American Thoracic Society

# Diagnostic Standards and Classification of Tuberculosis in Adults and Children

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## INTRODUCTION

The "Diagnostic Standards and Classification of Tuberculosis in Adults and Children" is a joint statement prepared by the American Thoracic Society and the Centers for Disease Control and endorsed by the Infectious Disease Society of America. The Diagnostic Standards are intended to provide a framework for and understanding of the diagnostic approaches to tuberculosis infection/disease and to present a classification scheme that facilitates management of all persons to whom diagnostic tests have been applied.

The specific objectives of this revision of the Diagnostic Standards are as follows.

1. To define diagnostic strategies for high- and low-risk patient populations based on current knowledge of tuberculosis epidemiology and information on newer technologies.
2. To provide a classification scheme for tuberculosis that is based on pathogenesis. Definitions of tuberculosis disease and latent infection have been selected that (*a*) aid in an accurate diagnosis; (*b*) coincide with the appropriate response of the health care team, whether it be no response, treatment of latent infection, or treatment of disease; (*c*) provide the most useful information that correlates with the prognosis; (*d*) provide the necessary information for appropriate public health action; and (*e*) provide a uniform, functional, and practical means of reporting. Because tuberculosis, even after it has been treated adequately, remains a pertinent and lifelong part of a person's medical history, previous as well as current disease is included in the classification.

This edition of the Diagnostic Standards has been prepared as a practical guide and statement of principles for all persons involved in the care of patients with tuberculosis. References have been included to guide the reader to texts and journal articles for more detailed information on each topic.

## I. EPIDEMIOLOGY

Tuberculosis remains one of the deadliest diseases in the world. The World Health Organization (WHO) estimates that each year more than 8 million new cases of tuberculosis occur and approximately 3 million persons die from the disease (1). Ninety-five percent of tuberculosis cases occur in developing countries, where few resources are available to ensure proper treatment and where human immunodeficiency virus (HIV) infection may be common. It is estimated that between 19 and 43% of the world's population is infected with *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis infection and disease (2).

In the United States, an estimated 15 million people are infected with *M. tuberculosis* (3). Although the tuberculosis case rate in the United States has declined during the past few years, there remains a huge reservoir of individuals who are infected with *M. tuberculosis.* Without application of effective treatment for latent infection, new cases of tuberculosis can be expected to develop from within this group.

Tuberculosis is a social disease with medical implications. It has always occurred disproportionately among disadvantaged populations such as the homeless, malnourished, and over-

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crowded. Within the past decade it also has become clear that the spread of HIV infection and the immigration of persons from areas of high incidence have resulted in increased numbers of tuberculosis cases.

## II. TRANSMISSION OF *Mycobacterium tuberculosis*

Tuberculosis is spread from person to person through the air by droplet nuclei, particles 1 to 5 μm in diameter that contain *M. tuberculosis* complex (4). Droplet nuclei are produced when persons with pulmonary or laryngeal tuberculosis cough, sneeze, speak, or sing. They also may be produced by aerosol treatments, sputum induction, aerosolization during bronchoscopy, and through manipulation of lesions or processing of tissue or secretions in the hospital or laboratory. Droplet nuclei, containing two to three *M. tuberculosis* organisms (5), are so small that air currents normally present in any indoor space can keep them airborne for long periods of time (6). Droplet nuclei are small enough to reach the alveoli within the lungs, where the organisms replicate. Although patients with tuberculosis also generate larger particles containing numerous bacilli, these particles do not serve as effective vehicles for transmission of infection because they do not remain airborne, and if inhaled, do not reach alveoli. Organisms deposited on intact mucosa or skin do not invade tissue. When large particles are inhaled, they impact on the wall of the upper airways, where they are trapped in the mucous blanket, carried to the oropharynx, and swallowed or expectorated (7).

Four factors determine the likelihood of transmission of *M. tuberculosis*: (*1*) the number of organisms being expelled into the air, (*2*) the concentration of organisms in the air determined by the volume of the space and its ventilation, (*3*) the length of time an exposed person breathes the contaminated air, and (*4*) presumably the immune status of the exposed individual. HIV-infected persons and others with impaired cell-mediated immunity are thought to be more likely to become infected with *M. tuberculosis* after exposure than persons with normal immunity; also, HIV-infected persons and others with impaired cell-mediated immunity are much more likely to develop disease if they are infected. However, they are no more likely to transmit *M. tuberculosis* (8).

Techniques that reduce the number of droplet nuclei in a given space are effective in limiting the airborne transmission of tuberculosis. Ventilation with fresh air is especially important, particularly in health care settings, where six or more room-air changes an hour is desirable (9). The number of viable airborne tubercle bacilli can be reduced by ultraviolet irradiation of air in the upper part of the room (5). The most important means to reduce the number of bacilli released into the air is by treating the patient with effective antituberculosis chemotherapy (10). If masks are to be used on coughing patients with infectious tuberculosis, they should be fabricated to filter droplet nuclei and molded to fit tightly around the nose and mouth. Measures such as disposing of such personal items as clothes and bedding, sterilizing fomites, using caps and gowns and gauze or paper masks, boiling dishes, and washing walls are unnecessary because they have no bearing on airborne transmission.

There are five closely related mycobacteria grouped in the *M. tuberculosis* complex: *M. tuberculosis, M. bovis, M. africanum, M. microti*, and *M. canetti* (11, 12). *Mycobacterium tuberculosis* is transmitted through the airborne route and there are no known animal reservoirs. *Mycobacterium bovis* may penetrate the gastrointestinal mucosa or invade the lymphatic tissue of the oropharynx when ingested in milk containing large numbers of organisms. Human infection with *M. bovis* has decreased significantly in developed countries as a result of the pasteurization of milk and effective tuberculosis control programs for cattle (13). Airborne transmission of both *M. bovis* and *M. africanum* can also occur (14–16). *Mycobacterium bovis* BCG is a live-attenuated strain of *M. bovis* and is widely used as a vaccine for tuberculosis. It may also be used as an agent to enhance immunity against transitional-cell carcinoma of the bladder. When used in this manner, adverse reactions such as dissemination may be encountered, and in such cases *M. bovis* BCG may be cultured from nonurinary tract system specimens, i.e., blood, sputum, bone marrow, etc. (17).

## III. PATHOGENESIS OF TUBERCULOSIS

After inhalation, the droplet nucleus is carried down the bronchial tree and implants in a respiratory bronchiole or alveolus. Whether or not an inhaled tubercle bacillus establishes an infection in the lung depends on both the bacterial virulence and the inherent microbicidal ability of the alveolar macrophage that ingests it (4, 18). If the bacillus is able to survive initial defenses, it can multiply within the alveolar macrophage. The tubercle bacillus grows slowly, dividing approximately every 25 to 32 h within the macrophage. *Mycobacterium tuberculosis* has no known endotoxins or exotoxins; therefore, there is no immediate host response to infection. The organisms grow for 2 to 12 wk, until they reach $10^3$ to $10^4$ in number, which is sufficient to elicit a cellular immune response (19, 20) that can be detected by a reaction to the tuberculin skin test.

Before the development of cellular immunity, tubercle bacilli spread via the lymphatics to the hilar lymph nodes and thence through the bloodstream to more distant sites. Certain organs and tissues are notably resistant to subsequent multiplication of these bacilli. The bone marrow, liver, and spleen are almost always seeded with mycobacteria, but uncontrolled multiplication of the bacteria in these sites is exceptional. Organisms deposited in the upper lung zones, kidneys, bones, and brain may find environments that favor their growth, and numerous bacterial divisions may occur before specific cellular immunity develops and limits multiplication.

In persons with intact cell-mediated immunity, collections of activated T cells and macrophages form granulomas that limit multiplication and spread of the organism. Antibodies against *M. tuberculosis* are formed but do not appear to be protective (21). The organisms tend to be localized in the center of the granuloma, which is often necrotic (22). For the majority of individuals with normal immune function, proliferation of *M. tuberculosis* is arrested once cell-mediated immunity develops, even though small numbers of viable bacilli may remain within the granuloma. Although a primary complex can sometimes be seen on chest radiograph, the majority of pulmonary tuberculosis infections are clinically and radiographically inapparent (18). Most commonly, a positive tuberculin skin test result is the only indication that infection with *M. tuberculosis* has taken place. Individuals with latent tuberculosis infection but not active disease are not infectious and thus cannot transmit the organism. It is estimated that approximately 10% of individuals who acquire tuberculosis infection and are not given preventive therapy will develop active tuberculosis. The risk is highest in the first 2 yr after infection, when half the cases will occur (23). The ability of the host to respond to the organism may be reduced by certain diseases such as silicosis, diabetes mellitus, and diseases associated with immunosuppression, e.g., HIV infection, as well as by corticosteroids and other immunosuppressive drugs. In these circumstances, the likelihood of developing tuberculosisdisease is greater. The risk of developing tuberculosis also appears to be greater during the first 2-yr of life.

HIV-infected persons, especially those with low $CD4^+$ cell counts, develop tuberculosis disease rapidly after becoming infected with *M. tuberculosis*; up to 50% of such persons may do so in the first 2 yr after infection with *M. tuberculosis* (24). Conversely, an individual who has a prior latent infection with *M. tuberculosis* (not treated) and then acquires HIV infection will develop tuberculosis disease at an approximate rate of 5–10% per year (25, 26).

In a person with intact cell-mediated immunity, the response to infection with the tubercle bacillus provides protection against reinfection. The likelihood of reinfection is a function of the risk of reexposure, the intensity of such exposure, and the integrity of the host's immune system. In the United States the risk of reexposure to an infectious case is low. Furthermore, in an otherwise healthy, previously infected person, any organisms that are deposited in the alveoli are likely to be killed by the cell-mediated immune response. Exceptions may occur, but in immunocompetent individuals, clinical and laboratory evidence indicates that disease produced by the inhalation of a second infecting strain is uncommon. However, reinfection has been documented to occur both in persons without recognized immune compromise and in persons with advanced HIV infection (27–29).

## IV. CLINICAL MANIFESTATIONS OF TUBERCULOSIS

The clinical manifestations of tuberculosis are quite variable and depend on a number of factors. Table 1 lists both host and microbe-related characteristics as well as their interactions that influence the clinical features of the disease. Before the beginning of the epidemic of infection with HIV, approximately 85% of reported tuberculosis cases were limited to the lungs, with the remaining 15% involving only nonpulmonary or both pulmonary and nonpulmonary sites (30). This proportional distribution is substantially different among persons with HIV infection. Although there are no national data that describe the sites of involvement in HIV-infected persons with tuberculosis, one large retrospective study of tuberculosis in patients with advanced HIV infection reported that 38% had only pulmonary involvement, 30% had only extrapulmonary sites, and 32% had both pulmonary and nonpulmonary involvement (31). Moreover, extrapulmonary involvement tends to increase in frequency with worsening immune compromise (32).

### A. Systemic Effects of Tuberculosis

Tuberculosis involving any site may produce symptoms and findings that are not specifically related to the organ or tissue involved but, rather, are systemic in nature. Of the systemic effects, fever is the most easily quantified. The frequency with which fever has been observed in patients with tuberculosis varies from approximately 37 to 80% (33, 34). In one study (33), 21% of patients had no fever at any point in the course of hospitalization for tuberculosis. Of the febrile patients, 34% were afebrile within 1 wk, and 64% in 2 wk, of beginning treatment. The median duration of fever after beginning treatment was 10 d, with a range of 1 to 109 d. Loss of appetite, weight loss, weakness, night sweats, and malaise are also common but are more difficult to quantify and may relate to coexisting diseases.

