigh the potential benefit [117]. Despite this, testing is often performed in conjunction with school enrollment, employee health testing, and other institutional settings. In such patients, many conversions are false results, which may lead to unnecessary therapy and, therefore, unnecessary and age-related risk of hepatotoxicity.

The evidence indicates that false-positive results are frequent (ie, more common than true-positive results) among individuals who are unlikely to be infected with *Mtb.* Use of a more specific test may result in fewer false-positive results and, therefore, fewer persons receiving unnecessary LTBI treatment and being placed at risk for adverse outcomes. In addition to the risk associated with isoniazid chemoprophylaxis, those with a positive

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

test for LTBI often undergo additional screening, including a chest radiograph. Avoiding such unnecessary screening has both cost and health benefits. The desire for a more specific test favors IGRA testing over TST, according to evidence described above from patients who are likely to be infected and who have a low or intermediate risk for progression. The notion of performing a second, confirmatory test following an initial positive result is based upon the evidence that false-positive results are common among individuals who are unlikely to be infected with *Mtb* and the committee's presumption that performing a second test on those whose initial test was positive will improve specificity.

The recommendations are both conditional because the quality of evidence provided the committee with limited confidence in the estimated test characteristics of IGRAs and TST in individuals who are unlikely to be infected; therefore, the committee could not be certain that the desirable consequences of performing IGRAs instead of TST, or of performing a second test following a positive result, outweigh the undesirable consequences in the vast majority of patients.

Cautions and Limitations (Testing for TB in Healthcare Workers)

Traditionally, once an individual has had a positive TST, future use of the TST for screening is not recommended due to the belief that the skin test will remain positive for life. In those who are TST negative, serial testing can be complicated by random variability, boosting (ie, increased reactions upon retesting due to immunological memory), conversions (ie, new reactions due to new infection), and reversions (ie, decreased reactions). Criteria for the placement and reading of the TST, as well as the effect of boosting with PPD, criteria for TST conversion have been established. Receiver operating characteristic (ROC) analysis has been used to establish criteria for positive and negative IGRA results in those thought to be unlikely to be infected or those with TB disease. However, IGRAs have not proven to be the solution to the problem of false-positive results associated with serial testing in low risk individuals. At present, there is insufficient information available to guide the establishment of definitive criteria for the conversion and possible reversion of IGRAs. The issue of interpreting IGRA conversions and reversions in the context of serial testing has proven especially problematic. For example, in a study of 216 Indian healthcare workers, a QFT conversion rate of 12% and a reversion rate of 24% were observed, with many of these apparent changes occurring near the cutoff values [118]. A longitudinal study involving 2563 in healthcare workers demonstrated an IGRA conversion (6%–8%) in those undergoing serial testing [116]. These rates were 6–9 times higher than that seen for the TST and were thought to have represent false conversions. Such studies have not yielded useful criteria that can be used to distinguish *Mtb* infection from a false-positive result [119]. As discussed above, there are a number of sources of variability in the IGRA assay related to laboratory technique such as sample agitation, time elapsed prior to incubation, duration of incubation, agitation technique, and blood volume that could result in variability around the cutoff value. In this instance, this variability may reflect the inherent variability of a biologic measurement, and is the rationale behind the committee's recommendation that quantitative values be reported. The optimal cut-points for IGRA testing are controversial. While results close to the cut-point tend to be less reliable than results substantially above or below the cut-point, this is not absolute; in many instances, positive values well above the threshold were not reproduced in subsequent testing [116]. It is for this reason that the committee felt that quantitative guidance regarding the interpretations of conversions and reversions in the context of healthcare worker screening could not be provided. Given the varied sources of IGRA variability [24], the committee thought that a positive test in a low-risk individual was likely to be a false-positive result, and recommended repeat testing.

Question 4: Should an IGRA or a TST be performed in healthy children <5 years of age in whom it has been decided that testing for LTBI is warranted?

Evidence

The body of evidence regarding IGRA performance in young children is limited. Compared with adults, a limited number of children have been enrolled in IGRA studies. Even fewer children from nonendemic countries have been studied, and many reports do not include a separate analysis of young children.

The sensitivity of IGRAs in young children with TB ranges from 52% to 100% when a final diagnosis of either microbiologically confirmed or clinical TB disease is used as the reference standard, which is comparable to adults [56, 59, 76, 102–105, 120]. The sensitivity of the TST has been reported as equivalent or increased compared with IGRAs in children [56, 59, 76, 101–105, 120], with young age associated with decreased IGRA positivity [107]. Important caveats to this comparison, however, are that some studies used earlier, less sensitive versions of the IGRA and results have been inconsistent. As examples of the inconsistencies, a study using an IGRA similar to a currently available IGRA test demonstrated increased sensitivity of the IGRA compared with TST in children aged <3 years, especially among those coinfected with HIV and/or malnourished [121]. None of these studies were performed in nonendemic countries.

The specificity of IGRAs appears to be excellent in children in the range of 90%–100% [122] according to a study conducted in children who had nontuberculous mycobacteria. The study found that IGRAs were more specific than TST in children with nontuberculous mycobacterial disease [102].

Our confidence in the estimated test characteristics of IGRAs and TST in children is very low because most of the studies did not report whether or not they enrolled consecutive patients,

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

were not performed in nonendemic countries, and have provided inconsistent results.

Recommendation: We suggest performing a TST rather than an IGRA in healthy children <5 years of age for whom it has been decided that diagnostic testing for LTBI is warranted (*conditional recommendation, very low-quality evidence*).

Rationale

The limited direct evidence described above suggests that the TST might be more sensitive than IGRAs in young children, and IGRAs may be more specific than the TST, particularly in those given BCG. Because young children have a high risk for progression to active TB disease, the committee believed that the sensitivity of the diagnostic test (ie, avoiding false-negative results, missed opportunities to treat) is more important than the specificity of the test (ie, avoiding false-positive results, unnecessary therapy). This is supported by the observations that the potential consequences of delayed treatment are high, while the risk of hepatotoxicity is greatly reduced in young children. An additional reason to favor TST testing over IGRA testing in young children is that the management of the most at-risk young children (ie, young household contacts) depends upon the results of serial testing for infection, for which there are no data for IGRAs in young children.

While there are theoretical benefits from IGRA testing (eg, improved acceptance of LTBI therapy), these benefits have not been proven. Therefore, there is insufficient evidence that the benefits of IGRA testing exceed the well-known limitations of the TST. For these reasons, it is too early to recommend replacing the TST with IGRA testing. The recommendation is conditional because the quality of evidence provided the committee with limited confidence in the estimated test characteristics of IGRAs and TSTs in children; therefore, the committee could not be certain that the desirable consequences of performing IGRAs instead of TSTs outweigh the undesirable consequences in the vast majority of patients.

Cautions and Limitations

In studies of young children that report rates of indeterminate IGRA results, the frequency ranges from 0 to 35%, which is generally higher than in studies that reported in adults. Several studies have reported an increased rate of indeterminate IGRA results in children <5 years of age [47, 56–60]. As phlebotomy is more difficult in young children, inability to perform the IGRA due to insufficient blood volumes represents an additional practical limitation to IGRA testing in young children. A relatively high incidence of failed phlebotomy has been documented in some studies [106, 123].

DIAGNOSTIC TESTS FOR TB

The diagnosis and management of TB disease rely on accurate laboratory tests, both for the benefit of individual patients and the control of TB in the community through public health services. Therefore, laboratory services are an essential component of effective TB control at the local, state, national, and global levels.

In the United States, up to 80% of all initial TB-related laboratory work (eg, AFB smear and culture inoculation) is performed in hospitals, clinics, and independent laboratories outside the public health system, whereas >50% of species identification and drug susceptibility testing (DST) is performed in public health laboratories [124]. Thus, effective TB control requires a network of public and private laboratories to optimize laboratory testing and the flow of information. Public health laboratory workers, as a component of the public health sector with a mandate for TB control, should take a leadership role in developing laboratory networks and in facilitating communication among laboratory workers, clinicians, and TB controllers.

Seven types of tests for the diagnosis of TB disease and detection of drug resistance are performed within the tuberculosis laboratory system and recommended for optimal TB control services (Table 2). These laboratory tests should be available to every clinician involved in TB diagnosis and management, and to jurisdictional public health agencies charged with TB control.

For suspected cases of pulmonary TB, sputum smears for AFB are correlated with the likelihood of transmission and then, for AFB smear–positive pulmonary cases, a nucleic acid amplification assay provides rapid confirmation that the infecting mycobacteria are from the *Mtb* complex. Both sputum smears for AFB and nucleic acid amplification tests (NAATs) should be available with rapid turnaround times from specimen collection. These tests facilitate decisions about initiating treatment for TB or a non-TB pulmonary infection, infection control measures (eg, patient isolation), and, if TB is diagnosed, for reporting the case and establishing priority for the contact investigation.

Table 2. Essential Laboratory Tests for the Detection of Mycobacterium tuberculosis

Test	Time Required
I. Nucleic acid amplification test, detection (NAAT-TB)	1 d
II. Nucleic acid amplification test, resistance markers (NAAT-R)	1–2 d
III. Acid-fast bacilli microscopy	1 d
IV. Growth detection Liquid Solid	Up to 6–8 wk (average 10–14 d) (average 3–4 wk)
V. Identification of Mycobacterium tuberculosis complex by DNA probe or HPLC	1 d[a]
VI. First-line drug susceptibility testing (liquid medium)	1 to 2 wk[a]
VII. Second-line and novel compound drug susceptibility testing	
i. Liquid (broth-based) medium	1 to 2 wk[a]
ii. Solid (agar- or egg-based) medium	3 to 4 wk[a]

Abbreviation: HPLC, high-performance liquid chromatography.[a]After detection of growth.

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

DIAGNOSTIC APPROACH: TESTING FOR SUSPECTED PULMONARY TB

Pulmonary TB is often first suspected on the basis of chest computed tomographic findings (Supplementary Table 8). Randomized trials and controlled observational studies that directly compared diagnostic tests for pulmonary tuberculosis and measured patient-important outcomes have not been performed. Therefore, the recommendations in this section are based upon data that describe how accurate a diagnostic test is at confirming or excluding pulmonary TB, coupled with the widely accepted knowledge that diagnosing pulmonary TB leads to therapy that dramatically improves patient-important outcomes and reduces disease transmission [125, 126].

Question 5: Should AFB smear microscopy be performed in persons suspected of having pulmonary TB?

Evidence

Performing 3 AFB smears confirms pulmonary TB with a sensitivity of approximately 70% when culture-confirmed TB disease is the reference standard. The reason for performing 3 AFB smears is that each specimen increases sensitivity. The sensitivity of the first specimen is 53.8%, which increases by a mean of 11.1% by obtaining a second specimen. Obtaining a third specimen increases the sensitivity by a mean of only 2%–5% (ie, false-positive results could exceed the additional true-positive results obtained from a third specimen).

The sensitivity of a first morning specimen is 12% greater than a single spot specimen [127]. Concentrated specimens have a mean increase in sensitivity of 18% compared with nonconcentrated specimens (using culture as the standard) and fluorescence microscopy is on average 10% more sensitive than conventional microscopy [128, 129]. The specificity of microscopy is relatively high (≥90%), but the positive predictive value (PPV) varies (70%–90%) depending upon the prevalence of tuberculosis versus nontuberculous mycobacterial disease [130, 131]. These accuracy studies provide moderate confidence in the estimated test characteristics because many did not report having enrolled consecutive patients.

Recommendation 5: We recommend that AFB smear microscopy be performed, rather than no AFB smear microscopy, in all patients suspected of having pulmonary TB (*strong recommendation, moderate-quality evidence*). Remarks: False-negative results are sufficiently common that a negative AFB smear result does not exclude pulmonary TB. Similarly, false-positive results are sufficiently common that a positive AFB smear result does not confirm pulmonary TB. Testing of 3 specimens is considered the normative practice in the United States and is strongly recommended by the CDC and the National Tuberculosis Controllers Association to improve sensitivity given the pervasive issue of poor sample quality. Providers should request a sputum volume of at least 3 mL, but the optimal volume is 5–10 mL. Concentrated respiratory specimens and fluorescence microscopy are preferred.

Rationale

AFB smear microscopy can be performed in hours, is inexpensive, and is technically simple. Our committee predetermined that AFB smear microscopy would be recommended if false-negative results occur <30% of the time (ie, sensitivity is ≥70%) and false-positive results occur <10% of the time (ie, specificity is ≥90%). The likely outcome of a false-negative result is additional diagnostic testing and/or delayed diagnosis due to the duration required for cultures to become positive, whereas the likely outcome of a false-positive result is unnecessary therapy with its associated risk for hepatotoxicity. The different thresholds for false results reflect the committee's recognition that the consequences of false-negative results are generally transient, whereas the consequences of false-positive results may be long lasting. In this case, the sensitivity and specificity of AFB smear microscopy were approximately 70% and ≥90%, respectively, so AFB smear microscopy is recommended.

The recommendation is strong because the quality of evidence provided the committee with moderate confidence in the estimated test characteristics of AFB smear microscopy, and the committee therefore felt certain that the desirable consequences of AFB smear microscopy (ie, an early presumptive diagnosis, initiation of therapy, and possibly less transmission) outweigh the undesirable consequences (ie, cost, burden, effects of false results) in the vast majority of patients.

Question 6: Should both liquid and solid mycobacterial cultures be performed in persons suspected of having pulmonary TB?

Evidence

A meta-analysis comparing 2 liquid culture methods with solid cultures found that both liquid culture methods were more sensitive (88% and 90%) than the solid culture method (76%) when a combination of conventional solid media with a broth-based method was the reference standard, and also had a shorter time to detection (13.2 and 15.2 days for liquid culture methods versus 25.8 days for the solid culture method) [132]. The specificity of all 3 methods exceeded 99%. Liquid culture medium has a higher contamination rate than solid culture medium due to the growth of bacteria other than mycobacteria (4%–9% in the meta-analysis), which interferes with obtaining a valid culture result. This evidence provides low confidence in the estimated test characteristics for 2 reasons. First, there may be selection bias, as many of the studies did not state whether they enrolled consecutive patients. Second, there is indirectness, since the studies address the test characteristics of either test alone but the question is about the tests combined.

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

Recommendation 6: We suggest that both liquid and solid mycobacterial cultures be performed, rather than either culture method alone, for every specimen obtained from an individual with suspected TB disease (*conditional recommendation, low-quality evidence*). Remarks: The conditional qualifier applies to performance of both liquid and solid culture methods on all specimens. At least liquid culture should be done on all specimens as culture is the gold standard microbiologic test for the diagnosis of TB disease. The isolate recovered should be identified according to the Clinical and Laboratory Standards Institute guidelines and the American Society for Microbiology Manual of Clinical Microbiology [133, 134].