The most common hematologic manifestations of tuberculosis are increases in the peripheral blood leukocyte count and anemia, each of which occurs in approximately 10% of patients with apparently localized tuberculosis (35, 36). The increase in white blood cell counts is usually slight, but leukemoid reactions may occur. Leukopenia has also been reported. An increase in the peripheral blood monocyte and eosinophil counts also may occur with tuberculosis. Anemia is common when the infection is disseminated. In some instances, anemia or pancytopenia may result from direct involvement of the bone marrow and, thus, be a local, rather than a remote, effect.

Hyponatremia, which in one series was found to occur in 11% of patients (37), has been determined to be caused by production of an antidiuretic hormone-like substance found within affected lung tissue (38).

In many patients tuberculosis is associated with other serious disorders. These include HIV infection, alcoholism, chronic renal failure, diabetes mellitus, neoplastic diseases, and drug abuse, to name but a few. The signs and symptoms of these diseases and their complications can easily obscure or modify those of tuberculosis and result in considerable delays in diagnosis or misdiagnoses for extended periods of time, especially in patients with HIV infection (39).

### B. Pulmonary Tuberculosis

*Symptoms and physical findings.* Cough is the most common symptom of pulmonary tuberculosis. Early in the course of the illness it may be nonproductive, but subsequently, as inflammation and tissue necrosis ensue, sputum is usually produced and is key to most of our diagnostic methods. Hemoptysis may rarely be a presenting symptom but usually is the result of previous disease and does not necessarily indicate active tuberculosis. Hemoptysis may result from residual tuberculous bronchiectasis, rupture of a dilated vessel in the wall of a cavity (Rasmussen's aneurysm), bacterial or fungal infection (especially *Aspergillus* in the form of a mycetoma) in a residual cavity, or from erosion of calcified lesions into the lumen of an airway (broncholithiasis). Inflammation of the lung parenchyma adjacent to a pleural surface may cause pleuritic pain. Dyspnea is unusual unless there is extensive disease. Tuberculosis may, however, cause severe respiratory failure (40, 41).

Physical findings in pulmonary tuberculosis are not generally helpful in defining the disease. Rales may be heard in the area of involvement as well as bronchial breath sounds if there is lung consolidation.

*Radiographic features of pulmonary tuberculosis.* Pulmonary tuberculosis nearly always causes abnormalities on the chest

**TABLE 1**

**FACTORS THAT INFLUENCE THE CLINICAL FEATURES OF TUBERCULOSIS**

| Host Factors | Microbial Factors | Host–Microbe Interaction |
|---|---|---|
| Age | Virulence of the organism | Sites of involvement |
| Immune status | Predilection (tropism) for specific tissues | Severity of disease |
| Specificic immunodeficiency states | | |
| Malnutrition | | |
| Genetic factors (not yet defined) | | |
| Coexisting diseases | | |
| Immunization with bacillus Calmette-Guérin (BCG) | | |

film, although an endobronchial lesion may not be associated with a radiographic finding. In addition, in patients with pulmonary tuberculosis disease and HIV infection, a normal chest film is more common than in persons with tuberculosis disease without immune suppression. In primary tuberculosis occurring as a result of recent infection, the process is generally seen as a middle or lower lung zone infiltrate, often associated with ipsilateral hilar adenopathy. Atelectasis may result from compression of airways by enlarged lymph nodes. This manifestation is more common in children. If the primary process persists beyond the time when specific cell-mediated immunity develops, cavitation may occur (so-called "progressive primary" tuberculosis) (42).

Tuberculosis that develops as a result of endogenous reactivation of latent infection usually causes abnormalities in the upper lobes of one or both lungs. Cavitation is common in this form of tuberculosis. The most frequent sites are the apical and posterior segments of the right upper lobe and the apical–posterior segment of the left upper lobe. Healing of the tuberculous lesions usually results in development of a scar with loss of lung parenchymal volume and, often, calcification. In the immunocompetent adult with tuberculosis, intrathoracic adenopathy is uncommon but may occur, especially with primary infection. In contrast, intrathoracic or extrathoracic lymphatic involvement is quite common in children. As tuberculosis progresses, infected material may be spread via the airways into other parts of the lungs, causing a patchy bronchopneumonia. Erosion of a parenchymal focus of tuberculosis into a blood or lymph vessel may lead to dissemination of the organism and a "miliary" (evenly distributed small nodules) pattern on the chest film. Disseminated tuberculosis can occur in primary disease and may be an early complication of tuberculosis in children (both immunocompetent and immunocompromised). When it occurs in children, it is most common in infants and the very young ($< 5$ yr).

Old, healed tuberculosis presents a different radiologic appearance from active tuberculosis. Dense pulmonary nodules, with or without visible calcification, may be seen in the hilar area or upper lobes. Smaller nodules, with or without fibrotic scars, are often seen in the upper lobes, and upper-lobe volume loss often accompanies these scars. Nodules and fibrotic lesions of old healed tuberculosis have well-demarcated, sharp margins and are often described as "hard." Bronchiectasis of the upper lobes is a nonspecific finding that sometimes occurs from previous pulmonary tuberculosis. Pleural scarring may be caused by old tuberculosis but is more commonly caused by trauma or other infections. Nodules and fibrotic scars may contain slowly multiplying tubercle bacilli with significant potential for future progression to active tuberculosis. Persons who have nodular or fibrotic lesions consistent with findings of old tuberculosis on chest radiograph and a positive tuberculin skin test reaction should be considered high-priority candidates for treatment of latent infection regardless of age. Conversely, calcified nodular lesions (calcified granuloma) or apical pleural thickening poses a much lower risk for future progression to active tuberculosis (42, 43).

In patients with HIV infection, the nature of the radiographic findings depends to a certain extent on the degree of immunocompromise produced by the HIV infection. Tuberculosis that occurs relatively early in the course of HIV infection tends to have the typical radiographic findings described above (44, 45). With more advanced HIV disease the radiographic findings become more "atypical": cavitation is uncommon, and lower lung zone or diffuse infiltrates and intrathoracic adenopathy are frequent.

### C. Extrapulmonary Tuberculosis

Extrapulmonary tuberculosis usually presents more of a diagnostic problem than pulmonary tuberculosis. In part this relates to its being less common and, therefore, less familiar to most clinicians (46, 47). In addition, extrapulmonary tuberculosis involves relatively inaccessible sites and, because of the nature of the sites involved, fewer bacilli can cause much greater damage. The combination of small numbers of bacilli and inaccessible sites causes bacteriologic confirmation of a diagnosis to be more difficult, and invasive procedures are frequently required to establish a diagnosis.

*Extrapulmonary tuberculosis in HIV-infected patients.* Presumably, the basis for the high frequency of extrapulmonary tuberculosis among patients with HIV infection is the failure of the immune response to contain *M. tuberculosis*, thereby enabling hematogenous dissemination and subsequent involvement of single or multiple nonpulmonary sites. Because of the frequency of extrapulmonary tuberculosis among HIV-infected patients, diagnostic specimens from any suspected site of disease should be examined for mycobacteria. Moreover, cultures of blood and bone marrow may reveal *M. tuberculosis* in patients who do not have an obvious localized site of disease but who are being evaluated because of fever.

*Disseminated tuberculosis.* Disseminated tuberculosis occurs because of the inadequacy of host defenses in containing tuberculous infection. This failure of containment may occur in either latent or recently acquired tuberculous infection. Because of HIV or other causes of immunosuppression, the organism proliferates and disseminates throughout the body. Multiorgan involvement is probably much more common than is recognized because, generally, once *M. tuberculosis* is identified in any specimen, other sites are not evaluated. The term "miliary" is derived from the visual similarity of some disseminated lesions to millet seeds. Grossly, these lesions are 1- to 2-mm yellowish nodules that, histologically, are granulomas. Thus disseminated tuberculosis is sometimes called "miliary" tuberculosis. When these small nodules occur in the lung, the resulting radiographic pattern is also termed "miliary."

Because of the multisystem involvement in disseminated tuberculosis, the clinical manifestations are protean. The presenting symptoms and signs are generally nonspecific and are dominated by systemic effects, particularly fever, weight loss, night sweats, anorexia, and weakness (48–52). Other symptoms depend on the relative severity of disease in the organs involved. A productive cough is common because most patients with disseminated disease also have pulmonary involvement. Headache and mental status changes are less frequent and are usually associated with meningeal involvement (49). Physical findings likewise are variable. Fever, wasting, hepatomegaly, pulmonary findings, lymphadenopathy, and splenomegaly occur in descending order of frequency. A finding that is strongly suggestive of disseminated tuberculosis is the choroidal tubercle, a granuloma located in the choroid of the retina (53).

The chest film is abnormal in most but not all patients with disseminated tuberculosis. In the series reported by Grieco and Chmel (48), only 14 of 28 patients (50%) had a miliary pattern on chest film, whereas 90% of 69 patients reported by Munt (49) had a miliary pattern. Overall, it appears that at the time of diagnosis approximately 85% of patients have the characteristic radiographic findings of miliary tuberculosis. Other radiographic abnormalities may be present as well. These include upper lobe infiltrates with or without cavitation, pleural effusion, and pericardial effusion. In patients with HIV infection the radiographic pattern is usually one of diffuse infiltration rather than discrete nodules.

*Lymph node tuberculosis.* Tuberculous lymphadenitis usually presents as painless swelling of one or more lymph nodes. The nodes involved most commonly are those of the posterior or anterior cervical chain or those in the supraclavicular fossa. Fre-

quently the process is bilateral and other noncontiguous groups of nodes can be involved (54). At least initially the nodes are discrete and the overlying skin is normal. With continuing disease the nodes may become matted and the overlying skin inflamed. Rupture of the node can result in formation of a sinus tract, which may be slow to heal. Intrathoracic adenopathy may compress bronchi, causing atelectasis leading to lung infection and perhaps bronchiectasis. This manifestation is particularly common in children. Needle biopsy or surgical resection of the node may be needed to obtain diagnostic material if the chest radiograph is normal and the sputum smear and culture are negative.

In persons not infected with HIV but with tuberculous lymphadenitis, systemic symptoms are not common unless there is concomitant tuberculosis elsewhere. The frequency of pulmonary involvement in reported series of patients with tuberculous lymphadenitis is quite variable, ranging from approximately 5 to 70%. In HIV-infected persons lymphadenitis is commonly associated with multiple organ involvement.

*Pleural tuberculosis.* There are two mechanisms by which the pleural space becomes involved in tuberculosis. The difference in pathogenesis results in different clinical presentations, approaches to diagnosis, treatment, and sequelae. Early in the course of a tuberculous infection a few organisms may gain access to the pleural space and, in the presence of cell-mediated immunity, cause a hypersensitivity response (55, 56). Commonly, this form of tuberculous pleuritis goes unnoticed, and the process resolves spontaneously. In some patients, however, tuberculous involvement of the pleura is manifested as an acute illness with fever and pleuritic pain. If the effusion is large enough, dyspnea may occur, although the effusions generally are small and rarely are bilateral. In approximately 30% of patients there is no radiographic evidence of involvement of the lung parenchyma; however, parenchymal disease is nearly always present, as evidenced by findings of lung dissections (57).

The second variety of tuberculous involvement of the pleura is empyema. This is much less common than tuberculous pleurisy with effusion and results from a large number of organisms spilling into the pleural space, usually from rupture of a cavity or an adjacent parenchymal focus via a bronchopleural fistula (58). A tuberculous empyema is usually associated with evident pulmonary parenchymal disease on chest films and air may be seen in the pleural space. In the absence of concurrent pulmonary tuberculosis, diagnosis of pleural tuberculosis requires thoracentesis and, usually, pleural biopsy.

*Genitourinary tuberculosis.* In patients with genitourinary tuberculosis, local symptoms predominate and systemic symptoms are less common (59, 60). Dysuria, hematuria, and frequent urination are common, and flank pain may also be noted. However, the symptoms may be subtle, and, often, there is advanced destruction of the kidneys by the time a diagnosis is established (61). In women genital involvement is more common without renal tuberculosis than in men and may cause pelvic pain, menstrual irregularities, and infertility as presenting complaints (60). In men a painless or only slightly painful scrotal mass is probably the most common presenting symptom of genital involvement, but symptoms of prostatitis, orchitis, or epididymitis may also occur (59). A substantial number of patients with any form of genitourinary tuberculosis are asymptomatic and are detected because of an evaluation for an abnormal routine urinalysis. In patients with renal or genital tuberculosis, urinalyses are abnormal in more than 90%, the main finding being pyuria, and/or hematuria. The finding of pyuria in an acid urine with no routine bacterial organisms isolated from a urine culture should prompt an evaluation for tuberculosis by culturing the urine for mycobacteria. Acid-fast bacillus (AFB) smears of the urine should be done, but the yield is low. The suspicion of genitourinary tuberculosis should be heightened by the presence of abnormalities on the chest film. In most series, approximately 40 to 75% of patients with genitourinary tuberculosis have chest radiographic abnormalities, although in many these may be the result of previous, not current, tuberculosis (59, 60).

*Skeletal tuberculosis.* The usual presenting symptom of skeletal tuberculosis is pain (62). Swelling of the involved joint may be noted, as may limitation of motion and, occasionally, sinus tracts. Systemic symptoms of infection are not common. Since the epiphyseal region of bones is highly vascularized in infants and young children, bone involvement with tuberculosis is much more common in children than adults. Approximately 1% of young children with tuberculosis disease will develop a bony focus (63). Because of the subtle nature of the symptoms, diagnostic evaluations often are not undertaken until the process is advanced. Delay in diagnosis can be especially catastrophic in vertebral tuberculosis, where compression of the spinal cord may cause severe and irreversible neurologic sequelae, including paraplegia.

Fortunately, such neurologic sequelae represent the more severe end of the spectrum. Early in the process the only abnormality noted may be soft tissue swelling. Subsequently, subchondral osteoporosis, cystic changes, and sclerosis may be noted before the joint space is actually narrowed. The early changes of spinal tuberculosis may be particularly difficult to detect by standard films of the spine. Computed tomographic scans and magnetic resonance imaging of the spine are considerably more sensitive than routine films and should be obtained when there is a high index of suspicion of tuberculosis. Bone biopsy may be needed to obtain diagnostic material if the chest radiograph is normal and the sputum smear and culture are negative.

*Central nervous system tuberculosis.* Tuberculous meningitis is a particularly devastating disease. Meningitis can result from direct meningeal seeding and proliferation during a tuberculous bacillemia either at the time of initial infection or at the time of breakdown of an old pulmonary focus, or can result from breakdown of an old parameningeal focus with rupture into the subarachnoid space. The consequences of subarachnoid space contamination can be diffuse meningitis or localized arteritis. In tuberculous meningitis the process is located primarily at the base of the brain (64). Symptoms, therefore, include those related to cranial nerve involvement as well as headache, decreased level of consciousness, and neck stiffness. The duration of illness before diagnosis is quite variable and relates in part to the presence or absence of other sites of involvement. In most series more than 50% of patients with meningitis have abnormalities on chest film, consistent with an old or current tuberculous process, often miliary tuberculosis.

Physical findings and screening laboratory studies are not particularly helpful in establishing a diagnosis. In the presence of meningeal signs on physical examination, lumbar puncture is usually the next step in the diagnostic sequence. If there are focal findings on physical examination or if there are suggestions of increased intracranial pressure, a computerized tomographic scan of the head, if it can be obtained expeditiously, should be performed before the lumbar puncture. With meningitis, the scan may be normal but can also show diffuse edema or obstructive hydrocephalus. Tuberculomas are generally seen as ring-enhancing mass lesions.

The other major central nervous system form of tuberculosis, the tuberculoma, presents a more subtle clinical picture than tuberculous meningitis (65). The usual presentation is that of a slowly growing focal lesion, although a few patients have increased intracranial pressure and no focal findings. The

cerebrospinal fluid is usually normal, and the diagnosis is established by computed tomographic or magnetic resonance scanning and subsequent resection, biopsy, or aspiration of any ring-enhancing lesion.

*Abdominal tuberculosis.* Tuberculosis can involve any intraabdominal organ as well as the peritoneum, and the clinical manifestations depend on the areas of involvement. In the gut itself tuberculosis may occur in any location from the mouth to the anus, although lesions proximal to the terminal ileum are unusual. The most common sites of involvement are the terminal ileum and cecum, with other portions of the colon and the rectum involved less frequently (66). In the terminal ileum or cecum the most common manifestations are pain, which may be misdiagnosed as appendicitis, and intestinal obstruction. A palpable mass may be noted that, together with the appearance of the abnormality on barium enema or small bowel films, can easily be mistaken for a carcinoma. Rectal lesions usually present as anal fissures, fistulae, or perirectal abscesses. Because of the concern with carcinoma, the diagnosis often is made at surgery. However, laparoscopy or colonoscopy with biopsy may be sufficient to obtain diagnostic material.

Tuberculous peritonitis frequently causes pain as its presenting manifestation, often accompanied by abdominal swelling (66–69). Fever, weight loss, and anorexia are also common. Active pulmonary tuberculosis is uncommon in patients with tuberculous peritonitis. Because the process frequently coexists with other disorders, especially hepatic cirrhosis with ascites, the symptoms of tuberculosis may be obscured. The combination of fever and abdominal tenderness in a person with ascites should always prompt an evaluation for intraabdominal infection, and a paracentesis should be performed. However, this is often not diagnostic, and laparoscopy with biopsy is recommended if tuberculosis is suspected.

*Pericardial tuberculosis.* The symptoms, physical findings, and laboratory abnormalities associated with tuberculous pericarditis may be the result of either the infectious process itself or the pericardial inflammation causing pain, effusion, and eventually hemodynamic effects. The systemic symptoms produced by the infection are quite nonspecific. Fever, weight loss, and night sweats are common in reported series (70–72). Symptoms of cardiopulmonary origin tend to occur later and include cough, dyspnea, orthopnea, ankle swelling, and chest pain. The chest pain may occasionally mimic angina but usually is described as being dull, aching, and often affected by position and by inspiration.

Apart from fever, the most common physical findings are those caused by the pericardial fluid or fibrosis–cardiac tamponade or constriction. Varying proportions of patients in reported series have signs of full-blown cardiac constriction when first evaluated. It is assumed that in these patients the acute phase of the process was unnoticed. In the absence of concurrent extracardiac tuberculosis, diagnosis of pericardial tuberculosis requires aspiration of pericardial fluid or, usually, pericardial biopsy.

## V. DIAGNOSTIC MICROBIOLOGY

The contribution of the microbiology laboratory to the diagnosis and management of tuberculosis involves the detection and isolation of mycobacteria, the identification of the mycobacterial species or complex isolated, and the determination of susceptibilities of the organisms to antimycobacterial drugs. Only laboratories having a sufficient volume of work and assured competence should provide clinical mycobacteriology services. Such procedures are time-consuming and employ reagents and special techniques not used routinely in the study of bacteria in other genera. Furthermore, handling of mycobacterial specimens requires special safety precautions and suitable isolation areas that may place a burden on some laboratories.

### A. Laboratory Services for Mycobacterial Diseases

With the closing of most tuberculosis sanatoria in the 1970s, treatment of patients with tuberculosis moved to general hospitals and outpatient clinics. The supporting mycobacteriology services were spread diffusely through more and more laboratories, each processing fewer and fewer specimens. Recently, managed care plans that centralize laboratory processing have increased the number of specimens that are sent to regional reference laboratories for processing, further decreasing the numbers of mycobacteriology specimens processed locally. Maintenance of laboratory proficiency requires continuing and frequent performance of the required tests. When tests are performed so infrequently that it is impractical to maintain the materials and expertise required for proficiency, a decision must be made concerning referral to another laboratory for testing. In addition, because tuberculosis can be transmitted to laboratory personnel who handle clinical specimens, adequate training in proper techniques and the availability of special containment areas are required for the safe manipulation of clinical specimens. Protection of laboratory personnel and environment can be achieved by observing standard laboratory practices and techniques, using appropriate safety equipment properly, and designing a safe laboratory layout that includes proper air handling (73, 74).

The laboratory and the clinicians requesting service must be confident of the results the laboratory provides. However, because of patients with multidrug-resistant tuberculosis and increased numbers of immunodeficient patients, results from diagnostic studies must also be timely. Waiting for well-grown subcultures of mycobacteria to send to reference laboratories may cause significant delays. Laboratories should use efficient procedures, refer specimens to specialized laboratories as early as possible, and ideally be staffed 7 d/wk to provide the most rapid results possible. A laboratory may choose to develop or maintain the skills for only some of the procedures required, depending on the frequency with which specimens are received for isolation of mycobacteria, the nature of the clinical community being served, and the availability of a specialized referral service. All laboratories doing clinical mycobacteriology must participate in recognized proficiency testing programs (Clinical Laboratory Improvement Amendments [CLIA 42 CFR-493]), and levels of service should be established and limited by the quality of performance demonstrated in these examinations. Laboratories with a low volume of work should refer specimens/cultures to laboratories that have chosen to maintain capabilities in mycobacteriology. This will save the time, effort, and expense of setting up and maintaining quality control standards for tests that are performed only rarely. The full spectrum of bacteriologic support should be concentrated in the laboratories in a given community or region where professional expertise and complete and safe facilities are available. Physicians and laboratories must cooperate to achieve the highest quality clinical mycobacteriology service (75).

### B. Collection of Specimens for Demonstration of Tubercle Bacilli

Because the identification of organisms is so critical in diagnosing tuberculosis, it is of utmost importance that careful attention be given to the collection and handling of specimens. Success in isolating mycobacteria from clinical materials depends on the manner in which specimens are handled after collection. For optimal results, specimens should be collected in clean, sterile containers and held under conditions that in-

hibit growth of contaminating organisms, since most specimens will contain bacteria other than mycobacteria.

Because mycobacterial disease may occur in almost any site in the body, a variety of clinical materials may be submitted to the laboratory for examination. In addition to the common specimens, such as sputum (natural or induced) and gastric aspirate, others include urine, cerebrospinal fluid, pleural fluid, bronchial washings, material from abscesses, endometrial scrapings, bone marrow, and other biopsy specimens or resected tissue. The methods for collecting specimens are briefly outlined below. All specimen collection procedures that produce aerosols that potentially contain *M. tuberculosis* (e.g., sputum, bronchoalveolar lavage, etc.) should be performed in properly ventilated areas or safety cabinets by personnel using adequate respiratory protection (76, 77). If the patient is ill, therapy should not be delayed as the diagnosis is being pursued.

*Sputum.* Patients need to be instructed as to the proper method of sputum collection. It is important that the patient be informed that nasopharyngeal discharge and saliva are not sputum; rather, the material brought up from the lungs after a productive cough constitutes the material desired. Whenever possible, attending personnel should observe the sputum collection. A series of at least three single specimens (but usually not more than six) should be collected initially (preferably on different days) from sputum-producing patients. For optimal results, sputum should be collected and processed in the same container. Commercially available sputum collection devices using a 50-ml plastic, single-use, disposable centrifuge tube is recommended (78). Alternatively, a sterile, wide-mouth specimen container with a tightly fitting screwtop lid is adequate. Specimens should be clearly labeled with patient-identifying information and the date of collection. Every effort should be made to prevent the exterior of the container from becoming contaminated during the collection. The container should be placed in a disposable watertight plastic bag before being transported to the laboratory.