Rationale

Mycobacterial culture is the laboratory gold standard for tuberculosis diagnosis, but the preferred type of cultures is uncertain. Liquid cultures alone are reasonably sensitive and highly specific, but limited by contamination. Solid cultures alone are not sufficiently sensitive to reliably diagnose TB and generally take longer to yield results; however, some *Mtb* isolates are detected only on solid medium. Performing both liquid and solid cultures likely improves the sensitivity of mycobacterial cultures, while the liquid cultures provide a more rapid answer and the solid cultures serve as a safeguard against contamination. The recommendation is conditional because the quality of evidence provided the committee with limited confidence in the estimated test characteristics of the culture methods; therefore, the committee could not be certain that the desirable consequences of performing both culture methods instead of only one method outweigh the undesirable consequences in the vast majority of patients.

Question 7: Should NAAT be performed on the initial respiratory specimen in persons suspected of having pulmonary TB?

Evidence

Three meta-analyses were identified and reviewed. The first stratified the performance characteristics of NAAT based upon AFB smear results [135]. When AFB smear microscopy was positive, the sensitivity and specificity of NAAT were 96% and 85%, respectively. Most studies used culture as the reference standard. When AFB smear microscopy was negative, the sensitivity decreased to 66% and the specificity increased to 98%. When further stratified by whether the patient received treatment, the specificity in untreated patients was 97%. The second meta-analysis reported an overall sensitivity of 85% and specificity of 97% and did not stratify according to the results of AFB smear microscopy [136]. There was significant heterogeneity in both meta-analyses. The third meta-analysis stratified the NAAT test characteristics in AFB smear microscopy–negative suspects according to clinical suspicion of tuberculosis [137]. It found that in AFB smear microscopy–negative individuals, a positive NAAT result is beneficial when the clinical suspicion of tuberculosis was intermediate or high (>30%) and a negative NAAT result is of little use in excluding the presence of *Mtb*. This evidence provides low confidence in the estimated test characteristics because there may be selection bias since many of the studies did not state whether they enrolled consecutive patients with legitimate diagnostic uncertainty and there was significant inconsistency in the meta-analyses.

Recommendation 7: We suggest performing a diagnostic NAAT, rather than not performing a NAAT, on the initial respiratory specimen from patients suspected of having pulmonary TB (*conditional recommendation, low-quality evidence*). Remarks: In AFB smear–positive patients, a negative NAAT makes TB disease unlikely. In AFB smear–negative patients with an intermediate to high level of suspicion for disease, a positive NAAT can be used as presumptive evidence of TB disease, but a negative NAAT cannot be used to exclude pulmonary TB. Appropriate NAATs include the Hologic Amplified Mycobacteria Tuberculosis Direct (MTD) test (San Diego, California) and the Cepheid Xpert MTB/Rif test (Sunnyvale, California).

Rationale

Mycobacterial culture results require at least 1–2 weeks; therefore, rapid diagnostic tests that can be performed within hours are desirable, such as AFB smear microscopy and diagnostic NAAT. Diagnostic NAAT has the added advantage over AFB smear microscopy of being able to distinguish *Mtb* from nontuberculous mycobacteria. However, NAAT is appropriate only as an adjunct to mycobacterial culture and AFB smear microscopy. It is used as an adjunct to mycobacterial culture because it is not sensitive enough to replace mycobacterial culture for diagnosis and does not produce an isolate, which is needed for phenotypic DST. It is used as an adjunct to AFB smear microscopy because the test characteristics of NAAT are highly variable depending upon the AFB smear results and clinical suspicion.

In AFB smear–positive patients, NAAT yields false-negative results only 4% of the time, indicating that it is reliable for excluding pulmonary TB. In AFB smear–negative patients, clinical suspicion needs to be considered. When there is an intermediate to high level of suspicion for disease, NAAT yields sufficiently few false-positive results that a positive NAAT result can be used as presumptive evidence of TB and guide therapeutic decisions; however, false-negative results are sufficiently common that NAAT cannot be used to exclude pulmonary TB. When the clinical suspicion for TB is low, NAAT is generally not performed because false-positive results are unacceptably frequent. An algorithm for interpretation and use of NAAT results in conjunction with AFB smear results has been published [138].

The recommendation is conditional because the quality of evidence provided the committee with limited confidence in

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

the estimated test characteristics of NAAT; therefore, the committee could not be certain that the desirable consequences of performing NAAT (ie, promptly diagnosing TB disease and initiating treatment), instead of not performing NAAT, outweigh the undesirable consequences (ie, cost, false-positive results leading to unnecessary treatment, and false-negative results provided false reassurance) in the vast majority of patients.

Cautions and Limitations

Laboratory-based diagnostic tests are not a replacement for clinical judgment and experience. A diagnosis of pulmonary tuberculosis can be made in the absence of laboratory confirmation, especially in children [139]. Although there appears to be little increase in accuracy achieved by routinely performing NAAT on multiple specimens rather than on a single specimen, some clinicians may find it beneficial in the diagnosis of individual patients [140, 141]. As an example, the presence of inhibitors can cause false-negative results for some NAATs [142] and, therefore, if a specimen has a positive AFB smear result and a negative NAAT result, evaluation of the sample for the presence of inhibitors should be considered if the NAAT being used is subject to inhibition. If inhibitors are detected, collection of a new specimen for NAAT should be considered. The recommendation for use of NAATs is based on studies of commercial test kits. The data on in-house tests show even greater heterogeneity [143]. If in-house tests are to be used, they should be validated and be shown to have analytical performance accuracy comparable to or better than that of commercial tests.

Question 8: Should rapid molecular drug susceptibility testing for isoniazid and rifampin be performed as part of the initial diagnostic evaluation for all patients suspected of having pulmonary TB or only in selected subgroups?

Evidence

Rapid molecular DST can be performed via line probe or molecular beacon assays. We evaluated systematic reviews with meta-analyses of 2 line probe assays [144, 145]. Both line probe assays detected rifampin resistance with a sensitivity and specificity of ≥97% and ≥98%, respectively, when conventional, culture-based DST was used as the reference standard. More recently, a molecular-beacon based method for rapid rifampin resistance detection was evaluated in a large international accuracy study [146]. This assay, Xpert MTB/RIF, was >92% sensitive and >99% specific for detection of rifampin resistance when performed on a single specimen; the sensitivity increased to >97% when performed on 3 specimens [146]. Despite its good sensitivity and specificity, the PPV of rapid molecular DST for the detection of rifampin resistance is low in populations with a low prevalence of drug resistance (Supplementary Table 9) [147].

One of the assays also detects isoniazid resistance. It identified isoniazid resistance with a sensitivity and specificity of 84% and 99%, respectively, when culture-based DST is used as the reference standard. However, when the meta-analysis was performed on a subgroup of studies that evaluated a newer version of the assay, the sensitivity increased to approximately 90%. This indicates than in appropriate subgroups of patients, false-positive and false-negative results occur in 1% and 10% of patients, respectively. In contrast to rifampin resistance, the PPV of a test indicating isoniazid resistance is quite high, a reflection of isoniazid resistance being fairly prevalent in the United States (approximately 8%) [144].

This evidence provides moderate confidence in the estimated sensitivities and specificities among patient subgroups with increased rates of drug resistance. The confidence is moderate instead of high because the absence of reporting that patients were enrolled consecutively suggests that there is a risk of bias.

Recommendation 8: We recommend performing rapid molecular DST for rifampin with or without isoniazid using the respiratory specimens of persons who are either AFB smear positive or Hologic Amplified MTD positive and who meet one of the following criteria: (1) have been treated for tuberculosis in the past, (2) were born in or have lived for at least 1 year in a foreign country with at least a moderate tuberculosis incidence (≥20 per 100 000) or a high primary MDR-TB prevalence (≥2%), (3) are contacts of patients with MDR-TB, or (4) are HIV infected (*strong recommendation, moderate-quality evidence*). Remarks: This recommendation specifically addresses patients who are Hologic Amplified MTD positive because the Hologic Amplified MTD NAAT only detects TB and not drug resistance; it is not applicable to patients who are positive for types of NAAT that detect drug resistance, including many line probe assays and Cepheid Xpert MTB/RIF.

Rationale

Conventional, culture-based DST is the laboratory gold standard [134, 148, 149]. It is performed routinely any time *Mtb* complex is isolated in culture. Drug susceptibility testing is essential because treatment success for patients with MDR-TB (can reach 75% or higher [150, 151]) is dependent upon patients being treated with an effective antimicrobial regimen [152]. An important limitation of culture-based DST, however, is that it can take >2 weeks to grow the isolate that is necessary for testing.

Rapid molecular DST addresses this limitation. It can be performed within hours, enabling earlier initiation of an appropriate antimicrobial regimen. Rapid molecular DST is an adjunct and not a replacement for culture-based DST because it only evaluates susceptibility to rifampin and occasionally isoniazid. Nonetheless, detection of rifampin resistance is helpful to clinicians because it is a good surrogate for MDR-TB in locations where rifampin monoresistance is uncommon. However,

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

an important limitation is that the PPV is expected to be lower in the United States than in areas where rifampin resistance is more common [153–155].

The committee recommends rapid molecular DST only for subgroups in which drug resistance is more likely, as the PPV for rifampin resistance testing is low in populations with a low prevalence of drug resistance. Examples of appropriate persons for testing include those who are NAAT or AFB smear positive and meet one of the following criteria: (1) have been treated for tuberculosis in the past, (2) were born in or have lived for at least 1 year in a foreign country with at least a moderate tuberculosis incidence (≥20 per 100 000) or a high primary MDR-TB prevalence (≥ 2%), (3) are contacts of patients with MDR-TB, or (4) are HIV infected [154, 156–158].

The sensitivity and specificity of rapid molecular DST for detecting rifampin resistance are both >97%, indicating that false-positive and false-negative results occur <3% of the time; thus, rapid molecular DST can be used confirm or exclude rifampin resistance in respiratory specimens. The sensitivity and specificity of rapid molecular DST for detecting isoniazid resistance are estimated to be 90% and 99%, respectively, indicating that false-positive and false-negative results occur roughly 1% and 10% of the time, respectively; thus, rapid molecular DST can be used to confirm isoniazid resistance in respiratory specimens, but not exclude it.

The recommendation is strong because the moderate-quality evidence provided the committee with sufficient confidence in the test characteristics to be certain that the benefits of rapid molecular DST (ie, early identification of possible MDR-TB and initiation of an appropriate antimicrobial regimen) outweigh the costs and burden of testing in the overwhelming majority of patients who have increased risk for drug resistance.

Cautions and Limitations

Line probe and molecular beacon assays have not been sufficiently validated for use on specimens other than respiratory specimens. The recommendation for line probe assays and molecular beacon on respiratory specimens is based upon studies of commercial test kits, only one of which is currently approved by the FDA: the molecular beacon–based method, Xpert MTB/RIF. It is the only FDA-approved assay and it integrates diagnosis of TB and detection of rifampin resistance. If this test is used for the diagnosis of TB, a rifampin resistance result is automatically provided regardless of patient risk.

Other assays for rapid detection of drug resistance using alternative molecular techniques (eg, automated real-time polymerase chain reaction [PCR] with sequencing, loop-mediated isothermal amplification [LAMP]) are being developed. These assays are promising, but are not yet commercially available [146, 159, 160]. The data on in-house tests show substantial heterogeneity [161]. If in-house tests are to be used, they should be validated and shown to have performance accuracy at least comparable to that of commercial tests. The same cautions also apply to new commercial assays that may become available in the near future.

Some clinicians and health departments may opt for broader use of the molecular detection of drug resistance assays than recommended above, especially in regions where MDR-TB is more common. Because the prevalence of rifampin resistance (and therefore MDR-TB) is low in the United States, the PPV of Xpert MTB/RIF and other assays for rifampin resistance will be lower than in settings where Xpert MTB/RIF has been predominantly studied. Therefore, confirmation of a positive test result for rifampin resistance has been recommended [147]. To confirm a positive result, genetic loci associated with rifampin resistance (to include *rpoB*), as well as isoniazid resistance (to include *inhA* and *katG*), should be sequenced to assess for MDR-TB. If mutations associated with rifampin resistance are confirmed, rapid molecular testing for other known mutations associated with drug resistance (to first-line and second-line drugs) is needed for healthcare providers to select an optimally effective treatment regimen. All molecular testing should prompt growth-based DST.

Alternative methods for rapid molecular DST are being developed and other technologies are likely to become available in the near future (eg, automated real-time PCR with sequencing, LAMP) [162]. It is possible that these techniques will be sufficiently sensitive to be used for AFB smear–negative specimens. Laboratories in the United States should only use tests approved by the FDA or tests that have been produced and validated in accord with applicable FDA and Clinical Laboratory Improvement Amendments regulations.

Question 9: Should respiratory specimens be collected from children with suspected pulmonary TB disease?

Evidence

Respiratory specimens that can be collected from children include gastric aspirates; sputum collected by spontaneous expectoration, induction, or nasopharyngeal aspiration; and bronchoalveolar lavage (BAL). Gastric aspirates involve intubating the stomach after an overnight fast to collect swallowed sputum before the stomach empties. Collection of specimens on 3 consecutive mornings from patients with suspected pulmonary TB provides a diagnostic yield of up to 40%–50%, with higher yields for infants (up to 90%), symptomatic children, and children with extensive disease (up to 77%), using a clinical diagnosis of TB disease in a low prevalence country as criteria for the diagnosis of TB disease [163–165]. Meticulous attention to detail during the collection and processing of the specimen can improve yield (details are provided at http://www.currytbcenter.ucsf.edu/pediatric_tb/). Sputum collected from children by nasopharyngeal aspiration or sputum induction with a bronchodilator has a yield of 20%–30% [166], whereas

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

BAL in children with pulmonary TB has a yield of 10%–22% [167]. These estimates of diagnostic yield are based upon moderate-quality evidence—accuracy studies for which it was not documented whether the subjects were enrolled consecutively.

Recommendation 9: We recommend mycobacterial culture of respiratory specimens for all children suspected of having pulmonary TB (*strong recommendation, moderate-quality evidence*).

Rationale

Despite the observation that less than half of pediatric specimens yield a positive culture, the committee judged that the desirable consequences of mycobacterial cultures of respiratory specimens outweigh the undesirable consequences of specimen collection in children for several reasons. First, a positive mycobacterial culture is likely to be reassuring to parents and staff that the diagnosis of tuberculosis is correct. Second, cultures are necessary for DST, which is particularly important because TB drug resistance has become more prevalent. Finally, after-the-fact culture collection in the face of treatment failure may have even lower yield than sampling a drug-naive child.

With respect to the need for DST, overtreatment for presumed drug-resistant TB may lead to unnecessary toxicities and cost, while undertreatment due to unidentified drug resistance may lead to treatment failure, risk of dissemination, and even death. While it is tempting to avoid culture collection from the child contact when a putative source case is identified (especially when susceptibility results are already available), prior case series indicate that 2%–10% of children have susceptibility patterns that differ from the presumed source case [168] and more recent US studies have found up to 15% discordance of molecular fingerprinting between the isolates collected from children with culture-proven TB compared to their presumed source case [169, 170]. In contrast, no discordance was found between pediatric TB cases and their presumed source cases from 2000 to 2004 in Houston [171].

The recommendation is strong because the moderate quality of evidence provided the committee with sufficient confidence in the estimated diagnostic yield that the committee felt certain that a diagnosis was rendered frequently enough that the desirable consequences of collecting respiratory specimens (ie, confirming the diagnosis of TB, obtaining an isolate for DST) outweigh the undesirable consequences (ie, cost, burden, effects of false results) in the vast majority of children with suspected pulmonary TB.

While in some circumstances, it is appropriate to use the susceptibility data from the mycobacterial isolate from a very close (ie, household contact) source case as a surrogate for an isolate from the child, some studies have revealed discordance between the susceptibility results and/or molecular fingerprint data of the child and presumed source case isolates (see below). Therefore, strong consideration should be given to culture collection from a child suspected to have TB, even if a possible source case is identified, especially if the child is particularly ill, is immunocompromised, or there is risk of drug-resistant TB in the child or presumed source case. Selection of an appropriate respiratory specimen (ie, gastric aspirates, spontaneous or induced sputa or, rarely, BAL) is based upon the expertise of the clinic and provider, the patient's age and developmental level, and the likelihood of an alternative diagnosis.

Cautions and Limitations

Gastric aspirates are rarely AFB smear positive and the yield of cultures is suboptimal in children with pulmonary TB; thus, gastric aspirate culture results are helpful only if they are positive. Negative results should not dissuade the provider from empirically treating tuberculosis in children in the appropriate clinical setting. Gastric aspirate, sputum induction, and nasopharyngeal aspiration in children are not comfortable and not without financial cost. The procedures have modest risk (bleeding from the nose, bronchospasm, airway intubation).

Question 10: Should sputum induction or flexible bronchoscopic sampling be the initial respiratory sampling method for individuals with suspected pulmonary TB who are either unable to expectorate sputum or whose expectorated sputum is AFB smear microscopy negative?

Evidence

We identified 6 studies [172–177] that compared the diagnostic yield of induced sputum with the yield of specimens obtained by flexible bronchoscopy, using a positive mycobacterial culture or evidence of a response the therapy as criteria for the diagnosis of pulmonary TB. Five of the 6 studies demonstrated a higher yield from induced sputum than bronchoscopy, with the remaining study [176] demonstrating a similar yield. The diagnostic yield of induced sputum increases with multiple specimens, with detection rates by AFB smear microscopy of 91%–98% and mycobacterial culture of 99%–100% reported when 3 or more specimens are obtained [178].

Two cost-analysis studies favored sputum induction over bronchoscopy [172, 174]. In the first study, direct costs for bronchoscopy measured in Canadian dollars were $187.60, compared with $22.22 for sputum induction [172]. In the second study, induced sputum was about one-third the cost of flexible bronchoscopy, and the most cost-effective strategy was 3 induced sputa without bronchoscopy [174].

Our confidence in the accuracy of the study results is low because there was a risk of bias and indirectness. With respect to risk of bias, most of the studies did not report whether or not consecutive patients were enrolled. Supporting this concern, the variability of prevalence among studies suggests that the degree of diagnostic uncertainty likely differed among studies. With respect to indirectness, there appeared to be indirectness

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

of the intervention because the studies varied in the number of specimens collected (from 1 to 3 per patient), the concentrations of hypertonic saline, the type of nebulizers, and the culture techniques.

Recommendation 10: We suggest sputum induction rather than flexible bronchoscopic sampling as the initial respiratory sampling method for individuals with suspected pulmonary TB who are either unable to expectorate sputum or whose expectorated sputum is AFB smear microscopy negative (*conditional recommendation, low-quality evidence*).

Rationale

Induced sputum has equal or greater diagnostic yield than bronchoscopic sampling, has fewer risks, and is less expensive. These features all favor induced sputum as the initial respiratory sampling method in patients with suspected pulmonary TB who are either unable to expectorate sputum or whose expectorated sputum is AFB smear microscopy negative. The committee recognizes that a potential advantage of bronchoscopy over sputum induction is the possibility of making a rapid presumptive diagnosis of tuberculosis by performing biopsies and identifying typical histopathologic findings, but felt that the balance of the upsides to downsides of induced sputum outweighed that of bronchoscopic sampling. The recommendation is conditional because the quality of evidence does not provide sufficient confidence in the study results for the committee to be absolutely certain that the balance of desirable to undesirable consequences favors induced sputum over bronchoscopy.

Question 11: Should flexible bronchoscopic sampling be performed in individuals with suspected pulmonary TB from whom a respiratory sample cannot be obtained via induced sputum?

Evidence

Numerous studies reported the diagnostic yield of respiratory specimens obtained by flexible bronchoscopy, using a positive mycobacterial culture or evidence of a response the therapy as criteria for the diagnosis of pulmonary TB [172–175, 178–182]. Generally speaking, bronchoscopic sampling appears to have a diagnostic yield of 50%–100% when based on culture in patients suspected of having pulmonary TB. This yield appears unaffected by HIV infection, with bronchoscopy leading to an early presumptive diagnosis of TB in 34%–48% of HIV-infected patients according to 2 studies [183, 184]. In one study, bronchial washings had the same culture yield (95%) as BAL but higher frequency of positive AFB smears (26% vs. 4%) [235]. Bronchoscopic brushings yield AFB smear–positive results in 9%–56% [180, 185].

Transbronchial biopsy (TBB) provides histopathologic findings suggestive of pulmonary TB in 42%–63% of specimens from smear-negative HIV-uninfected patients [183, 186]. HIV-infected patients are less likely (9%–19%) to demonstrate granulomas on TBB [183, 186], although in 2 studies TBB was the exclusive means of diagnosing pulmonary TB in 10%–23% of patients [183, 184].

Our confidence in the accuracy of these estimated diagnostic yields is very low because most of the studies did not report whether or not consecutive patients were enrolled, the range of reported diagnostic yields is wide, and the studies varied in how specimens were collected (bronchial aspirates and/or BAL and/or bronchial brushings and/or TBB) and the culture techniques.

Recommendation 11: We suggest flexible bronchoscopic sampling, rather than no bronchoscopic sampling, in individuals with suspected pulmonary TB from whom a respiratory sample cannot be obtained via induced sputum (*conditional recommendation, very low-quality evidence*). Remarks: In the committee members' clinical practices, BAL plus brushings alone are performed for most patients; however, for patients in whom a rapid diagnosis is essential, transbronchial biopsy is also performed.

Rationale

The committee judged that the desirable consequences of bronchoscopic sampling outweigh the undesirable consequences among patients with suspected pulmonary TB from whom respiratory samples could not be obtained noninvasively. The most important reason to perform bronchoscopy in a patient with possible pulmonary TB is to differentiate TB disease from alternative diseases. Another reason to perform bronchoscopy is to obtain specimens for cultures that provide isolates for DST. Empiric treatment for presumed drug-resistant TB may lead to unnecessary toxicities and cost if the patient actually has drug-sensitive TB, while empiric treatment for drug-sensitive TB may lead to treatment failure, risk of dissemination, and even death if the patient actually has drug-resistant TB. Moreover, delayed diagnosis of drug resistance will prolong therapy and increase risk of default. Bronchoscopy also provides the opportunity of obtaining a rapid presumptive diagnosis by identifying histopathologic findings consistent with tuberculosis. These benefits of bronchoscopic sampling were thought to outweigh the risks of bronchoscopy and the accompanying sedation, as well as the costs and burdens.

The recommendation is conditional, reflecting the guideline development committee's uncertainty that the desirable consequences of bronchoscopy outweigh the undesirable consequences in many situations. Reasons for the committee's uncertainty included the highly variable estimates of diagnostic yield, the very low quality of evidence, recognition that bronchoscopy is an invasive procedure and the risk of harm varies according to the patient's clinical condition, and recognition that the feasibility of timely bronchoscopy varies according to the clinical setting. For example, in the context of a public health clinic, the benefits of obtaining a bronchoscopy may not

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

justify the thousands of dollars that it will cost due to professional fees, hospital charges, pathology costs, and laboratory fees, or the days to weeks of delays that will be necessary to refer the patient to a pulmonologist for bronchoscopy. In some situations, the potential harm associated with delayed diagnosis may warrant empiric initiation of therapy based upon a reasonable suspicion of TB disease.

Question 12: Should postbronchoscopy sputum specimens be collected from individuals with suspected pulmonary TB?

Evidence

Postbronchoscopy sputum specimens are typically sent for AFB smear microscopy and mycobacterial culture. Postbronchoscopy AFB smears have a diagnostic yield of 9%–73% and postbronchoscopy mycobacterial cultures have a yield of 35%–71% according to multiple studies [182, 185, 187, 188]. In HIV-infected patients, the yield of postbronchoscopy sputum cultures was 80% in a single study [186]. Our confidence in the accuracy of the estimated diagnostic yields is low because most of the studies did not report whether or not consecutive patients were enrolled and the diagnostic yields reported varied greatly as indicated by the wide ranges described above.

Recommendation 12: We suggest that postbronchoscopy sputum specimens be collected from all individuals with suspected pulmonary TB who undergo bronchoscopy (*conditional recommendation, low-quality evidence*). Remarks: Postbronchoscopy sputum specimens are used to perform AFB smear microscopy and mycobacterial cultures.

Rationale

The rationale for postbronchoscopy sputum collection is the same as that described above for the bronchoscopic collection of respiratory specimens.

Question 13: Should flexible bronchoscopic sampling be performed in individuals with suspected miliary TB and no alternative lesions that are accessible for sampling whose induced sputum is AFB smear microscopy negative or from whom a respiratory sample cannot be obtained via induced sputum?

Evidence

Specimens obtained via bronchoscopy can undergo AFB smear microscopy, mycobacterial culture, NAAT, and histopathological analysis. There is a paucity of evidence regarding the diagnostic characteristics of various types of bronchoscopic specimens (ie, washings, BAL, brushings, TBB) obtained from patients with possible miliary TB. It has been reported that bronchial washings, brushings, and TBB have diagnostic yields of 14% [234], 27%–78% [181, 189, 190], and 32%–75% [181, 189, 190], respectively. The diagnostic yield of BAL has not been reported. Our confidence in the accuracy of the estimated diagnostic yields is very low because most of the studies did not report whether or not consecutive patients were enrolled (the prevalence of miliary TB ranged from 55% to 90% in the studies, suggesting that the degree of diagnostic uncertainty differed among the studies), the ranges of diagnostic yields were wide for both brushings and TBB, reflecting the variable results reported by the individual studies, and the studies varied in the technique used to perform the sampling (particularly TBB) and the number of specimens collected.

Recommendation 13: We suggest flexible bronchoscopic sampling, rather than no bronchoscopic sampling, in individuals with suspected miliary TB and no alternative lesions that are accessible for sampling whose induced sputum is AFB smear microscopy negative or from whom a respiratory sample cannot be obtained via induced sputum (*conditional recommendation, very low-quality evidence*). Remarks: Bronchoscopic sampling in patients with suspected miliary TB should include bronchial brushings and/or TBB, as the yield from washings is substantially less and the yield from BAL unknown. For patients in whom it is important to provide a rapid presumptive diagnosis of tuberculosis (ie, those who are too sick to wait for culture results), TBB is both necessary and appropriate.

Rationale

The rationale for bronchoscopic sampling in individuals with suspected miliary TB and no alternative lesions that are accessible for sampling (eg, enlarged lymph nodes or draining lesions) whose induced sputum is AFB smear negative or from whom a respiratory sample cannot be obtained via induced sputum is essentially the same as that described above for patients with suspected pulmonary TB from whom a respiratory sample cannot be obtained via induced sputum. That is, it is important to differentiate miliary TB from other diseases and also to obtain specimens for mycobacterial culture because cultures provide isolates for DST, which may prevent unnecessary drug toxicities and cost, mitigate treatment failure, and reduce the risk of dissemination and death. Moreover, bronchoscopy also provides the opportunity of obtaining a rapid presumptive diagnosis by identifying histopathologic findings consistent with tuberculosis. These benefits outweigh the risks of both bronchoscopy and the accompanying sedation.

The recommendation is conditional, reflecting the guideline development committee's uncertainty that the desirable consequences of bronchoscopy outweigh the undesirable consequences and its recognition that the balance of desirable and undesirable consequences depends upon clinical context. Reasons for the committee's uncertainty included the variable estimates of the diagnostic yield and very low quality of evidence. Clinical considerations that may affect the balance of desirable and undesirable effects include the patient's condition

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

(ie, bronchoscopy is an invasive procedure and the risk of harm varies according to the patient's clinical condition) and the availability, feasibility, and cost of timely bronchoscopy in a particular clinical setting.

DIAGNOSTIC APPROACH: TESTING FOR SUSPECTED EXTRAPULMONARY TB

Randomized trials and controlled observational studies that directly compared diagnostic tests or approaches for extrapulmonary TB and measured patient-important outcomes have not been performed. Therefore, the recommendations in this section are based upon data that describe how accurate a diagnostic test is at confirming or excluding extrapulmonary TB, coupled with evidence that the diagnosis of extrapulmonary TB leads to therapy that improves patient-important outcomes. Tests used to diagnose extrapulmonary TB are described in Supplementary Figure 1.

With respect to the evidence that the diagnosis of extrapulmonary TB leads to therapy that improves patient-important outcomes, trials directly comparing treatment with no treatment will never be done. However, indirect evidence from patients with pulmonary TB (described above) and evidence from patients with disseminated TB suggests that extrapulmonary TB is treatable with high cure rates in most drug-susceptible cases and that untreated extrapulmonary TB has significant morbidity and mortality, particularly meningeal TB [191]. As an example, an observational study that excluded patients with tuberculous meningitis found that mortality due to disseminated TB fell from 100% to <5% with the introduction of isoniazid-based antimicrobial regimens [192]. The large mortality reduction in this study would probably have been less dramatic if patients with tuberculous meningitis had been included, as 2 case series that included patients with tuberculous meningitis found that mortality due to disseminated tuberculosis is 20% [193, 194]. Nevertheless, mortality is clearly improved with a large magnitude of effect. This evidence constitutes moderate-quality evidence that treatment of extrapulmonary TB improves mortality because there are observational studies with a very large magnitude of effect, but the increase in confidence in the results due to the large magnitude of effect is mitigated by the indirectness of the population.

Question 14: Should cell counts and chemistries be performed on amenable (ie, liquid) specimens collected from sites of suspected extrapulmonary TB?

Evidence

We identified no studies that reported the sensitivity and specificity of cell counts and chemistries in the identification of extrapulmonary TB. Therefore, the committee used its collective clinical experience to inform its recommendation. Clinical experience constitutes very low-quality evidence.

Recommendation 14: We suggest that cell counts and chemistries be performed on amenable fluid specimens collected from sites of suspected extrapulmonary TB (*conditional recommendation, very low-quality evidence*). Remarks: Specimens that are amenable to cell counts and chemistries include pleural, cerebrospinal, ascitic, and joint fluids.