*Induced sputum.* For patients who have difficulty producing sputum, there are several methods of obtaining a specimen. Inhalation of an aerosol of sterile hypertonic saline (3–15%), usually produced by an ultrasonic nebulizer, can be used to stimulate the production of sputum (79). Even though aerosol-induced specimens may appear thin and watery, they should be processed. The specimen should be clearly labeled as "induced sputum"so it will not be discarded by the laboratory as an inadequate specimen. Because the cough induced by this method may be violent and uncontrolled, patients should be in areas with adequate environmental controls such as a hood or booth fitted with a high-efficiency particulate air (HEPA) filter to prevent transmission. They should be attended by qualified personnel using appropriate respiratory protection (9).

*Gastric aspiration.* Gastric aspiration may be necessary for those patients, particularly children, who cannot produce sputum even with aerosol inhalation. About 50 ml of gastric contents should be aspirated early in the morning, after the patient has fasted for at least 8 to 10 h, and preferably while the patient is still in bed. Gastric aspirates should be neutralized immediately on collection. For these reasons, gastric aspiration is best performed with hospitalized patients, according to a standard protocol (80). In children, *M. tuberculosis* can be recovered from gastric aspirates in about 40% of those with radiographic evidence of significant pulmonary disease (81, 82).

*Bronchial washings, bronchoalveolar lavage, transbronchial biopsy.* For patients in whom a diagnosis of tuberculosis has not been established from sputum, fiberoptic bronchoscopy performed with appropriate infection control precautions may be needed with bronchoalveolar lavage, and/or transbronchial biopsy (83). Even in the presence of significant pulmonary disease, the smears of bronchoalveolar lavage fluid may be negative. The topical agents used to anesthetize the airway mucosa may be lethal to *M. tuberculosis*, so these agents should be used judiciously. Patients should be placed in a room with appropriate infection controls during and after the procedure. Patient's sputum produced after bronchoscopy (during the recovery phase and the next morning) should also be collected and examined. The procedure may cause the patient to continue producing sputum for several days. These later specimens should also be collected and examined. Physical cleaning of the bronchoscope followed by chemical sterilization is absolutely essential since documented transmission through a contaminated bronchoscope has been reported (84–86).

*Urine.* The first morning-voided midstream specimen is preferred. Multiple specimens are advised to demonstrate the presence of mycobacteria. Smears of urine are usually negative and therefore may not be cost-effective to perform. It is preferable that the patient not be receiving broad-spectrum antibiotics at the time of collection because the antibiotics may inhibit growth of mycobacteria from urine.

*Blood.* Blood for mycobacterial culture should be anticoagulated with heparin and processed with a commercially available lysis centrifugation system or inoculated into commercially available broth media designed for mycobacterial blood cultures. Blood collected in ethylenediaminetetraacetic acid (EDTA/purple-topped tube) is not suited for mycobacterial culture.

*Cerebrospinal fluid.* Cerebrospinal fluid should be analyzed for protein and glucose (compared with simultaneous serum total protein and glucose). Total white blood cell and differential counts should also be obtained. A high protein ($>$ 50% of the serum protein concentration), lymphocytosis, and low glucose are typical of tuberculous meningitis. A minimum of 5 ml should be submitted to the laboratory in a sterile container for mycobacterial culture. The AFB smear of cerebrospinal fluid is usually negative; however, the culture may be positive. If the laboratory concentrates the fluid before smear and culture, a greater volume ($>$ 10 ml) can lead to increased yield, but may also increase complications of the procedure.

*Tissue and other body fluids.* Under a variety of circumstances, when noninvasive techniques have not provided a diagnosis, tissue or other body fluids should be obtained for histologic evaluation and culture (for both mycobacteria and fungi). Expeditious and appropriate handling of the specimen must be assured before the physician performs an invasive procedure to obtain the specimen. Especially important is rapid transportation to the laboratory in an appropriate container, either without preservative, or in the correct medium for the culture, according to the laboratory's instructions. The portion of the specimen put in formalin for histologic examination cannot be used for culture.

Pleural, peritoneal, and pericardial fluids may be analyzed for protein and glucose (compared with simultaneous serum total protein and glucose). Cell and differential counts should be obtained. A high protein ($>$ 50% of the serum protein concentration), lymphocytosis, and a low glucose are usually found in tuberculous infections, but neither their presence nor their absence is diagnostic. Adenosine deaminase (ADA), a purine-degrading enzyme that is necessary for the maturation and differentiation of lymphoid cells, has been reported in a number of studies to be elevated in these fluids when tuberculosis involves these sites (87, 88). However, the utility of the routine measurement of ADA has not been determined and this test is not generally available. The numbers of organisms

in the pleural fluid from most cases of tuberculous pleuritis is relatively low, with positive cultures found in less than 25% of cases. Pleural biopsy shows granulomatous inflammation in approximately 60% of patients. However, when culture of three biopsy specimens is combined with microscopic examination, the diagnosis can be made in up to 90% of cases (89). Pleuroscopy-guided biopsies increase the yield in pleural sampling. Peritoneal biopsies are best obtained via laparoscopy.

*Tissue biopsy*. Invasive procedures to obtain specimens from the lung, pericardium, lymph nodes, bones and joints, bowel, salpinges, and epididymis should be considered when noninvasive techniques do not provide a diagnosis. Many of these areas are amenable to closed techniques such as percutaneous needle biopsy or aspiration, transbronchial biopsy, or brushing, precluding a need for formal surgical procedures. In patients with hematogenous or disseminated disease, bone marrow biopsy, lung biopsy, and liver biopsy for histologic examination and culture should be considered. Appropriate measures must be taken when collecting these specimens to minimize aerosolation of *M. tuberculosis* organisms and prevent transmission of infection to personnel.

### C. Transport of Specimens to the Laboratory

Clinical specimens, which must be labeled clearly and accurately, should be transported or mailed to the laboratory and processed as soon as possible after collection. To minimize transit time, the use of overnight delivery should be considered. If a delay is anticipated, the specimen should be refrigerated until prompt delivery can be assured. The *Interstate Shipment of Etiologic Agents* (42 CFR, Part 72) provides specific instructions for packing and labeling of infectious agents. Authorized packaging and components include the following: (*1*) an inner package that contains a watertight primary receptacle, watertight secondary packaging, and absorbent material between the primary and secondary receptacle; and (*2*) outer packaging that is of adequate strength for its capacity, mass, and intended use. Clear labeling of packages specifying the contents is necessary. Standardized biohazard labels for this purpose are available. Additional information can be obtained from the Centers for Disease Control and Prevention, Office of Health and Safety (1600 Clifton Road, Atlanta, GA 30333; website: www.cdc.gov/od/ohs/). If the specimen cannot be shipped promptly to the laboratory, it should be refrigerated until shipped. No fixative or preservation agents should be used.

### D. Digestion and Decontamination of Specimens

Most clinical specimens contain an abundance of nonmycobacterial organisms. Unless an attempt is made to inhibit these usually fast-growing contaminants, they can quickly overgrow the generally more slowly reproducing (18- to 24-h generation time in culture) mycobacteria on the culture medium. It is also necessary to liquefy the organic debris (tissue, serum, and other proteinaceous material) surrounding the organisms in the specimen so that decontaminating agents may kill undesirable microbes, and surviving mycobacteria may gain access to the nutrients of the medium onto which they are subsequently inoculated. Because mycobacteria are more refractory to harsh chemicals than are most other microorganisms, chemical decontamination procedures have been successfully applied to ensure the recovery of acid-fast bacteria from clinical materials.

On arrival in the laboratory, most specimens are homogenized with a mucolytic agent (such as *N*-acetyl-L-cysteine) and decontaminant (such as a 1–2% sodium hydroxide solution) to render the bacterial contaminants nonviable. The mildest decontamination procedure that provides sufficient control of the contaminants without killing the mycobacteria is likely to yield the best results (78). However, even under optimal conditions, these procedures kill all but 10 to 20% of the mycobacteria in the specimen (90, 91). As a general rule for Löwenstein–Jensen medium, there should be approximately 2–5% of sputum specimens that are contaminated. If fewer than 2% of specimens are contaminated, the process may be killing many of the mycobacteria as well as contaminants. If more than 5% of the cultures are contaminated, the decontamination process is inadequate (92). Tissues may be ground in homogenizers and decontaminated. Specimens collected from normally sterile sites may be placed directly into the culture medium.

### E. Staining and Microscopic Examination

The detection of acid-fast bacilli (AFB) in stained smears examined microscopically is the first bacteriologic evidence of the presence of mycobacteria in a clinical specimen. It is the easiest and quickest procedure that can be performed, and it provides the physician with a preliminary confirmation of the diagnosis. Also, because it gives a quantitative estimation of the number of bacilli being excreted, the smear is of vital clinical and epidemiologic importance in assessing the patient's infectiousness.

Smears may be prepared directly from clinical specimens or from concentrated preparations. The acid-fast staining procedure depends on the ability of mycobacteria to retain dye when treated with mineral acid or an acid–alcohol solution. Two procedures are commonly used for acid–fast staining: the carbolfuchsin methods, which include the Ziehl–Neelsen and Kinyoun methods, and a fluorochrome procedure using auramine-O or auramine–rhodamine dyes. Several quantitative studies have shown that there must be 5,000 to 10,000 bacilli per milliliter of specimen to allow the detection of bacteria in stained smears (93). In contrast, 10 to 100 organisms are needed for a positive culture (94). Concentration procedures in which a liquefied specimen is centrifuged and the sediment is used for staining increases the sensitivity of the test; thus, smears of concentrated material are preferred. Negative smears, however, do not preclude tuberculosis disease. Various studies have indicated that 50 to 80% of patients with pulmonary tuberculosis will have positive sputum smears. Factors influencing the sensitivity of smears include staining technique, centrifugation speed, reader experience, and the prevalence of tuberculosis disease in the population being tested.

In reading smears, the microscopist should provide the clinician with a rough estimate of the number of AFB detected. Table 2 shows a frequently used scheme to quantify organisms seen on AFB smear.

Acid-fast bacteria seen on smear may represent either *M. tuberculosis* or nontuberculous mycobacteria. However, because of the infectious potential of *M. tuberculosis*, acid-fast

**TABLE 2**

**QUANTITATION SCALE FOR ACID-FAST BACILLUS SMEARS ACCORDING TO STAIN USED**

| Carbolfuchsin (× 1,000) | Fluorochrome (× 250) | Quantity Reported |
|---|---|---|
| No AFB/300 fields | No AFB/30 fields | No AFB seen |
| 1–2 AFB/300 fields | 1–2 AFB/30 fields | Doubtful, repeat test |
| 1–9 AFB/100 fields | 1–9 AFB/10 fields | Rare (1+) |
| 1–9 AFB/10 fields | 1–9 AFB/field | Few (2+) |
| 1–9 AFB/field | 10–90 AFB/field | Moderate (3+) |
| > 9 AFB/field | > 90 AFB/field | Numerous (4+) |

stains should be performed within 24 h of receipt in the laboratory and results should be reported to the physician immediately (95). Hospitalized patients should be placed in respiratory isolation until the absence of *M. tuberculosis* is definitely known or the patient has been treated with medications and is no longer thought to be infectious. There is no need to hospitalize a person solely because they are infectious. Outpatients should be instructed to remain at home, without visitors, until they are no longer thought to be infectious. Also, individuals who are particularly susceptible to developing tuberculosis disease if they become infected (small children, immunocompromised individuals) should not visit or live with the patient while they can transmit infection.

The percentage of specimens shown positive by smear but negative in culture should be less than 1% (96). Most smear-positive, culture-negative specimens are seen in patients who are taking antimycobacterial therapy. Laboratory errors, prolonged specimen decontamination, shortened incubation times of culture, cross-contamination of smears, and water or stains contaminated with acid-fast organisms can result in smear-positive, culture-negative specimens (78).