Rationale

Cell counts and chemistries can be performed in hours, are inexpensive, and are technically simple. Any risks are related to the sampling procedure. Although their sensitivity and specificity for extrapulmonary TB have not been reported, the committee suspects that the sensitivity is moderate to high and the specificity is poor if interpreted alone, but substantially better if interpreted in the context of the clinical setting, radiographic findings, and other laboratory results. Most importantly, it is believed that cell counts and chemistries can provide useful information to guide the clinician toward either confirmatory diagnostic testing for tuberculosis or diagnostic testing for alternative etiologies; this alone provides enough benefit to justify the costs of the additional tests. The strength of the recommendation is conditional because it is believed that the balance of the benefits of the additional information versus the cost of the testing may be finely balanced in some clinical situations, and the quality of evidence provides little confidence in the estimates upon which the committee based its judgments.

Question 15: Should adenosine deaminase (ADA) and free IFN-γ levels be measured on specimens collected from sites of suspected extrapulmonary TB?

Evidence

Test characteristics of ADA in the diagnostic evaluation of meningeal, pleural, peritoneal, and pericardial tuberculosis have been reported in meta-analyses of accuracy studies. Two meta-analyses estimated the sensitivity and specificity of an elevated ADA level in the cerebrospinal fluid [195, 196]. The first meta-analysis included 10 studies and found a sensitivity and specificity of 79% and 91%, respectively [195], using final clinical diagnosis, consistent pathology/cytology, or microbiologic confirmation as the reference standard. Most of the studies used a threshold of 9 U/L or 10 U/L to define an elevated ADA. The second meta-analysis included 13 studies and showed that the sensitivity and specificity are exquisitely sensitive to the threshold used to define an elevated ADA level [196]. If 4 U/L was used as the threshold, the sensitivity and specificity were >93% and <80%, respectively. In contrast, if 8 U/L was used as the threshold, the sensitivity and specificity were <59% and >96%, respectively.

Five meta-analyses that included 9–63 studies estimated that the sensitivity and specificity of an elevated ADA level in the pleural fluid are 89%–99% and 88%–97%, respectively, with all

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

but one of the meta-analyses estimating that the specificity is ≥90% [197–201]. A more recent meta-analysis reported similar sensitivity and specificity [202]. Final clinical diagnosis, consistent pathology/cytology, or microbiologic confirmation were used as the reference standard in most studies. Thresholds used to define an elevated ADA level ranged from 10 U/L to 71 U/L, with most clustering around 40 U/L.

A meta-analysis of 31 studies estimated that the sensitivity and specificity of an elevated ADA level in pericardial fluid are 88% and 83%, respectively [203]. The threshold to define an elevated ADA level was 40 U/L. Finally, a meta-analysis of 4 studies estimated that the sensitivity and specificity of an elevated ADA level in peritoneal fluid are 100% and 97%, respectively [204]. The threshold used to define an elevated ADA level ranged from 36 U/L to 40 U/L.

The test characteristics of free IFN-γ levels have not been as extensively studied. A meta-analysis of 6 studies estimated that the sensitivity and specificity of an elevated free IFN-γ level in peritoneal fluid are 93% and 99%, respectively [205]. The threshold used to define an elevated IFN-γ level ranged from 0.35 U/L to 9 U/L or 20 pg/mL to 112 pg/mL. A meta-analysis of 22 studies estimated that the sensitivity and specificity of an elevated free IFN-γ level in pleural fluid are 89% and 97%, respectively [206]. The threshold used to define an elevated IFN-γ level ranged from 0.3 U/L to 10 U/L or 12 pg/mL to 300 pg/mL. We did not identify any studies that looked at the test characteristics of free IFN-γ levels on pericardial fluid or cerebrospinal fluid.

No studies were identified that reported the test characteristics of using both ADA and free IFN-γ to evaluate specimens from patients with suspected extrapulmonary TB. This evidence provides low confidence in the accuracy of the estimated test characteristics for both ADA and free IFN-γ levels. The studies did not report whether consecutive patients were enrolled (ie, risk of bias) and the studies reported variable results (ie, inconsistency), probably due in large part to the different thresholds used to define an elevated level.

Recommendation 15a: We suggest that ADA levels be measured, rather than not measured, on fluid collected from patients with suspected pleural TB, TB meningitis, peritoneal TB, or pericardial TB (*conditional recommendation, low-quality evidence*).

Recommendation 15b: We suggest that free IFN-γ levels be measured, rather than not measured, on fluid collected from patients with suspected pleural TB or peritoneal TB (*conditional recommendation, low-quality evidence*).

Rationale

Neither the ADA level nor the IFN-γ level provide a definitive diagnosis of extrapulmonary TB disease; rather, they provide supportive evidence that must be interpreted in the entire clinical context. In any type of diagnostic testing for extrapulmonary TB, both false-negative results and false-positive results have important consequences. False-negative results delay diagnosis and treatment while diagnostic testing continues, whereas false-positive results may lead to unnecessary therapy and the associated risks of drug toxicity and cost. Therefore, it is desirable for diagnostic tests to be both sensitive and specific.

Our committee made the judgment that measurement of ADA levels and free IFN-γ levels are indicated if false-negative results occur <30% of the time (ie, sensitivity is ≥70%) and false-positive results occur <20% of the time (ie, specificity is ≥80%). The different thresholds for false results reflect the committee's recognition that the consequences of false-negative results are generally transient, whereas the consequences of false-positive results may be long lasting. In this case, the sensitivity and specificity of ADA were ≥79% and ≥83%, respectively, for detecting TB in cerebrospinal fluid, pleural fluid, peritoneal fluid, and pericardial fluid, so ADA measurements are recommended in these fluids. Similarly, the sensitivity and specificity of free IFN-γ measurements were ≥89% and ≥97%, respectively, for detecting TB in pleural fluid and peritoneal fluid, so free IFN-γ measurements are recommended in these fluids.

The recommendations are conditional because the low quality of evidence does not provide sufficient confidence in the estimated sensitivities and specificities for the committee to be certain that the balance of desirable to undesirable consequences favors testing and obtaining the specimens to test usually requires an invasive procedure and, therefore, the balance of benefits versus risks may vary substantially depending upon the clinical condition of the patient. Furthermore, the committee recognized that these tests often required the services of an off-site laboratory, and that standards were variable across laboratories and across published studies.

Question 16: Should AFB smear microscopy be performed on specimens collected from sites of suspected extrapulmonary TB?

Evidence

The diagnostic yield and sensitivity of AFB smear microscopy tend to be lower in extrapulmonary TB than pulmonary TB because the former is paucibacillary. Accuracy studies indicate that AFB smear microscopy has a sensitivity of 0–10%, 14%–39%, 10%–30%, <5%, and 0–42% in pleural fluid (Supplementary Table 10), pleural tissue (Supplementary Table 10), urine (Supplementary Table 11), cerebrospinal fluid (Supplementary Table 12), peritoneal fluid (Supplementary Table 13), and pericardial fluid (Supplementary Table 14), respectively [207–218], when final clinical diagnosis, consistent pathology/cytology, or microbiologic confirmation is used as the reference standard. In contrast, the specificity of AFB smear microscopy tends to be quite high as described for pulmonary TB (≥90%). This evidence provides very low confidence in the estimated test characteristics because many studies do not

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

report enrolling consecutive patients, most studies were small with few samples, and ranges are wide due to variable results from the individual studies.

Recommendation 16: We suggest that AFB smear microscopy be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB (*conditional recommendation, very low-quality evidence*). Remarks: A positive result can be used as evidence of extrapulmonary TB and guide decision making because false-positive results are unlikely. However, a negative result may not be used to exclude TB because false-negative results are exceedingly common.

Rationale

AFB smear microscopy provides the opportunity for early diagnosis and treatment. The estimated specificity of ≥90% for AFB smear in the diagnosis of extrapulmonary TB indicates that false-positive results occur only ≤10% of the time; thus, if a positive AFB smear result is obtained, it is reasonable to assume that infection is present and to act accordingly. In contrast, the estimated sensitivity of <50% for all specimen types indicates that false-negative results are more common that true-negative results and, thus, a negative AFB smear result should not be used to exclude extrapulmonary TB; additional diagnostic testing is indicated.

Even though a positive AFB smear result is infrequent, the committee judged the benefits of early diagnosis (early initiation of treatment, potential to reduce transmission) to outweigh the cost and burden of AFB smear microscopy. The recommendation is conditional because the very low quality of evidence does not provide sufficient confidence in the estimated sensitivities and specificities for the committee to be certain that the balance of desirable to undesirable consequences favors testing and obtaining the specimens to test usually requires an invasive procedure and, therefore, the balance of benefits versus risks may vary substantially depending upon the clinical condition of the patient.

Question 17: Should mycobacterial cultures be performed on specimens collected from sites of suspected extrapulmonary TB?

Evidence

Accuracy studies indicate that mycobacterial culture has a sensitivity of 23%–58%, 40%–58%, 80%–90%, 45%–70%, 45%–69%, and 50%–65% in pleural fluid (Supplementary Table 10), pleural tissue (Supplementary Table 10), urine (Supplementary Table 11), cerebrospinal fluid (Supplementary Table 12), peritoneal fluid (Supplementary Table 13), and pericardial fluid (Supplementary Table 14), respectively [207, 208, 211, 213–216, 219–223], when final clinical diagnosis, consistent pathology/cytology, or microbiologic confirmation is used as the reference standard. The specificity of mycobacterial culture tends to be comparatively higher than the sensitivity (>97%). This evidence provides low confidence in the estimated test characteristics because many studies do not report enrolling consecutive patients and most studies were small with few samples.

Recommendation 17: We recommend that mycobacterial cultures be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB (*strong recommendation, low-quality evidence*). Remarks: A positive result can be used as evidence of extrapulmonary TB and guide decision making because false-positive results are unlikely. However, a negative result may not be used to exclude TB because false-negative results are exceedingly common.

Rationale

The estimated specificity of >97% for mycobacterial cultures in the diagnosis of extrapulmonary TB indicates that false-positive results occur only <3% of the time; thus, a positive mycobacterial culture is a reliable indicator that infection is present. In contrast, the estimated sensitivity of mycobacterial culture is widely variable depending upon the specimen type. However, even the specimen that provides the highest sensitivity (urine has an 80%–90% sensitivity for the diagnosis of urinary TB) provides false-negative results 10%–20% of the time. Such frequent false-negative results suggest that mycobacterial cultures should not be used exclude extrapulmonary TB; additional diagnostic testing is indicated.

The committee judged the diagnostic yield and benefits of mycobacterial culture sufficient to outweigh the cost and burden. Importantly, positive mycobacterial cultures are the only way to obtain isolates for DST. Empiric treatment for presumed drug-resistant TB may lead to unnecessary toxicities and cost if the patient actually has drug-susceptible TB, whereas empiric treatment for drug-susceptible TB may lead to treatment failure, risk of dissemination, and even death if the patient actually has drug-resistant TB. Moreover, delayed diagnosis of drug resistance will prolong therapy and increase risk of default.

The recommendation is strong despite the low quality of evidence because the committee is certain that the balance of desirable to undesirable consequences favors mycobacterial culture. This certainty reflects the committee's recognition of the importance of obtaining mycobacterial isolates for DST compared with the costs and burdens of performing the cultures, and the belief that additional data would not alter the balance of desirable and undesirable consequences in the overwhelming majority of patients.

Question 18: Should NAAT be performed on specimens collected from sites of suspected extrapulmonary TB?

Evidence

Meta-analyses have been published for the use of NAAT in suspected pleural and meningeal tuberculosis [224, 225].

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

Most studies used final clinical diagnosis, consistent pathology/cytology, or microbiologic confirmation as the reference standard. Nucleic acid amplification performed on pleural fluid and cerebrospinal fluid has a sensitivity of 56% and 62%, respectively, indicating false-negative rates of 44% and 38%, respectively. In contrast, the specificity of NAAT is high for both pleural fluid and cerebrospinal fluid (98% for both), indicating that only about 2% of positive results are false-positives. Individual studies have been published describing the test characteristics of nucleic acid amplification on other body fluids and tissues. The studies showed considerable variability in the sensitivity and specificity based upon the disease site; generally speaking, the sensitivity was usually <90%, while the specificity was >95% (Supplementary Table 15) [191, 226]. This evidence provides very low confidence in the estimated test characteristics because many studies do not report enrolling consecutive patients, findings were inconsistent as exemplified by the wide ranges, and the studies were small with few samples.

Recommendation 18: We suggest that NAAT be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB (*conditional recommendation, very low-quality evidence*). Remarks: A positive NAAT result can be used as evidence of extrapulmonary TB and guide decision making because false-positive results are unlikely. However, a negative NAAT result may not be used to exclude TB because false-negative results are exceedingly common.

Rationale

NAAT cannot replace mycobacterial culture for diagnosis because it is not sensitive enough and it does not produce an isolate, which is needed for DST. However, NAAT is appropriate as an adjunct to mycobacterial culture because mycobacterial culture results require at least 1–2 weeks, but NAAT can be performed within hours, thereby offering the opportunity for early diagnosis and treatment. The committee felt that NAAT gives positive results frequently enough that the potential benefits outweigh the costs and burden of testing. Moreover, the committee felt that if the test results are applied correctly (ie, a positive NAAT result is considered adequate to confirm extrapulmonary TB, but a negative NAAT result is not used to exclude extrapulmonary TB), then the risks associated with false results are minimal.

The recommendation is conditional because the very low quality of evidence does not provide sufficient confidence in the estimated sensitivities and specificities for the committee to be certain that the balance of desirable to undesirable consequences favors testing and obtaining the specimens to test usually requires an invasive procedure and, therefore, the balance of benefits versus risks may vary substantially depending upon the clinical condition of the patient.

Cautions and Limitations

At this time there are no FDA-approved NAATs for use with extrapulmonary specimens.

Question 19: Should histological examination be performed on specimens collected from sites of suspected extrapulmonary TB?

Evidence

Accuracy studies indicate that histological examination has a sensitivity of 69%–97%, 86%–94%, 60%–70%, 79%–100%, and 73%–100% in pleural tissue (Supplementary Table 10), urologic tissue (Supplementary Table 11), endometrial curettage (Supplementary Table 11), peritoneal biopsy (Supplementary Table 13), and pericardial tissue (Supplementary Table 14), respectively, when final clinical diagnosis, consistent pathology/cytology, or microbiologic confirmation is used as the reference standard. The specificity of mycobacterial culture microscopy tends to be low because necrotizing and nonnecrotizing granulomas are seen in other infectious and noninfectious diseases. This evidence provides very low confidence in the estimated test characteristics because many studies do not report enrolling consecutive patients, the wide ranges of sensitivity are due to the variable results of individual studies, and the studies were small with few samples.

Recommendation 19: We suggest that histological examination be performed, rather than not performed, on specimens collected from sites of suspected extrapulmonary TB (*conditional recommendation, very low-quality evidence*). Remarks: Both positive and negative results should be interpreted in the context of the clinical scenario because neither false-positive nor false-negative results are rare.

Rationale

Tissue sampling with histological examination generally occurs after other types of diagnostic testing have failed to identify a definitive diagnosis. Thus, at this stage in the diagnostic process, the committee thought testing was worthwhile if sensitivity and specificity were both >50%, meaning that true results were more likely than false results. Histological examination surpassed these thresholds and, therefore, is recommended. However, the committee emphasizes the importance of interpreting the results within the clinical context, to lessen the impact of false results. The recommendation is conditional because the very low quality of evidence does not provide sufficient confidence in the estimated sensitivities and specificities for the committee to be certain that the balance of desirable to undesirable consequences favors testing and obtaining the specimens to test usually requires an invasive procedure and, therefore, the balance of benefits versus risks may vary substantially depending upon the clinical condition of the patient.

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

GENOTYPING OF *M. TUBERCULOSIS*

Over the past 2 decades, genotyping of TB strains has been shown to be a valuable tool in TB control. Molecular epidemiology has helped to identify unsuspected transmission, determine likely locations of transmission, measure the extent of transmission, and differentiate reactivation from newly acquired infection [227]. Often traditional contact investigations focus on persons in the household and workplace. Numerous reports describe TB cases linked through genotyping of *Mtb* isolates, when detection of transmission was initially missed by conventional contact investigation because the setting was nontraditional. This type of transmission occurs frequently among members of a "social network" that is centered around a specific activity, including illicit drug use, excess alcohol use, or gambling, or location such as a homeless shelter, adult entertainment club, or HIV residential care facility [228–231]. When genotyping detects previously unrecognized transmission of TB in a nonconventional setting, public health interventions to contain and subsequently end the outbreak can be redirected to focus on the social network or location associated with transmission.

Genotyping or DNA fingerprinting of *Mtb* can be used for determining the clonality of bacterial cultures. PCR-based, and sometimes Southern blotting, methods are used. The PCR-based methods are mycobacterial interspersed repetitive units (MIRU) and spacer oligonucleotide typing (spoligotyping) [232, 233]. A standardized protocol has been developed to permit comparison of genotypes from different laboratories [232].

Question 20: Should genotyping be performed on a culture isolate from culture-positive patients with TB?

Evidence

We identified no empirical evidence that estimated the frequency with which the availability of genotyped isolates changed public health practices or affected patient outcomes. Therefore, the recommendation is based upon the committee's collective clinical experience, which constitutes very low-quality evidence.

Recommendation 20: We recommend one culture isolate from each mycobacterial culture–positive patient be submitted to a regional genotyping laboratory for genotyping (*strong recommendation, very low-quality evidence*).

Rationale

Genotyping is useful in detecting false-positive results due to confirming laboratory cross-contamination [234, 235], investigating outbreaks of TB (both detecting unsuspected outbreaks and confirming suspected outbreaks) [236], evaluating contact investigations [237], and determining whether new episodes of TB are due to reinfection or reactivation [238]. In addition, genotyping is useful for elucidating sites and patterns of *Mtb* transmission within communities [237, 239]. This information is used by state and local tuberculosis control programs to focus interventions to interrupt further TB transmission. Genotyping is used to aid public health departments in the control of TB and poses no risk to individual patients.

Recently, whole-genome sequencing (WGS) has been applied to investigation of tuberculosis outbreaks [240]. This technique may add discriminatory power to strain identification, but the role of WGS in outbreak investigation is still being determined.

In response to nosocomial outbreaks and tuberculosis among HIV-infected patients, the CDC established a national universal tuberculosis genotyping system for the United States. The merger of modern molecular protocols for strain identification at the DNA level and conventional epidemiological methodologies has given birth to an enhanced collaborative strategy to impact tuberculosis control efforts. Regional TB genotyping laboratories can be contacted through the state public health laboratories or TB control programs.

The recommendation is strong because the committee felt certain that the public health benefits of genotyping far outweigh the modest costs and burdens of genotyping. Even though the evidence can provide very little confidence in the magnitude of the benefits, costs, and burdens used by the committee to make its decision, the differences seemed so overwhelming that the committee thought it extraordinarily unlikely that additional data would lead to a judgment that the costs and harms exceed the benefits.

RESEARCH NEEDS

As described by Abu-Raddad et al [241], improved detection of those with TB and improved identification of those at risk to progress once infected have the potential to substantially decrease the prevalence of TB and its associated mortality.

Tuberculosis

The ability to rapidly and accurately identify *Mtb* as well as drug resistance (eg, through NAAT, line probe, molecular beacon, and Xpert MTB/RIF assays) reflects substantial advances. While rapid tests for TB diagnosis still have a sensitivity of 70%–90%, they may fail to detect paucibacillary pulmonary TB. They also remain relatively expensive, making them difficult to implement in high-burden, low-resource settings. Ideally, what is needed is a simple, inexpensive, rapid (ie, hours) test that is highly accurate (>95% sensitivity and specificity). Rapid tests for detection of drug resistance are approaching the desired level of accuracy, at least for rifampin. However, these tests also are relatively expensive and need to be expanded to allow for detection of resistance to other TB medications. Such expansion is currently limited by gaps in knowledge of the molecular basis of resistance to most first- and second-line drugs. In this regard, improved functional tests for resistance may prove useful.

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

Other significant gaps remain in the diagnosis of pediatric and extrapulmonary TB. First, the yield of AFB smear and culture in children is low compared to that in adults, which leads to excessive morbidity and mortality due to delayed and missed diagnoses, especially in resource-limited settings. Conversely, the inability to exclude TB results in overtreatment when the diagnosis cannot be excluded. In areas of the world where TB is diagnosed entirely based on smear microscopy, children will be almost completely neglected and untreated for TB. In areas with greater resources, low yields of microbiologic specimens in children deter many clinicians from even attempting culture collection. This may result in prolonged treatment with extra TB drugs (in jurisdictions that use 4 drugs for 6 months in patients lacking susceptibility data). Alternatively, drug resistance will not be identified and the child could suffer dire consequences receiving inadequate care. Second, similar challenges exist for the accurate diagnosis of those with extrapulmonary TB. Finally, diagnostic approaches to the identification of those likely to fail TB treatment are needed. These limitations in the diagnosis of paucibacillary TB highlight the need to develop testing strategies based on either host or bacterial markers of infection that can be measured from readily available clinical sources such as plasma or urine.

Latent Tuberculosis Infection

Individuals with immunological evidence of exposure to *Mtb* antigens, but without evidence of clinical disease are termed "latently" infected. However, it is clear that there is considerable heterogeneity within this classification. As was described previously, those with recent infection (<2 years) are at increased risk for progression to clinical disease, and functionally might be considered acutely infected. Conversely, those more remotely infected are at substantially lower risk, but remote infection remains difficult to define operationally. Those with negative TSTs or IGRAs are unlikely to progress to TB. However, the PPV of either test is relatively modest, as current estimates would suggest that among household contacts, 20–40 people require treatment to prevent one case. While the number needed to treat based on IGRAs is not known with certainty, early evidence suggests that it is not likely to be dramatically different [24, 55]. Nonetheless, a careful evaluation of which diagnostic is more closely associated with the development of TB remains a research priority. Given the relatively poor PPVs of current diagnostics for the prediction of progression to TB disease, a diagnostic that can accurately identify those at risk is needed. It should be noted that both QFT and T-SPOT are largely measures of CD4 T-cell immunity, but additional markers of inflammation, cellular, or humoral immunity may prove useful. Clearly, the identification of biomarkers associated with the development of tuberculosis following infection will require carefully performed prospective investigation. In this regard, the prospective evaluation of populations at risk for disease progression, and the use of sophisticated imaging such as positron emission tomography–computed tomography (PET-CT) are likely to further delineate markers associated with either disease progression or subclinical infection.

Operationally, the intent of targeted testing is to identify those who would benefit from treatment. While much is now known about the accuracy of both the TST and IGRAs, much less is known about their performance with regard to treatment completion. Additional research on the use of IGRAs with regard to provider and patient perceptions is needed to establish optimal diagnostic and treatment strategies. Finally, the literature addressing the performance of IGRAs in children <5 years old is still limited and studies to inform the appropriate use of these tests to accurately diagnose LTBI in this age group are needed. Studies of young household contacts in low-incidence countries would be especially informative.

GUIDELINE STATEMENT

These guidelines are not intended to impose a standard of care. They provide the basis for rational decisions in the diagnostic evaluation of patients with possible latent tuberculosis or tuberculosis. Clinicians, patients, third-party payers, stakeholders, or the courts should never view the recommendations contained in these guidelines as dictates. Guidelines cannot take into account all of the often compelling unique individual clinical circumstances. Therefore, no one charged with evaluating clinicians' actions should attempt to apply the recommendations from these guidelines by rote or in a blanket fashion. Qualifying remarks accompanying each recommendation are its integral parts and serve to facilitate more accurate interpretation. They should never be omitted when quoting or translating recommendations from these guidelines.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

Acknowledgments. The writing committee thanks Drs Mike Iseman and Jeffrey Starke for their critical examination of the manuscript. The committee is particularly indebted to Kevin Wilson for his patience and his editing skills.

Potential conflicts of interest. D. L. C. has received speaking fees from Qiagen. C. L. D. serves on the data and safety monitoring board (DSMB) for Otsuka America Pharmaceutical, Inc, served on a DSMB for Sanofi Pasteur Inc, received research support from Insmed, and received travel support from Qiagen. L. R. serves as a speaker and on an advisory committee for Boehringer Ingelheim and F. Hoffmann-La Roche, serves on an advisory committee and received research support from Biogen Inc, served on an advisory committee for AstraZeneca, GlaxoSmithKline, and Sanofi Pasteur, served as a consultant for Bayer HealthCare, served as a speaker for Cipla. T.M.S. is employed by the US Centers for Disease Control and Prevention (CDC). T. R. S. serves on a DSMB for Otsuka. P. A. L. is employed by the CDC. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Schünemann HJ, Osborne M, Moss J, et al. ATS Ethics and Conflict of Interest Committee and the Documents Development and Implementation Committee. An official American Thoracic Society Policy statement: managing conflict of interest in professional societies. Am J Respir Crit Care Med **2009**; 180:564–80.