### F. Identification of Mycobacteria Directly from Clinical Specimens

A dramatic improvement in the direct detection and identification of *M. tuberculosis* has resulted from methods using nucleic acid amplification techniques. These technologies allow for the amplification of specific target sequences of nucleic acids that can then be detected through the use of a nucleic acid probe. Both RNA and DNA amplification systems (97, 98) are commercially available. The uses of nucleic acid amplification for the diagnosis of tuberculosis are rapidly evolving and the discussion below represents a cautious view that should be modified as additional information becomes available.

Nucleic acid amplification methods can be applied to clinical specimens within hours. In the research laboratory, these procedures can produce a positive result from specimens containing as few as 10 bacilli (79); however, in clinical laboratories, the sensitivity is somewhat less. When evaluating the utility of these tests, it is important to keep in mind that initial studies were performed from the perspective of the laboratory, not from the clinical perspective. In clinical respiratory specimens that are AFB smear positive, the sensitivity of the amplification methods is approximately 95%, with a specificity of 98%. In specimens that contain fewer organisms and are AFB smear negative, the nucleic acid amplification test is positive in 48–53% of patients with culture-positive tuberculosis and the specificity remains approximately 95% (99). Thus, the CDC included a positive nucleic acid amplification test in the setting of a positive smear as confirmation of the diagnosis of tuberculosis (100). An "enhanced" nucleic acid amplification test has been approved by a Food and Drug Administration (FDA) advisory panel for use on both smear-positive and smear-negative respiratory specimens from patients who are clinically suspected of having tuberculosis. This recommendation was based on a clinical trial in which the "suspicion" of tuberculosis disease was quantified (101). In patients (AFB smear positive and negative) where the clinician had an intermediate or high suspicion of tuberculosis disease, the sensitivity of the enhanced nucleic acid amplification test was 75–88% and the specificity was 100%. The clinical use of nucleic acid amplification in this setting needs to be confirmed and efforts to clarify appropriate uses are underway (100, 102). On the basis of available information, decisions about when and how to use nucleic amplification tests should be individualized. These tests may enhance diagnostic certainty, particularly in patients where prompt treatment is imperative (e.g., immunocompromised) (103), but should be interpreted in a clinical context and on the basis of local laboratory performance (100). Newer methods for nucleic acid amplification are being developed to increase the sensitivity and specificity of the test.

Nucleic acid amplification methods do not replace the need for routine AFB smear and culture, especially when drug susceptibility tests are to be performed. However, these tests can greatly increase confidence in the clinical diagnosis pending culture results. As with other laboratory techniques, results from nucleic acid amplification methods must be interpreted in the context of the patient's signs and symptoms, and the prevalence of tuberculosis within the community. Laboratory contamination, and technician and sampling errors, can cause false-positive results. Also, nucleic acid amplification procedures can detect nucleic acids from dead as well as live *M. tuberculosis* and, therefore, can remain positive for long periods in patients who have completed tuberculosis therapy. Thus, this method should be used only for initial diagnosis and not follow-up evaluations of patients who are receiving antimycobacterial drugs.

### G. Cultivation of Mycobacteria

All clinical specimens suspected of containing mycobacteria should be inoculated (after appropriate digestion and decontamination, if required) onto culture media for four reasons: (1) culture is much more sensitive than microscopy, being able to detect as few as 10 bacteria/ml of material (94); (2) growth of the organisms is necessary for precise species identification; (3) drug susceptibility testing requires culture of the organisms; and (4) genotyping of cultured organisms may be useful to identify epidemiological links between patients or to detect laboratory cross-contamination. In general, the sensitivity of culture is 80–85% with a specificity of approximately 98% (104, 105).

Although the diagnosis of tuberculosis disease is mainly bacteriologic in adults, it is usually epidemiologic and, thus, indirect in children (106). For example, from 1985 to 1988 in the United States, 90% of tuberculosis cases in adults were bacteriologically confirmed, compared with 28% in children (107). In HIV-infected pediatric tuberculosis cases, the bacteriologic confirmation of disease appears to be much higher, possibly because of the dissemination of the organism or higher bacterial burdens.

Three different types of traditional culture media are available: egg based (Löwenstein–Jensen), agar based (Middlebrook 7H10 or 7H11 medium), and liquid (Middlebrook 7H12 and other commercially available broths), and each can be made into selective media by adding antibiotics. Of the solid media, growth of mycobacteria tends to be slightly better on the egg-based medium but more rapid on the agar medium. Growth in liquid media is faster than growth on solid media. However, liquid media can be used for primary isolation of mycobacteria from nonsterile sites only if supplemented with an antibiotic cocktail.

A major improvement in mycobacteriology has been the development of commercial broth systems for mycobacterial growth detection. Automated culture systems such as BACTEC 460 (Becton Dickinson Microbiology Systems, Sparks, MD), mycobacterial growth indicator tube (MGIT) systems, ESP (Extra Sensing Power) Myco-ESPculture System II (Trek Diagnostic Systems, Inc., Westlake, OH), and BacT/ALERT MB Susceptibility Kit (Organon Teknika, Durham, NC) use Middlebrook 7H12 media with added material for detection of mycobacteria (radiometric or colorimetric systems). Liquid systems allow for rapid growth [detection of mycobacterial

growth within 1–3 wk compared with solid media, where growth takes 3–8 wk (104)], whereas agar media provide an opportunity to examine colony morphology and detect mixed cultures. At least one container of solid medium should be inoculated and used in conjunction with broth culture systems. Egg-based media such as Löwenstein–Jensen slants are an important backup for rare *M. tuberculosis* strains that may not grow on the other media. Automated liquid systems should be checked at least every 2–3 d for growth while solid media should be checked once or twice a week.

Mycobacterial growth observed on solid culture media should be quantified. Growth in liquid culture systems cannot be similarly quantitated although a qualitative measure of organisms in the inoculum can be made by noting the time required for liquid culture to turn positive. Table 3 presents a widely used scale for quantitating growth on agar plates.

### H. Identification of Mycobacteria from Culture

The genus *Mycobacterium* consists of more than 80 different species of organisms, all of which appear similar on acid-fast staining. More than half of them, both saprophytes and potential pathogens, may be isolated from humans. The specialized laboratory, through the use of a variety of *in vitro* tests, should be able to provide a precise species identification of most acid-fast bacilli isolated from patients. A clear-cut distinction between pathogen and saprophyte is not always possible for the individual isolate. Isolation of a nontuberculous organism of potential clinical significance is not *ipso facto* evidence that the patient has disease caused by the organism; conversely, all such isolates, which are usually clinically insignificant, should not be regarded as saprophytes. Each mycobacterial isolate, like each patient, must be evaluated individually (108).

Historically, *M. tuberculosis* could readily be identified by its rough, nonpigmented, corded colonies on oleic acid–albumin agars; a positive niacin test; generally weak catalase activity, that is lost completely by heating to 68° C; and a positive nitrate reduction test. Observation of mycobacterial colonial morphology remains a valuable tool. Although the colonial morphologies of mycobacteria on various egg-based media may be quite similar, their appearance on Middlebrook 7H10 or 7H11 agar is distinctive (109).

Biochemical methods can distinguish mycobacterial species, but are time-consuming and laborious. Two identification procedures that are based on distinctive molecular characteristics of *M. tuberculosis* have gained widespread use: nucleic acid hybridization and high performance liquid chromatography (HPLC). Nucleic acid hybridization uses molecular probes that can hybridize specifically with *M. tuberculosis* complex, *M. avium* complex, *M. kansasii*, and *M. gordonae* (79). Nucleic acid probes for other specific mycobacterial species are not yet commercially available. These assays can be completed within hours and have sensitivities and specificities approaching 100% when at least $10^5$ organisms are present. This requirement is easily met when pure cultures are used but is rarely achieved with clinical specimens. Thus nucleic acid hybridization is typically used after the organisms are grown in culture.

HPLC is based on the observation that each *Mycobacterium* species synthesizes a unique set of mycolic acids, β-hydroxy-α-fatty acids that are components of the cell wall (110). HPLC can produce a pattern that reliably identifies and distinguishes > 50 mycobacterial species. It can be performed in a few hours but requires organisms from pure cultures. HPLC is particularly useful since it can replace an entire battery of biochemical tests. However, it cannot differentiate *M. tuberculosis* from *M. bovis*, although it can differentiate *M. bovis* BCG from *M. tuberculosis* complex. The initial equipment cost for HPLC limits its availability. Newer molecular techniques for species identification, such as spoligotyping, polymerase chain reaction restriction analysis (PRA), and DNA sequencing, are on the horizon.

**TABLE 3**

**QUANTITATION SCALE FOR MYCOBACTERIAL GROWTH ON AGAR PLATES**

| No. of Colonies Seen | Quantity Reported |
|---|---|
| No colonies seen | Negative |
| Fewer than 50 colonies | Report actual number seen |
| 50–100 colonies | 1+ |
| 100–200 colonies | 2+ |
| 200–500 colonies (almost confluence) | 3+ |
| > 500 colonies (confluence) | 4+ |

### I. Drug Susceptibility Testing

Drug susceptibility tests should be performed on initial isolates from all patients in order to identify what should be an effective antituberculous regimen. In addition, drug susceptibility tests should be repeated if the patient continues to produce culture-positive sputum after 3 mo of treatment or develops positive cultures after a period of negative cultures. There are four conventional laboratory methods for detecting mycobacterial resistance: (1) the agar proportion method, (2) the liquid radiometric method, (3) the absolute concentration method, and (4) the resistance ratio method (91). The absolute concentration methodology and the resistance ratio method (111) are not used in the United States.

*Direct and indirect methods.* The source of the inoculum for a susceptibility test may be either a smear-positive specimen (direct method) or growth from a primary culture or subculture (indirect method). The direct method can be used only when large numbers of organisms are seen on stained smears. The indirect method is considered the standard method for inoculum preparation and results of the direct method are usually confirmed by subsequent testing using the indirect method. With both the direct and indirect methods, careful attention must be given to avoid over- or underinoculation. For the direct method, the inoculum is either a digested, decontaminated clinical specimen, or an untreated normally sterile body fluid. To ensure adequate but not excessive growth in the direct susceptibility test on solid medium, specimens are diluted according to the number of organisms observed in the stained smear of the clinical specimen. Theoretically, this type of inoculum is more representative of the population of the tubercle bacilli in a particular lesion in the host. If the smear-positive specimen is from a patient who is receiving antimicrobial therapy, it is prudent to include an undiluted inoculum because a significant proportion of the bacilli seen on the smear may be nonviable.

Use of the direct method may be warranted when there is a high prevalence of drug resistance, especially when second-line drugs are included in the initial test panel. Because direct susceptibility testing is done immediately on receipt of the specimen and does not require prior growth of organisms, the time required to identify susceptibility patterns may be reduced. However, cost can be a critical factor when there is a high incidence of smear-positive specimens containing nontuberculous mycobacteria. The direct method is not recommended for routine use with liquid medium methods at this time, since this application has had only limited evaluation.

For the indirect method the source of the inoculum is a

subculture usually from the primary isolation medium. Careful attention should be given to the selection of colony types so that the final inoculum is representative of all types present, to ensure that there is a balance of potentially resistant and susceptible bacilli. The source of inoculum for the BACTEC method can be growth on Middlebrook 7H10 or 7H11 agar or on an egg-based medium. Turbid growth in a liquid medium or sufficient growth in BACTEC 12B medium is also acceptable; however, one should remember that a mixed culture (*M. tuberculosis* and nontuberculous mycobacteria) can result in apparent resistance, especially when the inoculum is derived from a broth culture. Smear and subculture of BACTEC bottles showing growth in the presence of drug is therefore recommended.

The agar and radiometric BACTEC proportion methods are used by most laboratories in the United States that perform mycobacterial susceptibility testing. The agar proportion method allows for the quantitation of the proportion of organisms that is resistant to a given drug at a specified concentration. For a test to be valid, isolated, countable colonies (50 to 150) must be obtained on the drug-free medium. The number of colonies observed on the drug-containing medium is then compared with the number on the drug-free medium. The proportion of bacilli that is resistant to a given drug can be determined and expressed as a percentage of the total population tested. This proportion has been set at 1%, because when 1% or more of the mycobacterial population is resistant to the critical concentration of a drug, that agent is not, or soon will not be, useful for therapy. The critical concentration of a drug is the level of drug that inhibits the growth of most cells within the population of a "wild" type strain of tubercle bacilli without appreciably affecting the growth of the resistant mutant cells that might be present. Wild strains are those that have never come into contact with antituberculosis agents. These strains have a similar degree of susceptibility and show a narrow range of minimal inhibitory concentrations (MICs) to antituberculous drugs (112). The critical concentrations of the drugs being tested should be included when drug susceptibility test results are reported (91).

To generate rapid testing and faster turnaround time for better patient management, the radiometric BACTEC 460 system can be used to test all primary drugs (isoniazid, rifampin, pyrazinamide, and ethambutol). The rapid availability of results using the BACTEC procedure may take precedence over cost considerations. Many laboratories will follow up detection of resistance by the BACTEC method by testing the same patient's culture by the agar proportion method. The additional testing may include the equivalent critical concentrations of primary drugs in agar proportion to confirm the BACTEC results and higher concentrations of primary drugs to determine the level of drug resistance. In comparing the reported results between the two methods, clinicians should differentiate which are the equivalent critical concentrations and which are the higher concentrations of primary drugs (Table 4).

Testing of pyrazinamide using the BACTEC method is different from the BACTEC method used with the other primary drugs because pyrazinamide activity must be measured at pH 5.5 rather than pH 6.8, the usual pH of the growth medium. However, most strains of *M. tuberculosis* grow poorly at pH 5.5 and some fail to grow altogether. As a compromise between testing at the pH for optimum pyrazinamide activity versus optimum growth, pH 6.0 has been chosen for testing pyrazinamide. In addition, to accurately and reliably test pyrazinamide several other adjustments are made to the standard radiometric method (113). If an isolate tests resistant to pyrazinamide, especially if the isolate is resistant to pyrazinamide alone, the identity of the isolate should be confirmed since *M. bovis* and *M. bovis* BCG are pyrazinamide resistant whereas the majority of *M. tuberculosis* isolates are pyrazinamide susceptible. This is especially important if the laboratory identifies isolates only to the level of the *M. tuberculosis* complex.

Susceptibility test results should be reported without delay. The report should include the method used, the name of the drug, the concentration tested, and the result (susceptible or resistant for the BACTEC method, susceptible or percent resistant for the agar proportion method). Drug resistance should be reported by telephone and/or facsimile to the requesting physician, infection control staff, and the local tuberculosis control program with a follow-up hard copy report. It is prudent to confirm the receipt of a facsimile report by direct communication with the physician or public health official.

Clinician concerns about discrepancies between susceptibility test results and clinical response or status must be communicated back to the laboratory as part of an effective quality assurance program. Commonly, an *M. tuberculosis* isolate may test by either the agar or BACTEC proportion method as resistant to isoniazid, ethambutol, or streptomycin at the concentration of the drug tested. Although the agar proportion and BACTEC methods represent break-point susceptibility testing with a single critical concentration of drug, some laboratories test an additional, higher concentration of drug for isoniazid, ethambutol, and streptomycin. The critical concentration of

**TABLE 4**

**RECOMMENDED DRUG CONCENTRATIONS FOR DRUG PANEL SUSCEPTIBILITY TESTING***

| | | | | Nonradiometric Broth Systems | | |
|---|---|---|---|---|---|---|
| Drug | Radiometric (BACTEC) | Agar Proportion | Löwenstein–Jensen | ESP Myco | MGIT† | BacT/ ALERT MB |
| Isoniazid | 0.1‡ | 0.2‡ | 0.2‡ | 0.1‡ | 0.1 | 0.09 |
| Isoniazid (high) | 0.4 | 1.0 | 1.0 | 0.4 | 0.4 | 0.4 |
| Rifampin | 2.0‡ | 1.0‡ | 40.0‡ | 1.0‡ | 1.0 | 0.9 |
| Ethambutol | 2.5‡ | 5.0‡ | 2.0‡ | 5.0‡ | 5.0 | 2.3 |
| Ethambutol (high) | 7.5 | 10.0 | 8.0 | — | 7.5 | — |
| Pyrazinamide | 100‡ | 25.0‡ | — | — | 100 | 200 |
| Streptomycin | 2.0‡ | 2.0‡ | 4.0‡ | — | 1.0 | 0.9 |
| Streptomycin (high) | 6.0 | 10.0 | — | — | 4.0 | — |

*Definition of abbreviations*: ESP Myco = ESP (Extra Sensing Power) Culture System II; MGIT = mycobacterial growth indicator tube; BacT/ALERT MB = BacT/ALERT MB Susceptibility Kit.

* Micrograms per milliliter.

† MGIT and BacT/ALERT MB are not FDA approved.

‡ Critical concentration of the drug in this medium.

drug determines whether the isolate is considered resistant. The additional higher concentration of drugs, however, can provide the physician with information about the level of drug resistance in deciding whether to continue therapy with a drug either at the recommended dose or at an increased dose. When drug resistance is noted, it is important that the clinician with less experience seek assistance from experts in the field or from the local tuberculosis control program and the strain should be retested and confirmed as to its resistance pattern.

Investigations into mechanisms of antimicrobial drug action and of resistance in *M. tuberculosis* have benefited from studies in other bacterial species, especially *Escherichia coli* (114). A particular focus of such studies has been rifampin resistance because of the pivotal role of rifampin in the treatment of tuberculosis, the conserved nature of the genetic basis for resistance (> 96% of rifampin resistance in *M. tuberculosis* correlates with mutations in an 81-bp segment of the *rpoB* gene), and the use of rifampin resistance as a marker of multidrug-resistant *M. tuberculosis*. The methods employed to detect *rpoB* mutations include PCR amplification of the target sequence and detection by DNA sequencing, line probe assay, single-strand conformation polymorphism, and other molecular procedures. Before these techniques become widespread, technical simplification or automation, as well as outcome analysis in order to justify the anticipated increased costs compared with conventional approaches, will be required.

#### J. Genotyping of *Mycobacterium tuberculosis*

Genotyping or DNA fingerprinting of *M. tuberculosis* has replaced phage typing as a method for determining the clonality of bacterial cultures. The Southern blotting method is used, whereby cultured organisms are heat killed and their DNA is isolated, cut with specific restriction enzymes, separated in an agarose gel by electrophoresis, transferred to a membrane, and probed for specific genetic sequences. A standardized protocol has been developed to permit comparison of genotypes from different laboratories around the world (115). Genotyping is useful in confirming laboratory cross-contamination (116), investigating outbreaks of tuberculosis (117), evaluating contact investigations (118), and determining whether new episodes of tuberculosis are due to reinfection or reactivation (26). In addition, genotyping is useful for elucidating sites and patterns of *M. tuberculosis* transmission within communities (119, 120).

In response to several nosocomial outbreaks and a dramatic increase in tuberculosis among HIV-infected patients in the early 1990s, the Centers for Disease Control and Prevention established a National Tuberculosis Genotyping and Surveillance Network. 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 tuberculosis genotyping laboratories can be contacted through the state public health laboratories or tuberculosis control programs.

#### K. Assessment of Laboratory Performance

There has been a growing body of new and exciting methods in mycobacteriology, but there is still no single test that is diagnostic in all situations. Complementary techniques should be used to generate complete and rapid information. The laboratory director needs to decide which tests will be best performed in-house, taking into consideration the need for rapid results, particularly AFB smear results. Possible options for small laboratories would include splitting a specimen, with one portion processed locally for AFB smear and the other portion sent for culture to a reference laboratory; or obtaining two specimens, with one processed locally so that smear results can be obtained within 24 h and the other specimen shipped to a reference laboratory for further processing. Decisions as to which specimens should be sent to a reference laboratory should be based on the community served and the resources available, as well as in consultation with infectious disease, pulmonary, or other affected physicians. With this partnership, physicians will then share the responsibility for the quality and timeliness of laboratory results.

## VI. TUBERCULIN SKIN TEST

The tuberculin skin test is currently the only widely used method for identifying infection with *M. tuberculosis* in persons who do not have tuberculosis disease. Although currently the tuberculin skin test antigens that are available are substantially less than 100% sensitive and specific for detection of infection with *M. tuberculosis*, no better diagnostic method is widely available. Proper use of the tuberculin skin test requires a knowledge of the antigen used (tuberculin), the immunologic basis for the reaction to this antigen, the technique(s) of administering and reading the test, and the results of epidemiologic and clinical experience with the test.

#### A. Tuberculin

The tuberculin test is based on the fact that infection with *M. tuberculosis* produces a delayed-type hypersensitivity reaction to certain antigenic components of the organism that are contained in extracts of culture filtrates called "tuberculins." Two companies manufacture tuberculin purified protein derivative (PPD) in the United States: Parke-Davis Pharmaceuticals (Aplisol) and Pasteur Mérieux-Connaught Laboratories (Tubersol). Tuberculin PPD is isolated from a culture filtrate of tubercle bacilli by protein precipitation. The most commonly employed methods of precipitation use either ammonium sulfate (AS) or trichloroacetic acid (TCA). Aplisol is isolated by the AS method; Tubersol is isolated by the TCA method. Standardization should guarantee equivalent potency between PPD preparations isolated by different precipitation methods and between successive lots isolated by a single method. However, the difference in the chemical composition of the PPDs produced by the different precipitation methods implies that the actual antigens in the two preparations do differ. Most of the constituents of PPD are small proteins with molecular masses of approximately 10,000 Da, but there are also polysaccharides and some lipids present (121). The relatively small size of the protein constituents in PPD is the reason that PPD does not sensitize individuals who have not been exposed to mycobacteria (121).

A batch of PPD (lot 49608) called PPD-S, which was produced by Seibert and Glenn in 1939, has continued to serve as the international standard as well as the standard reference material in the United States (122). All PPD lots must be bioassayed to demonstrate equal potency to PPD-S (123). "Tuberculins" and "PPDs" have been prepared from other mycobacterial species, but these materials are less sensitive and specific for diagnosis of nontuberculous mycobacterial infections than is PPD for *M. tuberculosis* infections. These preparations are occasionally used for epidemiologic purposes (124), but have little clinical utility.

The standard 5-tuberculin unit (TU) dose of PPD-S is defined as the delayed skin test activity contained in a PPD-S dose of 0.1 mg/0.1 ml. The standard test dose of a commercial PPD preparation is defined as the dose of the product that is biologi-

cally equivalent to that contained in 5 TU of PPD-S (i.e., it elicits reactions of equivalent size ± 20%). Other doses of tuberculin, such as 1 and 250 TU, are remnants of the old graduated system of administration (125) and represent the smallest and largest doses of tuberculin that were administered. These strengths of tuberculin are not commonly available and, even if available, are not standardized by bioassay. The clinical utility of these concentrations of tuberculin is minimal.