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

2. Schunemann HJ, Oxman AD, Brozek J, et al. Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. BMJ **2008**; 336:1106–10.
3. Schünemann HJ, Jaeschke R, Cook DJ, et al. ATS Documents Development and Implementation Committee. An official ATS statement: grading the quality of evidence and strength of recommendations in ATS guidelines and recommendations. Am J Respir Crit Care Med **2006**; 174:605–14.
4. World Health Organization. Global tuberculosis report 2015. Geneva, Switzerland: WHO, **2015**.
5. Edlin BR, Tokars JI, Grieco MH, et al. An outbreak of multidrug-resistant tuberculosis among hospitalized patients with the acquired immunodeficiency syndrome. N Engl J Med **1992**; 326:1514–21.
6. Gandhi NR, Moll A, Sturm AW, et al. 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 **2006**; 368:1575–80.
7. Scott C, Kirking HL, Jeffries C, et al. Tuberculosis trends—United States, 2014. MMWR Morb Mortal Wkly Rep **2015**; 64: 265–9.
8. Bennett DE, Courval JM, Onorato I, et al. Prevalence of tuberculosis infection in the United States population: the national health and nutrition examination survey, 1999–2000. Am J Respir Crit Care Med **2008**; 177:348–55.
9. US Institute of Medicine; Committee on the Elimination of Tuberculosis in the United States, Geiter L, ebrary Inc. Ending neglect the elimination of tuberculosis in the United States. Washington, DC: National Academy Press, **2000**:xvi, 269 p.
10. Reichman LB, Hershfield ES; ebrary Inc. Tuberculosis: a comprehensive international approach. Lung biology in health and disease v 144. 2nd ed. New York: Dekker, **2000**:xxviii, 898 p.
11. Targeted tuberculin testing and treatment of latent tuberculosis infection. American Thoracic Society. MMWR Recomm Rep **2000**; 49:1–51.
12. Reichler MR, Reves R, Bur S, et al. Evaluation of investigations conducted to detect and prevent transmission of tuberculosis. JAMA **2002**; 287:991–5.
13. Catanzaro A. Nosocomial tuberculosis. Am Rev Respir Dis **1982**; 125:559–62.
14. Schluger NW, Rom WN. The host immune response to tuberculosis. Am J Respir Crit Care Med **1998**; 157:679–91.
15. Balasubramanian V, Wiegeshaus EH, Taylor BT, Smith DW. Pathogenesis of tuberculosis: pathway to apical localization. Tuber Lung Dis **1994**; 75:168–78.
16. Dannenberg AM Jr. Immune mechanisms in the pathogenesis of pulmonary tuberculosis. Rev Infect Dis **1989**; 11 Suppl 2:S369–78.
17. Horsburgh CR Jr. Priorities for the treatment of latent tuberculosis infection in the United States. N Engl J Med **2004**; 350:2060–7.
18. Selwyn PA, Hartel D, Lewis VA, et al. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. N Engl J Med **1989**; 320:545–50.
19. Markowitz N, Hansen NI, Wilcosky TC, et al. Tuberculin and anergy testing in HIV-seropositive and HIV-seronegative persons. Pulmonary Complications of HIV Infection Study Group. Ann Intern Med **1993**; 119:185–93.
20. Marks SM, Taylor Z, Qualls NL, Shrestha-Kuwahara RJ, Wilce MA, Nguyen CH. Outcomes of contact investigations of infectious tuberculosis patients. Am J Respir Crit Care Med **2000**; 162:2033–8.
21. Reichler MR, Reves R, Bur S, et al. Contact Investigation Study Group. Treatment of latent tuberculosis infection in contacts of new tuberculosis cases in the United States. South Med J **2002**; 95:414–20.
22. Hirsch-Moverman Y, Daftary A, Franks J, Colson PW. Adherence to treatment for latent tuberculosis infection: systematic review of studies in the US and Canada. Int J Tuberc Lung Dis **2008**; 12:1235–54.
23. Huebner RE, Schein MF, Bass JB Jr. The tuberculin skin test. Clin Infect Dis **1993**; 17:968–75.
24. Pai M, Denkinger CM, Kik SV, et al. Gamma interferon release assays for detection of *Mycobacterium tuberculosis* infection. Clin Microbiol Rev **2014**; 27:3–20.
25. Kendig EL Jr, Kirkpatrick BV, Carter WH, Hill FA, Caldwell K, Entwistle M. Underreading of the tuberculin skin test reaction. Chest **1998**; 113:1175–7.
26. Centers for Disease Control and Prevention. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in health-care settings, 2005. MMWR Recomm Rep **2005**; 54:1–141.
27. Black CA. Delayed type hypersensitivity: current theories with an historic perspective. Dermatol Online J **1999**; 5:7.
28. Cole ST, Brosch R, Parkhill J, et al. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature **1998**; 393:537–44.
29. Andersen P, Andersen AB, Sørensen AL, Nagai S. Recall of long-lived immunity to *Mycobacterium tuberculosis* infection in mice. J Immunol **1995**; 154:3359–72.
30. Mustafa AS, Amoudy HA, Wiker HG, et al. Comparison of antigen-specific T-cell responses of tuberculosis patients using complex or single antigens of *Mycobacterium tuberculosis*. Scand J Immunol **1998**; 48:535–43.
31. Mustafa AS, Oftung F, Amoudy HA, et al. Multiple epitopes from the *Mycobacterium tuberculosis* ESAT-6 antigen are recognized by antigen-specific human T cell lines. Clin Infect Dis **2000**; 30(suppl 3):S201–5.
32. Harboe M, Oettinger T, Wiker HG, Rosenkrands I, Andersen P. Evidence for occurrence of the ESAT-6 protein in *Mycobacterium tuberculosis* and virulent *Mycobacterium bovis* and for its absence in *Mycobacterium bovis* BCG. Infect Immun **1996**; 64:16–22.
33. Mahairas GG, Sabo PJ, Hickey MJ, Singh DC, Stover CK. Molecular analysis of genetic differences between *Mycobacterium bovis* BCG and virulent *M. bovis*. J Bacteriol **1996**; 178:1274–82.
34. Berthet FX, Rasmussen PB, Rosenkrands I, Andersen P, Gicquel B. A *Mycobacterium tuberculosis* operon encoding ESAT-6 and a novel low-molecular-mass culture filtrate protein (CFP-10). Microbiology **1998**; 144 (pt 11):3195–203.
35. Bothamley GH, Rudd RM. Clinical evaluation of a serological assay using a monoclonal antibody (TB72) to the 38 kDa antigen of *Mycobacterium tuberculosis*. Eur Respir J **1994**; 7:240–6.
36. Colangeli R, Spencer JS, Bifani P, et al. MTSA-10, the product of the Rv3874 gene of *Mycobacterium tuberculosis*, elicits tuberculosis-specific, delayed-type hypersensitivity in guinea pigs. Infect Immun **2000**; 68:990–3.
37. Dillon DC, Alderson MR, Day CH, et al. Molecular and immunological characterization of *Mycobacterium tuberculosis* CFP-10, an immunodiagnostic antigen missing in *Mycobacterium bovis* BCG. J Clin Microbiol **2000**; 38:3285–90.
38. Arend SM, de Haas P, Leyten E, et al. ESAT-6 and CFP-10 in clinical versus environmental isolates of *Mycobacterium kansasii*. J Infect Dis **2005**; 191:1301–10.
39. Geluk A, van Meijgaarden KE, Franken KL, et al. Identification and characterization of the ESAT-6 homologue of *Mycobacterium leprae* and T-cell cross-reactivity with *Mycobacterium tuberculosis*. Infect Immun **2002**; 70:2544–8.
40. Geluk A, van Meijgaarden KE, Franken KL, et al. Immunological crossreactivity of the *Mycobacterium leprae* CFP-10 with its homologue in *Mycobacterium tuberculosis*. Scand J Immunol **2004**; 59:66–70.
41. Millington KA, Gooding S, Hinks TS, Reynolds DJ, Lalvani A. *Mycobacterium tuberculosis*-specific cellular immune profiles suggest bacillary persistence decades after spontaneous cure in untreated tuberculosis. J Infect Dis **2010**; 202:1685–9.
42. Hinks TS, Dosanjh DP, Innes JA, et al. Frequencies of region of difference 1 antigen-specific but not purified protein derivative-specific gamma interferon-secreting T cells correlate with the presence of tuberculosis disease but do not distinguish recent from remote latent infections. Infect Immun **2009**; 77:5486–95.
43. Kik SV, Franken WP, Arend SM, et al. Interferon-gamma release assays in immigrant contacts and effect of remote exposure to *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis **2009**; 13:820–8.
44. Lalvani A, Nagvenkar P, Udwadia Z, et al. Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent *Mycobacterium tuberculosis* infection in healthy urban Indians. J Infect Dis **2001**; 183:469–77.
45. T-SPOT.TB: an aid in the diagosis of tuberculosis infection; package insert for in vitro diagnositic use. Abingdon, UK: Oxford Immunotec Ltd.
46. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This Official Statement of the American Thoracic Society and the Centers for Disease Control and Prevention was Adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Diseases Society of America, September 1999. Am J Respir Crit Care Med **2000**; 161:1376–95.
47. Ferrara G, Losi M, D'Amico R, et al. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with *Mycobacterium tuberculosis*: a prospective study. Lancet **2006**; 367:1328–34.
48. Ferrara G, Losi M, Meacci M, et al. Routine hospital use of a new commercial whole blood interferon-gamma assay for the diagnosis of tuberculosis infection. Am J Respir Crit Care Med **2005**; 172:631–5.
49. Lee JY, Choi HJ, Park IN, et al. Comparison of two commercial interferon-gamma assays for diagnosing *Mycobacterium tuberculosis* infection. Eur Respir J **2006**; 28:24–30.
50. van Zyl-Smit RN, Zwerling A, Dheda K, Pai M. Within-subject variability of interferon-g assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review. PLoS One **2009**; 4:e8517.
51. van Zyl-Smit RN, Pai M, Peprah K, et al. Within-subject variability and boosting of T-cell interferon-gamma responses after tuberculin skin testing. Am J Respir Crit Care Med **2009**; 180:49–58.
52. Metcalfe JZ, Cattamanchi A, McCulloch CE, Lew JD, Ha NP, Graviss EA. Test variability of the QuantiFERON-TB gold in-tube assay in clinical practice. Am J Respir Crit Care Med **2013**; 187:206–11.
53. Richeldi L. An update on the diagnosis of tuberculosis infection. Am J Respir Crit Care Med **2006**; 174:736–42.
54. Leyten EM, Prins C, Bossink AW, et al. Effect of tuberculin skin testing on a *Mycobacterium tuberculosis*-specific IFN-{gamma} assay. Eur Respir J **2007**; 29:1212–6.
55. Zellweger JP, Sotgiu G, Block M, et al. TBNET. Risk assessment of tuberculosis in contacts by IFN-γ release assays. A Tuberculosis Network European Trials Group Study. Am J Respir Crit Care Med **2015**; 191:1176–84.

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

56. Connell TG, Curtis N, Ranganathan SC, Buttery JP. Performance of a whole blood interferon gamma assay for detecting latent infection with *Mycobacterium tuberculosis* in children. Thorax **2006**; 61:616–20.
57. Richeldi L, Bergamini BM, Vaienti F. Prior tuberculin skin testing does not boost QuantiFERON-TB results in paediatric contacts. Eur Respir J **2008**; 32:524–5.
58. Bergamini BM, Losi M, Vaienti F, et al. Performance of commercial blood tests for the diagnosis of latent tuberculosis infection in children and adolescents. Pediatrics **2009**; 123:e419–24.
59. Kampmann B, Whittaker E, Williams A, et al. Interferon-gamma release assays do not identify more children with active tuberculosis than the tuberculin skin test. Eur Respir J **2009**; 33:1374–82.
60. Lighter J, Rigaud M, Eduardo R, Peng CH, Pollack H. Latent tuberculosis diagnosis in children by using the QuantiFERON-TB Gold In-Tube test. Pediatrics **2009**; 123:30–7.
61. Talati NJ, Seybold U, Humphrey B, et al. Poor concordance between interferon-gamma release assays and tuberculin skin tests in diagnosis of latent tuberculosis infection among HIV-infected individuals. BMC Infect Dis **2009**; 9:15.
62. Efficacy of various durations of isoniazid preventive therapy for tuberculosis: five years of follow-up in the IUAT trial. International Union Against Tuberculosis Committee on Prophylaxis. Bull World Health Organ **1982**; 60:555–64.
63. Ferebee SH, Mount FW. Tuberculosis morbidity in a controlled trial of the prophylactic use of isoniazid among household contacts. Am Rev Respir Dis **1962**; 85:490–510.
64. Comstock GW, Ferebee SH, Hammes LM. A controlled trial of community-wide isoniazid prophylaxis in Alaska. Am Rev Respir Dis **1967**; 95:935–43.
65. Ferebee SH, Mount FW, Murray FJ, Livesay VT. A controlled trial of isoniazid prophylaxis in mental institutions. Am Rev Respir Dis **1963**; 88:161–75.
66. Pape JW, Jean SS, Ho JL, Hafner A, Johnson WD Jr. Effect of isoniazid prophylaxis on incidence of active tuberculosis and progression of HIV infection. Lancet **1993**; 342:268–72.
67. Whalen CC, Johnson JL, Okwera A, et al. A trial of three regimens to prevent tuberculosis in Ugandan adults infected with the human immunodeficiency virus. Uganda-Case Western Reserve University Research Collaboration. N Engl J Med **1997**; 337:801–8.
68. Wilkinson D, Squire SB, Garner P. Effect of preventive treatment for tuberculosis in adults infected with HIV: systematic review of randomised placebo controlled trials. BMJ **1998**; 317:625–9.
69. Martinson NA, Barnes GL, Moulton LH, et al. New regimens to prevent tuberculosis in adults with HIV infection. N Engl J Med **2011**; 365:11–20.
70. Carmona L, Gomez-Reino JJ, Rodriguez-Valverde V, et al. Effectiveness of recommendations to prevent reactivation of latent tuberculosis infection in patients treated with tumor necrosis factor antagonists. Arthritis Rheum **2005**; 52:1766–72.
71. Perez J, Kupper H, Spencer-Green G. Impact of screening for latent TB before initiating anti-TNF therapy in North America and Europe. Ann Rheum Dis **2005**; 64:265.
72. Bartu V, Havelkova M, Kopecka E. QuantiFERON-TB Gold in the diagnosis of active tuberculosis. J Int Med Res **2008**; 36:434–7.
73. Bosshard V, Roux-Lombard P, Perneger T, et al. Do results of the T-SPOT.TB interferon-gamma release assay change after treatment of tuberculosis? Respir Med **2009**; 103:30–4.
74. Chee CB, Gan SH, Khinmar KW, et al. Comparison of sensitivities of two commercial gamma interferon release assays for pulmonary tuberculosis. J Clin Microbiol **2008**; 46:1935–40.
75. Chou CH, Hsu HL, Lee LN, Hsueh PR, Luh KT. Comparison of 2 interferon-gamma assays and Roche Cobas Amplicor *Mycobacterium tuberculosis* assay for rapid diagnosis of tuberculosis among patients with suspected tuberculosis in Taiwan. J Microbiol Immunol Infect **2009**; 42:251–7.
76. Domínguez J, Ruiz-Manzano J, De Souza-Galvão M, et al. Comparison of two commercially available gamma interferon blood tests for immunodiagnosis of tuberculosis. Clin Vaccine Immunol **2008**; 15:168–71.
77. Dosanjh DP, Hinks TS, Innes JA, et al. Improved diagnostic evaluation of suspected tuberculosis. Ann Intern Med **2008**; 148:325–36.
78. US Food and Drug Administration. Medical devices: T-SPOT-TB—P070006. Silver Spring, MD: FDA, **2008**.
79. Gerogianni I, Papala M, Klapsa D, Zinzaras E, Petinaki E, Gourgoulianis KI. Whole-blood interferon-gamma assay for the diagnosis of tuberculosis infection in an unselected Greek population. Respirology **2008**; 13:270–4.
80. Goletti D, Carrara S, Butera O, et al. Accuracy of immunodiagnostic tests for active tuberculosis using single and combined results: a multicenter TBNET-Study. PLoS One **2008**; 3:e3417.
81. Harada N, Higuchi K, Yoshiyama T, et al. Comparison of the sensitivity and specificity of two whole blood interferon-gamma assays for *M. tuberculosis* infection. J Infect **2008**; 56:348–53.
82. Higuchi K, Kawabe Y, Mitarai S, Yoshiyama T, Harada N, Mori T. Comparison of performance in two diagnostic methods for tuberculosis infection. Med Microbiol Immunol **2009**; 198:33–7.
83. Jafari C, Ernst M, Diel R, et al. Rapid diagnosis of smear-negative tuberculosis by bronchoalveolar lavage enzyme-linked immunospot. Am J Respir Crit Care Med **2006**; 174:1048–54.
84. Janssens JP. Interferon-gamma release assay tests to rule out active tuberculosis. Eur Respir J **2007**; 30:183–4; author reply 184–5.
85. Kang YA, Lee HW, Hwang SS, et al. Usefulness of whole-blood interferon-gamma assay and interferon-gamma enzyme-linked immunospot assay in the diagnosis of active pulmonary tuberculosis. Chest **2007**; 132:959–65.
86. Latorre I, De Souza-Galvão M, Ruiz-Manzano J, et al. Quantitative evaluation of T-cell response after specific antigen stimulation in active and latent tuberculosis infection in adults and children. Diagn Microbiol Infect Dis **2009**; 65:236–46.
87. Leung WL, Law KL, Leung VS, et al. Comparison of intracellular cytokine flow cytometry and an enzyme immunoassay for evaluation of cellular immune response to active tuberculosis. Clin Vaccine Immunol **2009**; 16:344–51.
88. Liao CH, Lai CC, Tan CK, et al. False-negative results by enzyme-linked immunospot assay for interferon-gamma among patients with culture-confirmed tuberculosis. J Infect **2009**; 59:421–3.
89. Meier T, Eulenbruch HP, Wrighton-Smith P, Enders G, Regnath T. Sensitivity of a new commercial enzyme-linked immunospot assay (T SPOT-TB) for diagnosis of tuberculosis in clinical practice. Eur J Clin Microbiol Infect Dis **2005**; 24:529–36.
90. Palazzo R, Spensieri F, Massari M, et al. Use of whole-blood samples in in-house bulk and single-cell antigen-specific gamma interferon assays for surveillance of *Mycobacterium tuberculosis* infections. Clin Vaccine Immunol **2008**; 15:327–37.
91. Park SY, Jeon K, Um SW, Kwon OJ, Kang ES, Koh WJ. Clinical utility of the QuantiFERON-TB Gold In-Tube test for the diagnosis of active pulmonary tuberculosis. Scand J Infect Dis **2009**; 41:818–22.
92. Ruhwald M, Bodmer T, Maier C, et al. TBNET. Evaluating the potential of IP-10 and MCP-2 as biomarkers for the diagnosis of tuberculosis. Eur Respir J **2008**; 32:1607–15.
93. Tan CK, Lai CC, Liao CH, et al. Rapid diagnosis of active pulmonary tuberculosis in the elderly using enzyme-linked immunospot assay for interferon-gamma. J Am Geriatr Soc **2009**; 57:2361–2.
94. Wang JY, Chou CH, Lee LN, et al. Diagnosis of tuberculosis by an enzyme-linked immunospot assay for interferon-gamma. Emerg Infect Dis **2007**; 13:553–8.
95. Bienek DR, Chang CK. Evaluation of an interferon-gamma release assay, T-SPOT.TB, in a population with a low prevalence of tuberculosis. Int J Tuberc Lung Dis **2009**; 13:1416–21.
96. LoBue PA, Moser KS. Use of isoniazid for latent tuberculosis infection in a public health clinic. Am J Respir Crit Care Med **2003**; 168:443–7.
97. Nolan CM, Goldberg SV, Buskin SE. Hepatotoxicity associated with isoniazid preventive therapy: a 7-year survey from a public health tuberculosis clinic. JAMA **1999**; 281:1014–8.
98. Yee D, Valiquette C, Pelletier M, Parisien I, Rocher I, Menzies D. Incidence of serious side effects from first-line antituberculosis drugs among patients treated for active tuberculosis. Am J Respir Crit Care Med **2003**; 167:1472–7.
99. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. Am J Epidemiol **1974**; 99:131–8.
100. Marais BJ, Gie RP, Schaaf HS, et al. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. Int J Tuberc Lung Dis **2004**; 8:392–402.
101. Connell T, Bar-Zeev N, Curtis N. Early detection of perinatal tuberculosis using a whole blood interferon-gamma release assay. Clin Infect Dis **2006**; 42:e82–5.
102. Detjen AK, Keil T, Roll S, et al. Interferon-gamma release assays improve the diagnosis of tuberculosis and nontuberculous mycobacterial disease in children in a country with a low incidence of tuberculosis. Clin Infect Dis **2007**; 45:322–8.
103. Dogra S, Narang P, Mendiratta DK, et al. Comparison of a whole blood interferon-gamma assay with tuberculin skin testing for the detection of tuberculosis infection in hospitalized children in rural India. J Infect **2007**; 54:267–76.
104. Connell TG, Ritz N, Paxton GA, Buttery JP, Curtis N, Ranganathan SC. A three-way comparison of tuberculin skin testing, QuantiFERON-TB gold and T-SPOT.TB in children. PLoS One **2008**; 3:e2624.
105. Nicol MP, Davies MA, Wood K, et al. Comparison of T-SPOT.TB assay and tuberculin skin test for the evaluation of young children at high risk for tuberculosis in a community setting. Pediatrics **2009**; 123:38–43.
106. Hesseling AC, Mandalakas AM, Kirchner HL, et al. Highly discordant T cell responses in individuals with recent exposure to household tuberculosis. Thorax **2009**; 64:840–6.
107. Nakaoka H, Lawson L, Squire SB, et al. Risk for tuberculosis among children. Emerg Infect Dis **2006**; 12:1383–8.