PPD, when diluted in a buffered diluent, is adsorbed in varying amounts by glass and plastics. A small amount of the detergent Tween 80 is added by the manufacturer to the diluent for PPD to reduce adsorption (126). To minimize reduction in potency by adsorption, tuberculin should never be transferred from one container to another, and skin tests should be given as soon after the syringe has been filled as possible. Following these procedures will also help avoid contamination: test doses should always be removed from the vial under strictly aseptic conditions, and the remaining solution should be kept refrigerated (not frozen). Tuberculin should be stored in the dark as much as possible and exposure to strong light should be avoided.

#### B. Immunologic Basis for the Tuberculin Reaction

The reaction to intracutaneously injected tuberculin is the classic example of a delayed (cellular) hypersensitivity reaction. T cells sensitized by prior infection are recruited to the skin site where they release lymphokines (127). These lymphokines induce induration through local vasodilatation, edema, fibrin deposition, and recruitment of other inflammatory cells to the area (128). Features of the reaction include (*1*) its delayed course, reaching a peak more than 24 h after injection of the antigen; (*2*) its indurated character; and (*3*) its occasional vesiculation and necrosis. Reactivity of the PPD provides a general measure of a person's cellular immune responsiveness (121).

Typically, the reaction to tuberculin begins 5 to 6 h after injection, causes maximal induration at 48 to 72 h, and subsides over a period of days. In a few individuals (the elderly and those who are being tested for the first time), the reaction may not peak until after 72 h (129). Such delayed reactions do not alter the intrepretation of the test. Immediate hypersensitivity reactions to tuberculin or constituents of the diluent can also occur. These reactions disappear by 24 h, and should not be confused with delayed hypersensitivity reactions. However, if the immediate reaction is severe, it may be prudent not to retest. Symptom screening is suggested if these individuals are part of a regular tuberculin skin testing program (e.g., healthcare workers).

#### C. Administration and Reading of Tests

The tuberculin test, like all medical tests, is subject to variability, but many of the inherent variations in administration and reading of tests can be avoided by careful attention to details. The test is administered by injecting 0.1 ml of 5-TU PPD intradermally (Mantoux method) into the volar or dorsal surface of the forearm. Other areas may be used, but the forearm is preferred. The use of a skin area free of lesions and away from veins is recommended. The injection is made using a one-quarter- to one-half-inch, 27-gauge needle and a tuberculin syringe. The tuberculin should be injected just beneath the surface of the skin, with the needle bevel upward or downward (130). A discrete, pale elevation of the skin (a wheal) 6 to 10 mm in diameter should be produced when the injection is done correctly. If it is recognized that the first test was improperly administered, another test dose can be given at once, selecting a site several centimeters away from the original injection. A note in the record should indicate the site chosen for the second test.

Tests should be read between 48 and 72 h after injection, when the induration is maximum. Tests read after 72 h tend to underestimate the true size of induration. Reading should be performed in a good light, with the forearm slightly flexed at the elbow. The basis of reading is the presence or absence of induration, which may be determined by inspection (from a side view against the light as well as by direct light) and by palpation. For standardization, the diameter of induration should be measured transversely to the long axis of the forearm and recorded in millimeters (130, 131). The absence of induration should be recorded as "0 mm," not "negative." Interobserver variability may be decreased by using the ball-point pen method of Sokal to measure induration (132, 133).

The multiple puncture test introduces tuberculin into the skin either by puncture with an applicator with points coated with dried tuberculin or by puncturing through a film of liquid tuberculin. All multiple puncture tests presently available use concentrated tuberculin. The quantity of tuberculin introduced into the skin by the multiple puncture test cannot be precisely controlled. For these reasons, multiple-puncture tests are not as reliable as the Mantoux method of skin testing (123) and should not be used as a diagnostic test. If a multiple puncture test has been placed, the test should be repeated using the Mantoux method, regardless of the result of the multiple puncture test unless there is vesiculation. In this case, the test should be considered positive and no Mantoux skin test placed. For each tuberculin test, a record should be made of the technique of administration (Mantoux or multiple-puncture), the kind and dose of tuberculin, and the size of reaction in millimeters of induration.

#### D. Interpretation of Skin Test Reactions

To interpret the tuberculin skin test appropriately, one must understand the sensitivity and specificity of the test as well as the positive and negative predictive value of the test. The sensitivity of a test is the percentage of people with the condition who have a positive test. If false-negative results are uncommon, the sensitivity is high. The PPD skin test has a reported false-negative rate of 25% during the initial evaluation of persons with active tuberculosis (134). This high false-negative rate appears to be due to poor nutrition and general health, overwhelming acute illness, or immunosuppression.

Immunosuppression can be either specific, which may be seen early during disease, or nonspecific, which can be the result of medications, malignancy, or HIV infection (135). Because of the low sensitivity of the test, especially in acutely ill patients and those who are infected with HIV, the tuberculin test cannot be used to eliminate the possibility of active tuberculosis (136). Other factors that may result in a false-negative test are shown in Table 5.

Vaccination with live-attenuated virus can cause suppression of the PPD response in patients known to be infected with *M. tuberculosis.* Live-attenuated vaccines that may cause false-negative PPD results are measles, mumps, rubella, oral polio, varicella, yellow fever, BCG, and oral typhoid (TY21a). This suppression does not appear within the first 48 h after measles vaccination, so the Advisory Committee on Immunization Practices recommends that tuberculin testing be done either on the same day as vaccination with live virus or 4–6 wk later (137–140).

The specificity of the test is the percentage of people without the condition who have a negative test. False-positive results decrease the specificity of a test. False-positive tuberculin tests occur in individuals who have been infected with other

**TABLE 5**

**FACTORS CAUSING FALSE-NEGATIVE TUBERCULIN SKIN TESTS**

| |
|---|
| Factors related to the person being tested |
| Infections |
| Viral (measles, mumps, chicken pox, HIV) |
| Bacterial (typhoid fever, brucellosis, typhus, leprosy, pertussis, overwhelming tuberculosis, tuberculous pleurisy) |
| Fungal (South American blastomycosis) |
| Live virus vaccinations (measles, mumps, polio, varicella) |
| Metabolic derangements (chronic renal failure) |
| Low protein states (severe protein depletion, afibrinogenemia) |
| Diseases affecting lymphoid organs (Hodgkin's disease, lymphoma, chronic leukemia, sarcoidosis) |
| Drugs (corticosteroids and many other immumosuppressive agents) |
| Age (newborns, elderly patients with "waned" sensitivity) |
| Stress (surgery, burns, mental illness, graft-versus-host reactions) |
| Factors related to the tuberculin used |
| Improper storage (exposure to light and heat) |
| Improper dilutions |
| Chemical denaturation |
| Contamination |
| Adsorption (partially controlled by adding Tween 80) |
| Factors related to the method of administration |
| Injection of too little antigen |
| Subcutaneous injection |
| Delayed administration after drawing into syringe |
| Injection too close to other skin tests |
| Factors related to reading the test and recording results |
| Inexperienced reader |
| Conscious or unconscious bias |
| Error in recording |

mycobacteria, including vaccination with BCG. Some antigens in PPD are shared with the other mycobacteria (141, 142) and thus can elicit a skin test response. These cross-reactions tend to result in smaller amounts of induration than reactions due to *M. tuberculosis*, but the overlap may be considerable in areas of the world where the other mycobacteria are common (136). In these populations, the specificity of the test is highly dependent on the criterion used to define a "positive" test. Thus, the specificity of the test can be improved by progressively increasing the cut point for positivity.

In any population, the likelihood that a positive test represents a true infection is influenced by the prevalence of infection with *M. tuberculosis.* Table 6 shows how the prevalence of infection influences the predictive value of a positive tuberculin test (positive predictive value). The tuberculin skin test has a specificity of approximately 99% in populations that have no other mycobacterial exposures or BCG vaccination, but the specificity decreases to 95% in populations where cross-reactivity with other mycobacteria is common. The general population of the United States as a whole currently has an estimated *M. tuberculosis* infection rate of 5–10%, thus causing the tuberculin skin test to have a low positive predictive value. Children entering school in many areas of the country have a 0.1–1% likelihood of being infected. The yearly incidence of new tuberculosis infection in the general U.S. population without known exposure to tuberculosis is estimated to be 0.1–0.01%. Therefore, screening of groups without a known or likely exposure to *M. tuberculosis* is not recommended. In these groups, a false-positive result is more likely than a true-positive result.

**TABLE 6**

**POSITIVE PREDICTIVE VALUE OF A TUBERCULIN TEST**

| Prevalence of TB infection (%) | Positive Predictive Value | |
|---|---|---|
| | Specificity of 0.95 | Specificity of 0.99 |
| 90 | 0.99 | 0.999 |
| 50 | 0.95 | 0.99 |
| 25 | 0.86 | 0.97 |
| 10 | 0.67 | 0.91 |
| 5 | 0.50 | 0.83 |
| 1 | 0.16 | 0.49 |
| 0.1 | 0.03 | 0.10 |
| 0.01 | 0.002 | 0.09 |

In contrast, among persons who have been in close contact to individuals with infectious tuberculosis, there is a 25–50% chance of being infected with *M. tuberculosis.* Likewise, in high-prevalence countries, adults have a similarly high prevalence of infection. In such individuals or populations, the tuberculin skin test is highly specific and a positive test is highly likely to indicate tuberculosis infection.

On the basis of the sensitivity, specificity, and the prevalence of tuberculosis in different groups, three cut points have been recommended for defining a positive tuberculin reaction. For individuals who are at great risk of developing tuberculosis disease if they become infected with *M. tuberculosis* (143), a cut point of $\geqslant$ 5 mm is recommended. Reactions in persons who have had recent close contact with tuberculosis and in persons with abnormal chest radiographs consistent with tuberculosis are more likely to represent infection with *M. tuberculosis* than cross-reactions. Persons who are immunosuppressed because of disease (e.g., HIV infection) or drugs (e.g., corticosteroids) are more likely to progress to tuberculosis disease if they are infected with *M. tuberculosis.* Therefore, using a lower cut point (e.g., 5 mm) for separating positive from negative reactions is appropriate in these groups. This will ensure that few persons infected with *M. tuberculosis* will be classified as having negative reactions, although a few persons not infected with tubercle bacilli will be classified as having positive reactions.

A cut point of $\geqslant$ 10 mm is suggested for individuals who have normal or mildly impaired immunity and a high likelihood of being infected with *M. tuberculosis* but are without other risk factors that would increase their likelihood of developing active disease (Table 7). In addition to those groups listed, other high-prevalence populations may be identified locally.

Persons who are not likely to be infected with *M. tuberculosis* should generally not be tuberculin tested since the predictive value of a positive test in low-prevalence populations is

**TABLE 7**

**HIGH-PREVALENCE AND HIGH-RISK GROUPS**

| High-prevalence Groups | High-risk Groups |
|---|---|
| Persons born in countries with high prevalence of TB | Children less than 4 yr of age |
| Groups with poor access to health care | Persons with HIV coinfection |
| Persons who live or spend time in certain facilities (e.g., nursing homes, correctional institutions, homeless shelters, drug treatment centers) | Persons who are close contacts of persons with infectious TB |
| Persons who inject drugs | Persons whose tuberculin skin test results converted to positive in the past 1–2 yr |
| | Persons who have chest radiographs suggestive of old TB |
| | Persons with certain medical conditions* |

* Diabetes mellitus, silicosis, prolonged therapy with corticosteroids, immunosuppressive therapy, leukemia, Hodgkin's disease, head and neck cancers, severe kidney disease, certain intestinal conditions, malnutrition (143).

poor. However, if a skin test is done, e.g., at entry into a work site where some risk of exposure to tuberculosis is anticipated and a longitudinal tuberculin testing program is in place, a higher cut point of ⩾ 15 mm is suggested in order to improve the specificity of the test (136) (for additional information, please refer to the ATS Supplement: Targeted tuberculin testing and treatment of latent tuberculosis infection. *Am. J. Respir. Crit. Care Med.* 2000;161:S221–S247). These guidelines are summarized in Table 8.