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

108. Higuchi K, Harada N, Mori T, Sekiya Y. Use of QuantiFERON-TB Gold to investigate tuberculosis contacts in a high school. Respirology **2007**; 12:88–92.
109. Chee CB, Soh CH, Boudville IC, Chor SS, Wang YT. Interpretation of the tuberculin skin test in *Mycobacterium bovis* BCG-vaccinated Singaporean schoolchildren. Am J Respir Crit Care Med **2001**; 164:958–61.
110. Radhakrishna S, Frieden TR, Subramani R; Tuberculosis Research Centre (ICMR). Association of initial tuberculin sensitivity, age and sex with the incidence of tuberculosis in south India: a 15-year follow-up. Int J Tuberc Lung Dis **2003**; 7:1083–91.
111. Mancuso JD, Tribble D, Mazurek GH, et al. Impact of targeted testing for latent tuberculosis infection using commercially available diagnostics. Clin Infect Dis **2011**; 53:234–44.
112. Clark SA, Martin SL, Pozniak A, et al. Tuberculosis antigen-specific immune responses can be detected using enzyme-linked immunospot technology in human immunodeficiency virus (HIV)-1 patients with advanced disease. Clin Exp Immunol **2007**; 150:238–44.
113. Sauzullo I, Mengoni F, Lichtner M, et al. QuantiFERON-TB Gold and selected region of difference 1 peptide-based assays for the detection of *Mycobacterium tuberculosis* infection in a cohort of patients enrolled with suspected tuberculosis. Diagn Microbiol Infect Dis **2008**; 62:395–401.
114. Vincenti D, Carrara S, Butera O, et al. Response to region of difference 1 (RD1) epitopes in human immunodeficiency virus (HIV)-infected individuals enrolled with suspected active tuberculosis: a pilot study. Clin Exp Immunol **2007**; 150:91–8.
115. Cattamanchi A, Smith R, Steingart KR, et al. Interferon-gamma release assays for the diagnosis of latent tuberculosis infection in HIV-infected individuals: a systematic review and meta-analysis. J Acquir Immune Defic Syndr **2011**; 56:230–8.
116. Dorman SE, Belknap R, Graviss EA, et al. Tuberculosis Epidemiologic Studies Consortium. Interferon-γ release assays and tuberculin skin testing for diagnosis of latent tuberculosis infection in healthcare workers in the United States. Am J Respir Crit Care Med **2014**; 189:77–87.
117. Lobue P, Menzies D. Treatment of latent tuberculosis infection: an update. Respirology **2010**; 15:603–22.
118. Pai M, Joshi R, Dogra S, et al. Serial testing of health care workers for tuberculosis using interferon-gamma assay. Am J Respir Crit Care Med **2006**; 174:349–55.
119. Daley CL, Reves RR, Beard MA, et al. A summary of meeting proceedings on addressing variability around the cut point in serial interferon-gamma release assay testing. Infect Control Hosp Epidemiol **2013**; 34:625–30.
120. Okada K, Mao TE, Mori T, et al. Performance of an interferon-gamma release assay for diagnosing latent tuberculosis infection in children. Epidemiol Infect **2007**:1–9.
121. Liebeschuetz S, Bamber S, Ewer K, Deeks J, Pathan AA, Lalvani A. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. Lancet **2004**; 364:2196–203.
122. Sun L, Xiao J, Miao Q, et al. Interferon gamma release assay in diagnosis of pediatric tuberculosis: a meta-analysis. FEMS Immunol Med Microbiol **2011**; 63:165–73.
123. Tsiouris SJ, Austin J, Toro P, et al. Results of a tuberculosis-specific IFN-gamma assay in children at high risk for tuberculosis infection. Int J Tuberc Lung Dis **2006**; 10:939–41.
124. Taylor Z, Nolan CM, Blumberg HM; American Thoracic Society; Centers for Disease Control and Prevention; Infectious Diseases Society of America. Controlling tuberculosis in the United States. Recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society of America. MMWR Recomm Rep **2005**; 54:1–81.
125. Greenaway C, Menzies D, Fanning A, Grewal R, Yuan L, FitzGerald JM; Canadian Collaborative Group in Nosocomial Transmission of Tuberculosis. Delay in diagnosis among hospitalized patients with active tuberculosis–predictors and outcomes. Am J Respir Crit Care Med **2002**; 165:927–33.
126. Golub JE, Bur S, Cronin WA, et al. Delayed tuberculosis diagnosis and tuberculosis transmission. Int J Tuberc Lung Dis **2006**; 10:24–30.
127. Mase SR, Ramsay A, Ng V, et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. Int J Tuberc Lung Dis **2007**; 11:485–95.
128. Steingart KR, Ng V, Henry M, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis **2006**; 6:664–74.
129. Steingart KR, Henry M, Ng V, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis **2006**; 6:570–81.
130. Gordin F, Slutkin G. The validity of acid-fast smears in the diagnosis of pulmonary tuberculosis. Arch Pathol Lab Med **1990**; 114:1025–7.
131. Yajko DM, Nassos PS, Sanders CA, Madej JJ, Hadley WK. High predictive value of the acid-fast smear for *Mycobacterium tuberculosis* despite the high prevalence of *Mycobacterium avium* complex in respiratory specimens. Clin Infect Dis **1994**; 19:334–6.
132. Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, Mengoli C. Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycobacteria. J Clin Microbiol **2004**; 42:2321–5.
133. Forbes B, Banaiee N, Beavis K, et al. Laboratory detection and identification of mycobacteria; approved guideline. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI publication no. M48A), **2008**.
134. Woods G, Lin G, Desmond E. Susceptibility test methods: mycobacteria, *Nocardia*, and other actinomycetes. Washington, DC: ASM Press, **2011**.
135. Greco S, Girardi E, Navarra A, Saltini C. Current evidence on diagnostic accuracy of commercially based nucleic acid amplification tests for the diagnosis of pulmonary tuberculosis. Thorax **2006**; 61:783–90.
136. Ling DI, Flores LL, Riley LW, Pai M. Commercial nucleic-acid amplification tests for diagnosis of pulmonary tuberculosis in respiratory specimens: meta-analysis and meta-regression. PLoS One **2008**; 3:e1536.
137. Dinnes J, Deeks J, Kunst H, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technol Assess **2007**; 11:1–196.
138. Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. MMWR Morb Mortal Wkly Rep **2009**; 58:7–10.
139. Shingadia D, Novelli V. Diagnosis and treatment of tuberculosis in children. Lancet Infect Dis **2003**; 3:624–32.
140. Moore DF, Guzman JA, Mikhail LT. Reduction in turnaround time for laboratory diagnosis of pulmonary tuberculosis by routine use of a nucleic acid amplification test. Diagn Microbiol Infect Dis **2005**; 52:247–54.
141. Campos M, Quartin A, Mendes E, et al. Feasibility of shortening respiratory isolation with a single sputum nucleic acid amplification test. Am J Respir Crit Care Med **2008**; 178:300–5.
142. Sloutsky A, Han LL, Werner BG. Practical strategies for performance optimization of the enhanced Gen-Probe amplified *Mycobacterium tuberculosis* direct test. J Clin Microbiol **2004**; 42:1547–51.
143. Flores LL, Pai M, Colford JM Jr, Riley LW. In-house nucleic acid amplification tests for the detection of *Mycobacterium tuberculosis* in sputum specimens: meta-analysis and meta-regression. BMC Microbiol **2005**; 5:55.
144. Ling DI, Zwerling AA, Pai M. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. Eur Respir J **2008**; 32:1165–74.
145. Morgan M, Kalantri S, Flores L, Pai M. A commercial line probe assay for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: a systematic review and meta-analysis. BMC Infect Dis **2005**; 5:62.
146. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med **2010**; 363:1005–15.
147. Centers for Disease Control and Prevention. Availability of an assay for detecting *Mycobacterium tuberculosis*, including rifampin-resistant strains, and considerations for its use—United States, 2013. MMWR Morb Mortal Wkly Rep **2013**; 62:821–7.
148. Woods G, Brown-Elliott B, Conville P, et al. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard—2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI publication no. M24-A2), **2011**.
149. Francis J. Curry National Tuberculosis Center and California Department of Public Health. Drug-resistant tuberculosis: a survival guide for clinicians. 2nd ed. San Francisco, CA: Francis J. Curry National Tuberculosis Center, **2008**.
150. Chan ED, Laurel V, Strand MJ, et al. Treatment and outcome analysis of 205 patients with multidrug-resistant tuberculosis. Am J Respir Crit Care Med **2004**; 169:1103–9.
151. Narita M, Alonso P, Lauzardo M, Hollender ES, Pitchenik AE, Ashkin D. Treatment experience of multidrug-resistant tuberculosis in Florida, 1994–1997. Chest **2001**; 120:343–8.
152. Lew W, Pai M, Oxlade O, Martin D, Menzies D. Initial drug resistance and tuberculosis treatment outcomes: systematic review and meta-analysis. Ann Intern Med **2008**; 149:123–34.
153. Traore H, Fissette K, Bastian I, Devleeschouwer M, Portaels F. Detection of rifampicin resistance in *Mycobacterium tuberculosis* isolates from diverse countries by a commercial line probe assay as an initial indicator of multidrug resistance. Int J Tuberc Lung Dis **2000**; 4:481–4.
154. Moore M, Onorato IM, McCray E, Castro KG. Trends in drug-resistant tuberculosis in the United States, 1993–1996. JAMA **1997**; 278:833–7.
155. LoBue PA, Moser KS. Isoniazid- and rifampin-resistant tuberculosis in San Diego County, California, United States, 1993–2002. Int J Tuberc Lung Dis **2005**; 9:501–6.
156. Clark CM, Li J, Driver CR, Munsiff SS. Risk factors for drug-resistant tuberculosis among non-US-born persons in New York City. Int J Tuberc Lung Dis **2005**; 9:964–9.

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

157. Granich RM, Oh P, Lewis B, Porco TC, Flood J. Multidrug resistance among persons with tuberculosis in California, 1994–2003. JAMA **2005**; 293:2732–9.
158. Weiner M, Benator D, Burman W, et al. Tuberculosis Trials Consortium. Association between acquired rifamycin resistance and the pharmacokinetics of rifabutin and isoniazid among patients with HIV and tuberculosis. Clin Infect Dis **2005**; 40:1481–91.
159. Helb D, Jones M, Story E, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol **2010**; 48:229–37.
160. Zhu RY, Zhang KX, Zhao MQ, et al. Use of visual loop-mediated isotheral amplification of rimM sequence for rapid detection of *Mycobacterium tuberculosis* and *Mycobacterium bovis*. J Microbiol Methods **2009**; 78:339–43.
161. World Health Organization. Molecular line probe assays for rapid screening of patients at risk of multi-drug resistant tuberculosis (MDR-TB). Geneva, Switzerland: WHO, **2008**.
162. Marais BJ, Brittle W, Painczyk K, et al. Use of light-emitting diode fluorescence microscopy to detect acid-fast bacilli in sputum. Clin Infect Dis **2008**; 47:203–7.
163. Loeffler AM. Pediatric tuberculosis. Semin Respir Infect **2003**; 18:272–91.
164. Marais BJ, Gie RP, Schaaf HS, Beyers N, Donald PR, Starke JR. Childhood pulmonary tuberculosis: old wisdom and new challenges. Am J Respir Crit Care Med **2006**; 173:1078–90.
165. Berggren Palme I, Gudetta B, Bruchfeld J, Eriksson M, Giesecke J. Detection of *Mycobacterium tuberculosis* in gastric aspirate and sputum collected from Ethiopian HIV-positive and HIV-negative children in a mixed in- and outpatient setting. Acta Paediatr **2004**; 93:311–5.
166. Hatherill M, Hawkridge T, Zar HJ, et al. Induced sputum or gastric lavage for community-based diagnosis of childhood pulmonary tuberculosis? Arch Dis Child **2009**; 94:195–201.
167. Abadco DL, Steiner P. Gastric lavage is better than bronchoalveolar lavage for isolation of *Mycobacterium tuberculosis* in childhood pulmonary tuberculosis. Pediatr Infect Dis J **1992**; 11:735–8.
168. Steiner M, Zimmerman R, Park BH, Shirali SR, Schmidt H. Primary tuberculosis in children. 2. Correlation of susceptibility patterns of *M. tuberculosis* isolated from children with those isolated from source cases as an index of drug-resistant infection in a community. Am Rev Respir Dis **1968**; 98:201–9.
169. Steiner P, Rao M, Mitchell M, Steiner M. Primary drug-resistant tuberculosis in children. Correlation of drug-susceptibility patterns of matched patient and source case strains of *Mycobacterium tuberculosis*. Am J Dis Child **1985**; 139:780–2.
170. Sun SJ, Bennett DE, Flood J, Loeffler AM, Kammerer S, Ellis BA. Identifying the sources of tuberculosis in young children: a multistate investigation. Emerg Infect Dis **2002**; 8:1216–23.
171. Marquez L, Feske ML, Teeter LD, Musser JM, Graviss EA. Pediatric tuberculosis: the litmus test for tuberculosis control. Pediatr Infect Dis J **2012**; 31:1144–7.
172. Anderson C, Inhaber N, Menzies D. Comparison of sputum induction with fiber-optic bronchoscopy in the diagnosis of tuberculosis. Am J Respir Crit Care Med **1995**; 152(5 pt 1):1570–4.
173. Conde MB, Soares SL, Mello FC, et al. Comparison of sputum induction with fiberoptic bronchoscopy in the diagnosis of tuberculosis: experience at an acquired immune deficiency syndrome reference center in Rio de Janeiro, Brazil. Am J Respir Crit Care Med **2000**; 162:2238–40.
174. McWilliams T, Wells AU, Harrison AC, Lindstrom S, Cameron RJ, Foskin E. Induced sputum and bronchoscopy in the diagnosis of pulmonary tuberculosis. Thorax **2002**; 57:1010–4.
175. Saglam L, Akgun M, Aktas E. Usefulness of induced sputum and fibreoptic bronchoscopy specimens in the diagnosis of pulmonary tuberculosis. J Int Med Res **2005**; 33:260–5.
176. Brown M, Varia H, Bassett P, Davidson RN, Wall R, Pasvol G. Prospective study of sputum induction, gastric washing, and bronchoalveolar lavage for the diagnosis of pulmonary tuberculosis in patients who are unable to expectorate. Clin Infect Dis **2007**; 44:1415–20.
177. Al Zahrani K, Al Jahdali H, Poirier L, René P, Menzies D. Yield of smear, culture and amplification tests from repeated sputum induction for the diagnosis of pulmonary tuberculosis. Int J Tuberc Lung Dis **2001**; 5:855–60.
178. Charoenratanakul S, Dejsomritrutai W, Chaiprasert A. Diagnostic role of fiberoptic bronchoscopy in suspected smear negative pulmonary tuberculosis. Respir Med **1995**; 89:621–3.
179. Wongthim S, Udompanich V, Limthongkul S, Charoenlap P, Nuchprayoon C. Fiberoptic bronchoscopy in diagnosis of patients with suspected active pulmonary tuberculosis. J Med Assoc Thai **1989**; 72:154–9.
180. Chawla R, Pant K, Jaggi OP, Chandrashekhar S, Thukral SS. Fibreoptic bronchoscopy in smear-negative pulmonary tuberculosis. Eur Respir J **1988**; 1:804–6.
181. Willcox PA, Benatar SR, Potgieter PD. Use of the flexible fibreoptic bronchoscope in diagnosis of sputum-negative pulmonary tuberculosis. Thorax **1982**; 37:598–601.
182. Danek SJ, Bower JS. Diagnosis of pulmonary tuberculosis by flexible fiberoptic bronchoscopy. Am Rev Respir Dis **1979**; 119:677–9.
183. Miro AM, Gibilara E, Powell S, Kamholz SL. The role of fiberoptic bronchoscopy for diagnosis of pulmonary tuberculosis in patients at risk for AIDS. Chest **1992**; 101:1211–4.
184. Salzman SH, Schindel ML, Aranda CP, Smith RL, Lewis ML. The role of bronchoscopy in the diagnosis of pulmonary tuberculosis in patients at risk for HIV infection. Chest **1992**; 102:143–6.
185. Wallace JM, Deutsch AL, Harrell JH, Moser KM. Bronchoscopy and transbronchial biopsy in evaluation of patients with suspected active tuberculosis. Am J Med **1981**; 70:1189–94.
186. Kennedy DJ, Lewis WP, Barnes PF. Yield of bronchoscopy for the diagnosis of tuberculosis in patients with human immunodeficiency virus infection. Chest **1992**; 102:1040–4.
187. Sarkar SK, Sharma GS, Gupta PR, Sharma RK. Fiberoptic bronchoscopy in the diagnosis of pulmonary tuberculosis. Tubercle **1980**; 61:97–9.
188. Schoch OD, Rieder P, Tueller C, et al. Diagnostic yield of sputum, induced sputum, and bronchoscopy after radiologic tuberculosis screening. Am J Respir Crit Care Med **2007**; 175:80–6.
189. Willcox PA, Potgieter PD, Bateman ED, Benatar SR. Rapid diagnosis of sputum negative miliary tuberculosis using the flexible fibreoptic bronchoscope. Thorax **1986**; 41:681–4.
190. Pant K, Chawla R, Mann PS, Jaggi OP. Fiberbronchoscopy in smear-negative miliary tuberculosis. Chest **1989**; 95:1151–2.
191. Cheng VC, Yew WW, Yuen KY. Molecular diagnostics in tuberculosis. Eur J Clin Microbiol Infect Dis **2005**; 24:711–20.
192. Falk A. U.S. veterans administration-armed forces cooperative study on the chemotherapy of tuberculosis. 13. Tuberculous meningitis in adults, with special reference to survival, neurologic residuals, and work status. Am Rev Respir Dis **1965**; 91:823–31.
193. Maartens G, Willcox PA, Benatar SR. Miliary tuberculosis: rapid diagnosis, hematologic abnormalities, and outcome in 109 treated adults. Am J Med **1990**; 89:291–6.
194. Kim JH, Langston AA, Gallis HA. Miliary tuberculosis: epidemiology, clinical manifestations, diagnosis, and outcome. Rev Infect Dis **1990**; 12:583–90.
195. Xu HB, Jiang RH, Li L, Sha W, Xiao HP. Diagnostic value of adenosine deaminase in cerebrospinal fluid for tuberculous meningitis: a meta-analysis. Int J Tuberc Lung Dis **2010**; 14:1382–7.
196. Tuon FF, Higashino HR, Lopes MI, et al. Adenosine deaminase and tuberculous meningitis–a systematic review with meta-analysis. Scand J Infect Dis **2010**; 42:198–207.
197. Morisson P, Neves DD. Evaluation of adenosine deaminase in the diagnosis of pleural tuberculosis: a Brazilian meta-analysis. J Bras Pneumol **2008**; 34:217–24.
198. Liang QL, Shi HZ, Wang K, Qin SM, Qin XJ. Diagnostic accuracy of adenosine deaminase in tuberculous pleurisy: a meta-analysis. Respir Med **2008**; 102:744–54.
199. Greco S, Girardi E, Masciangelo R, Capoccetta GB, Saltini C. Adenosine deaminase and interferon gamma measurements for the diagnosis of tuberculous pleurisy: a meta-analysis. Int J Tuberc Lung Dis **2003**; 7:777–86.
200. Goto M, Noguchi Y, Koyama H, Hira K, Shimbo T, Fukui T. Diagnostic value of adenosine deaminase in tuberculous pleural effusion: a meta-analysis. Ann Clin Biochem **2003**; 40(pt 4):374–81.
201. Ena J, Valls V, Pérez de Oteyza C, Enríquez de Salamanca R. The usefulness and limitations of adenosine deaminase in the diagnosis of tubercular pleurisy. A meta-analytical study [in Spanish]. Med Clin (Barc) **1990**; 95:333–5.
202. Gui X, Xiao H. Diagnosis of tuberculosis pleurisy with adenosine deaminase (ADA): a systematic review and meta-analysis. Int J Clin Exp Med **2014**; 7:3126–35.
203. Tuon FF, Litvoc MN, Lopes MI. Adenosine deaminase and tuberculous pericarditis—a systematic review with meta-analysis. Acta Trop **2006**; 99:67–74.
204. Riquelme A, Calvo M, Salech F, et al. Value of adenosine deaminase (ADA) in ascitic fluid for the diagnosis of tuberculous peritonitis: a meta-analysis. J Clin Gastroenterol **2006**; 40:705–10.
205. Su SB, Qin SY, Guo XY, Luo W, Jiang HX. Assessment by meta-analysis of interferon-gamma for the diagnosis of tuberculous peritonitis. World J Gastroenterol **2013**; 19:1645–51.
206. Jiang J, Shi HZ, Liang QL, Qin SM, Qin XJ. Diagnostic value of interferon-gamma in tuberculous pleurisy: a metaanalysis. Chest **2007**; 131:1133–41.
207. Epstein DM, Kline LR, Albelda SM, Miller WT. Tuberculous pleural effusions. Chest **1987**; 91:106–9.
208. Chan CH, Arnold M, Chan CY, Mak TW, Hoheisel GB. Clinical and pathological features of tuberculous pleural effusion and its long-term consequences. Respiration **1991**; 58(3–4):171–5.

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016

209. Berenguer J, Moreno S, Laguna F, et al. Tuberculous meningitis in patients infected with the human immunodeficiency virus. N Engl J Med **1992**; 326:668–72.
210. Ogawa SK, Smith MA, Brennessel DJ, Lowy FD. Tuberculous meningitis in an urban medical center. Medicine (Baltimore) **1987**; 66:317–26.
211. Haas EJ, Madhavan T, Quinn EL, Cox F, Fisher E, Burch K. Tuberculous meningitis in an urban general hospital. Arch Intern Med **1977**; 137:1518–21.
212. Monteyne P, Sindic CJ. The diagnosis of tuberculous meningitis. Acta Neurol Belg **1995**; 95:80–7.
213. Karney WW, O'Donoghue JM, Ostrow JH, Holmes KK, Beaty HN. The spectrum of tuberculous peritonitis. Chest **1977**; 72:310–5.
214. Shakil AO, Korula J, Kanel GC, Murray NG, Reynolds TB. Diagnostic features of tuberculous peritonitis in the absence and presence of chronic liver disease: a case control study. Am J Med **1996**; 100:179–85.
215. Sherman S, Rohwedder JJ, Ravikrishnan KP, Weg JG. Tuberculous enteritis and peritonitis. Report of 36 general hospital cases. Arch Intern Med **1980**; 140:506–8.
216. Singh MM, Bhargava AN, Jain KP. Tuberculous peritonitis. An evaluation of pathogenetic mechanisms, diagnostic procedures and therapeutic measures. N Engl J Med **1969**; 281:1091–4.
217. Quale JM, Lipschik GY, Heurich AE. Management of tuberculous pericarditis. Ann Thorac Surg **1987**; 43:653–5.
218. Fowler NO, Manitsas GT. Infectious pericarditis. Prog Cardiovasc Dis **1973**; 16:323–36.
219. Berger HW, Mejia E. Tuberculous pleurisy. Chest **1973**; 63:88–92.
220. Seibert AF, Haynes J Jr, Middleton R, Bass JB Jr. Tuberculous pleural effusion. Twenty-year experience. Chest **1991**; 99:883–6.
221. Simon HB, Weinstein AJ, Pasternak MS, Swartz MN, Kunz LJ. Genitourinary tuberculosis. Clinical features in a general hospital population. Am J Med **1977**; 63:410–20.
222. Christensen WI. Genitourinary tuberculosis: review of 102 cases. Medicine (Baltimore) **1974**; 53:377–90.
223. Alvarez S, McCabe WR. Extrapulmonary tuberculosis revisited: a review of experience at Boston City and other hospitals. Medicine (Baltimore) **1984**; 63:25–55.
224. Pai M, Flores LL, Hubbard A, Riley LW, Colford JM Jr. Nucleic acid amplification tests in the diagnosis of tuberculous pleuritis: a systematic review and meta-analysis. BMC Infect Dis **2004**; 4:6.
225. Pai M, Flores LL, Pai N, Hubbard A, Riley LW, Colford JM Jr. Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis. Lancet Infect Dis **2003**; 3:633–43.
226. Pai M, Ling DI. Rapid diagnosis of extrapulmonary tuberculosis using nucleic acid amplification tests: what is the evidence? Future Microbiol **2008**; 3:1–4.
227. Daley CL, Kawamura LM. The role of molecular epidemiology in contact investigations: a US perspective. Int J Tuberc Lung Dis **2003**; 7(12 suppl 3):S458–62.
228. Daley CL, Small PM, Schecter GF, et al. An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus. An analysis using restriction-fragment-length polymorphisms. N Engl J Med **1992**; 326:231–5.
229. Cluster of tuberculosis cases among exotic dancers and their close contacts—Kansas, 1994–2000. MMWR Morb Mortal Wkly Rep **2001**; 50:291–3.
230. Bock NN, Mallory JP, Mobley N, DeVoe B, Taylor BB. Outbreak of tuberculosis associated with a floating card game in the rural south: lessons for tuberculosis contact investigations. Clin Infect Dis **1998**; 27:1221–6.
231. Increase in African immigrants and refugees with tuberculosis—Seattle-King County, Washington, 1998–2001. MMWR Morb Mortal Wkly Rep **2002**; 51:882–3.
232. Cowan LS, Diem L, Monson T, et al. Evaluation of a two-step approach for large-scale, prospective genotyping of *Mycobacterium tuberculosis* isolates in the United States. J Clin Microbiol **2005**; 43:688–95.
233. Oelemann MC, Diel R, Vatin V, et al. Assessment of an optimized mycobacterial interspersed repetitive-unit-variable-number tandem-repeat typing system combined with spoligotyping for population-based molecular epidemiology studies of tuberculosis. J Clin Microbiol **2007**; 45:691–7.
234. Breese PE, Burman WJ, Hildred M, et al. The effect of changes in laboratory practices on the rate of false-positive cultures for *Mycobacterium tuberculosis*. Arch Pathol Lab Med **2001**; 125:1213–6.
235. Burman WJ, Reves RR. Review of false-positive cultures for *Mycobacterium tuberculosis* and recommendations for avoiding unnecessary treatment. Clin Infect Dis **2000**; 31:1390–5.
236. Daley CL. Molecular epidemiology: a tool for understanding control of tuberculosis transmission. Clin Chest Med **2005**; 26:217–31, vi.
237. Malakmadze N, González IM, Oemig T, et al. Unsuspected recent transmission of tuberculosis among high-risk groups: implications of universal tuberculosis genotyping in its detection. Clin Infect Dis **2005**; 40:366–73.
238. Behr MA, Small PM. Molecular fingerprinting of *Mycobacterium tuberculosis*: how can it help the clinician? Clin Infect Dis **1997**; 25:806–10.
239. Kimerling ME, Benjamin WH, Lok KH, Curtis G, Dunlap NE. Restriction fragment length polymorphism screening of *Mycobacterium tuberculosis* isolates: population surveillance for targeting disease transmission in a community. Int J Tuberc Lung Dis **1998**; 2:655–62.
240. Gardy JL, Johnston JC, Ho Sui SJ, et al. Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. N Engl J Med **2011**; 364:730–9.
241. Abu-Raddad LJ, Sabatelli L, Achterberg JT, et al. Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. Proc Natl Acad Sci U S A **2009**; 106:13980–5.

Downloaded from http://cid.oxfordjournals.org/ at Government on December 8, 2016