### E. Boosted Reactions and Serial Tuberculin Testing

In most individuals, PPD skin test sensitivity persists throughout life. However, over time, the size of the skin test may decrease and may disappear. If PPD is administered to infected individuals whose skin tests have waned, the reaction of the initial test may be small or absent; however, there may be an accentuation of response on repeated testing. This is called the "booster effect" and can be misinterpreted as a skin test conversion. Boosted reactions also are particularly common in individuals exposed to other mycobacteria or who have been vaccinated with BCG. If repeated tuberculin testing is anticipated, as in health care workers, for example, a two-step method is recommended. In this method, persons who have a negative initial PPD skin test undergo a second tuberculin test 1–3 wk after the first. The results from the second test should be considered to be the "correct" result, i.e., those individuals with a positive reaction on the second test should be considered to be previously infected, and those with a negative reaction on the second test should be considered uninfected. In these uninfected persons, a positive result on any future PPD skin test should be interpreted as a skin test conversion (144). Repeated skin testing with tuberculin will not induce a positive skin test reaction in individuals who have no cellular immunity to the antigens in PPD (145).

### F. Previous Vaccination with BCG

Immunization with bacillus Calmette–Guerin (BCG) is currently used in many parts of the world as a means of preventing tuberculosis. BCG is named after the two French investigators responsible for developing the vaccine from an attenuated strain of *M. bovis*. Millions of people around the world have been vaccinated with BCG, but even so, the efficacy of the vaccine is uncertain. There are ample data indicating that BCG vaccine protects against disseminated tuberculosis and meningitis in children (146). However, the protection afforded by BCG against pulmonary disease in both children and adults is not proven (147). Interestingly, skin test reactivity resulting from vaccination does not correlate with protection against tuberculosis (148–150). Genetic variability of the subjects vaccinated, the nature of the mycobacteria endemic in different parts of the world, the use of different strains of BCG for immunization, and the use of different doses of vaccine and different schedules of immunization may all contribute to this variability in efficacy (151).

There is no reliable method of distinguishing tuberculin reactions caused by vaccination with BCG from those caused by natural mycobacterial infections. It is usually prudent to consider "positive" reactions to 5 TU of PPD tuberculin in BCG-vaccinated persons as indicating infection with *M. tuberculosis*, especially among persons from countries with a high prevalence of tuberculosis. There are several reasons for not assuming that a large reaction to tuberculin is due to BCG vaccination: (*1*) tuberculin test conversion rates after vaccination may be much less than 100%; (*2*) the mean reaction size among persons who have received BCG is often < 10 mm; and (*3*) tuberculin sensitivity tends to wane after vaccination. Although a positive tuberculin skin test due to BCG vaccination can wane over time, it can be "boosted" by serial testing. Because most persons who have received BCG are from high-prevalence areas of the world, it is important that vaccinated persons who have a positive reaction to a tuberculin skin test be evaluated for tuberculosis and treated accordingly (152, 153).

### G. Definition of Skin Test Conversions

It may be difficult to determine the relevance of a tuberculin skin test if there are two readings with different degrees of induration recorded. Because there are unavoidable errors in even the most carefully performed tests, small increases in reaction size may not be meaningful. For persons with negative tuberculin skin test reactions who undergo repeat skin testing (e.g., health care workers), an increase in reaction size of 10 mm or more within a period of 2 yr should be considered a skin test conversion indicative of recent infection with *M. tuberculosis*.

In some individuals who have been infected with nontuberculous mycobacteria or have undergone BCG vaccination, the skin test may show some degree of induration. For these individuals, a conversion to "positive" is defined as an increase in induration by 10 mm on subsequent tests.

### H. Anergy Testing in Individuals Infected with HIV

HIV-infected persons may have a compromised ability to react to tuberculin skin tests because of cutaneous anergy (26, 154).

**TABLE 8**

**GUIDELINES FOR DETERMINING A POSITIVE TUBERCULIN SKIN TEST REACTION**

| Induration ⩾ 5 mm | Induration ⩾ 10 mm | Induration ⩾ 15 mm |
|---|---|---|
| HIV-positive persons | Recent arrivals (< 5 yr) from high-prevalence countries | Persons with no risk factors for TB |
| Recent contacts of TB case | Injection drug users | |
| | Residents and employees* of high-risk congregate settings: prisons and jails nursing homes and other health care facilities, residential facilities for AIDS patients, and homeless shelters | |
| | Mycobacteriology laboratory personnel | |
| Fibrotic changes on chest radiograph consistent with old TB | Persons with clinical conditions that make them high-risk: silicosis diabetes mellitus, chronic renal failure, some hematologic disorders (e.g., leukemias and lymphomas), other specific malignancies (e.g., carcinoma of the head or neck and lung), weight loss of > 10% of ideal body weight, gastrectomy, jejunoileal bypass | |
| Patients with organ transplants and other immunosuppressed patients (receiving the equivalent of > 15 mg/d Prednisone for > 1 mo) | Children < 4 yr of age or infants, children, and adolescents exposed to adults in high-risk categories | |

* For persons who are otherwise at low risk and are tested at entry into employment, a reaction of > 15 mm induration is considered positive.

Anergy testing is the assessment of response to skin test antigens to which a cell-mediated delayed-type hypersensitivity (DTH) is expected. An impaired DTH response is directly related to decreasing $CD4^+$ T cell counts and, therefore, as HIV infection causes decreasing $CD4^+$ counts, the incidence of anergy increases. Some studies have shown that anergy testing provides additional prognostic information for HIV complications and death after stratification of $CD4^+$counts (155, 156).

Skin tests to identify anergy are placed by intradermal injection, using the Mantoux method, but there is no standard convention for classifying a positive response. Individuals who mount a response to any antigen are considered to have relatively intact cellular immunity, whereas those who cannot mount any responses are considered "anergic." The PPD tuberculin test elicits a DTH response and therefore people with a "positive" PPD are not anergic (157). Unfortunately, the validity of anergy testing in the diagnosis of tuberculosis is questionable. First, selective nonreactivity to PPD is a recognized phenomenon (158). Second, mumps reactivity may remain after loss of PPD reactivity (159). Third, PPD boosting can occur in people with an initial positive reaction to control antigens (160). Because of these findings, a positive DTH response to antigens other than PPD is not proof that a negative PPD applied at the same time indicates absence of TB infection. Conversely, failure to respond to anergy tests does not mean that an individual will not respond to PPD. In addition, serial anergy testing among HIV-infected persons has shown unpredictable variations over time (159), and how much variation is due to changes in immune competence and how much is due to differences in the tests themselves cannot be determined (157). Thus, anergy testing is not recommended for use in identifying tuberculosis infection in individuals, including those who are HIV infected (157).

## VII. CLASSIFICATION OF PERSONS EXPOSED TO AND/OR INFECTED WITH *Mycobacterium tuberculosis*

This classification is based on the broad host–parasite relationships as described by exposure history, infection, and disease. It is intended mainly as an operational framework for public health programs. The HIV status of an individual should be known, since HIV infection may change the approach to diagnosis and therapy for tuberculosis.

*0. No tuberculosis exposure, not infected.* Persons in this class have no history of exposure and a negative reaction to the tuberculin skin test (if tested).

*1. Tuberculosis exposure, no evidence of infection.* Persons in class 1 do have a history of exposure but have a negative reaction to the tuberculin skin test. Action taken for persons in this class depends mainly on the degree and recency of exposure to *M. tuberculosis,* as well as the immune status of the exposed person. If there has been significant exposure within 3 mo, a follow-up skin test should be performed 10 wk after the last exposure and in the interim, treatment of latent tuberculosis infection should be considered, especially for children less than 15 yr of age and persons with HIV infection.

*2. Latent tuberculosis infection, no disease.* Persons in class 2 have a positive reaction to the tuberculin skin test (indicate mm in duration), negative bacteriologic studies (if done), and no clinical, bacteriological, or radiographic evidence of active tuberculosis. Treatment of latent tuberculosis infection may be indicated for some persons in this group.

*Chemotherapy status*
- Never received therapy
- Currently receiving chemotherapy (date and regimen)
- Therapy complete (dates and prescribed course of therapy)
- Therapy incomplete (dates and regimen)

*3. Tuberculosis, clinically active.* Class 3 includes all patients with clinically active tuberculosis whose diagnostic procedures are complete. If the diagnosis is still pending, the person should be classified as a tuberculosis suspect (Class 5). To fit into Class 3, a person must have clinical, bacteriological, and/or radiographic evidence of current tuberculosis. This is established most definitively by isolation of *M. tuberculosis.* A person who had past tuberculosis and who also currently has clinically active disease belongs in Class 3. A person remains in Class 3 until treatment for the current episode of disease is completed. This group is further defined by the following features:

*Location of disease*
- Pulmonary
- Pleural
- Lymphatic
- Bone and/or joint
- Genitourinary
- Disseminated (miliary)
- Meningeal
- Peritoneal
- Other

The predominant site should be listed. Other sites may also be listed. Anatomic sites may be specified more precisely.

*Bacteriologic status*
- *Negative*
  - Not done
  - Microscopy (date)
  - Nucleic acid amplification (date)
  - Culture (date)
- *Positive*
  - Microscopy (date)
  - Nucleic acid amplification (date)
  - Culture (date)
  - Susceptibility results with method and concentrations used (date)

The following data are necessary under certain circumstances:

*Chest radiograph findings*
- Normal
- Abnormal
- Cavitary or noncavitary
- Stable or worsening or improving

*Tuberculin skin test reaction*
- Positive (mm induration)
- Negative (mm induration)

*4. Tuberculosis: not clinically active.* This classification is defined by a history of previous episode(s) of tuberculosis or abnormal stable radiographic findings in a person with a positive reaction to tuberculin skin test (indicate mm induration), negative bacteriologic studies (if done), and no clinical and/or radiographic evidence of current disease. Persons in Class 4 may never have received chemotherapy, may be receiving treatment for latent infection, or may have completed a previously prescribed course of chemotherapy. If current clinically active disease has not been ruled out, especially in persons not adequately treated in the past, this person should be classified

as a tuberculosis suspect (Class 5) until diagnostic evaluation permits classification as Class 3 or Class 4.

*5. Tuberculosis suspect (diagnosis pending).* Persons should be so classified when a diagnosis of tuberculosis is being considered, whether or not treatment has been started, until diagnostic procedures have been completed. Persons should not remain in this class for more than 3 mo. When diagnostic procedures have been completed, the person should be placed in one of the preceding classes.

## VIII. REPORTING OF TUBERCULOSIS

By law and regulation, a case of active tuberculosis in the United States must be reported to the local health department. Reporting is essential for action by the tuberculosis control program at local, state, and national levels, and for understanding of the magnitude and the distribution of the tuberculosis problem.

Reporting makes the resources of the health department available to assist the physician in the proper management of the case. Public health services are available for epidemiologic evaluation, including the identification and the examination of source cases and contacts. Therefore, reporting of tuberculosis suspects promptly (prior to bacteriologic confirmation) is important. Health department laboratory and radiographic services and consultation are generally available to assist the physician in carrying out responsibilities in the treatment of tuberculosis (161). All positive smear, culture, susceptibility, and nucleic acid amplification results should be reported to the health department and treating physicians within one working day. Ideally, the actual *M. tuberculosis* culture should be sent to the state laboratory so that genotyping can be performed when needed.

This statement was prepared by an ad-hoc committee of the Scientific Assembly on Microbiology, Tuberculosis, and Pulmonary Infections. Members of the committee are:

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