American Thoracic Society

MEDICAL SECTION OF THE AMERICAN LUNG ASSOCIATION

DIAGNOSTIC STANDARDS AND CLASSIFICATION OF TUBERCULOSIS

This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, June 1989. This is a joint statement of the American Thoracic Society and the Centers for Disease Control.

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Introduction

Historically, the American Thoracic Society (ATS) and the Centers for Disease Control (CDC) have provided guidance on the diagnosis, treatment, prevention, and control of tuberculosis in the United States and Canada. The ATS-CDC recommendations are contained, for the most part, in three official joint statements: "Diagnostic Standards and Classification of Tuberculosis," "Treatment of Tuberculosis and Tuberculosis Infection in Adults and Children," and "Control of Tuberculosis." Because the technology applicable to the diagnosis, treatment, and control of tuberculosis continues to evolve, periodically it is necessary to revise these statements. This revision has been made primarily to update the information on tuberculin skin testing and laboratory services for the diagnosis of tuberculosis, and to remove material pertaining to nontuberculous mycobacterial diseases, which will henceforth be dealt with separately.

The development of specific chemotherapeutic agents has revolutionized the prognosis of tuberculosis and tuberculous infection, making tuberculosis truly curable and preventable. However, the realization of the promise of chemotherapy must, of necessity, depend on prompt and accurate diagnosis. The objectives of the Diagnostic Standards continue to be:

1. To describe the general principles of the transmission, pathogenesis, and diagnosis of tuberculosis. The approach to diagnosis of tuberculosis follows from the concepts of its pathogenesis.

2. To provide a classification scheme for tuberculosis that is based on pathogenesis, is consistent with current chemotherapy and chemopreventive recommendations, and is applicable to all in whom the diagnosis is or should be considered. Definitions have been selected that:

- assure an accurate diagnosis;
- coincide with the appropriate response of the health-care team, whether it be no response, preventive therapy, or chemotherapy;
- provide the most useful information that correlates with the prognosis;
- provide the necessary information for appropriate public health action; and
- 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.

As with previous editions, this edition of Diagnostic Standards has been prepared as a basic guide and statement of principles for all persons involved in the care of patients with tuberculosis. To encourage the use of this publication as a primary resource, the content has been limited to the essential material appropriate to the divergent educational backgrounds and varied needs of the intended audience. The reader should refer to standard texts, monographs, and journal articles for more basic, detailed, or technical information concerning anatomy, physiology, pathology, radiology, bacteriology, and epidemiology as related to tuberculosis.

Transmission and Pathogenesis

Transmission of Mycobacterium tuberculosis

The infectious agent of tuberculosis, Mycobacterium tuberculosis, is carried on airborne droplet nuclei. Droplet nuclei are produced when persons with pulmonary tuberculosis cough, sneeze, speak, or sing. They also may be produced through manipulation of lesions or processing of tissue or secretions in the hospital or laboratory. Droplet nuclei are so small (1 to 5 μm) that air currents normally present in any indoor space can keep them airborne for long periods of time. Once released from the host, they are dispersed throughout the room.

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 airway or trachea, where they are trapped in the mucous blanket, carried to the oropharynx, and swallowed or expectorated.

Techniques that reduce the number of droplet nuclei in the room air are effective in preventing the airborne transmission of tuberculosis. Ventilation with fresh air is especially important, with six or more room-air changes an hour being desirable. The number of viable airborne tubercle bacilli can be reduced by ultraviolet irradiation of air in the upper part of the room. Effective antituberculosis chemotherapy reduces the number of bacilli released into the air by reducing the number of organisms in the sputum and the frequency of coughing. Covering the mouth and the nose with tissues while coughing or sneezing, or more effectively with a mask, reduces the number of organisms by reducing the number of droplet nuclei put into the air. If masks are to be used, they should be fabricated to fit tightly around the nose and mouth. Methods once thought to be important in preventing the transmission of tuberculosis - 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.

M. 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 essentially been eliminated in developed countries as a result of the pasteurization of milk and effective tuberculosis control programs for cattle. Airborne transmission of M. bovis can also occur.

Pathogenesis of Tuberculosis

After inhalation of a droplet nucleus, it passes down the bronchial tree without settling in the bronchiole or alveolus of the mucociliary system. Here the bacilli may multiply with no initial resistance from the host. The organisms are slowly engulfed by macrophages, but they may remain viable and even multiply within the cells.

The tubercle bacilli are spread through the lymphatic channels to regional lymph nodes and 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 immunity develops and limits multiplication.

Specific immunity is usually adequate to limit further multiplication of the bacilli; the host remains asymptomatic, and the lesions heal. Overall, in approximately 5% of all infected persons, control of the replicating organisms is inadequate, and disease occurs within 1 yr of infection. In another 5%, containment of the organism fails at a time more remote from the acquisition of infection, and disease results. Thus, overall, approximately 10% of persons infected with M. tuberculosis will develop clinical tuberculosis sometime during their lives. 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., human immunodeficiency virus infection (HIV), as well as by corticosteroids and other immunosuppressive drugs, and by gastrectomy. In these circumstances, the likelihood of tuberculosis developing is greater. Susceptibility to tuberculosis also may be greater during the first 2 yr of life, at puberty, and during adolescence.

The inflammatory response in tissues and subsequent necrosis, along with some of the systemic symptoms of tuberculosis, are the host's immunologic response to the bacilli. The host's immune system eliminates the bacilli that have spread through the body to distant sites. Certain organs and tissues are notably predisposed to much more severe forms of tuberculosis. In HIV-infected persons with tuberculosis, dissemination of tubercle bacilli and a variety of extrapulmonary manifestations are common. Unusual clinical presentations of tuberculosis in HIV-infected persons thus present a special diagnostic challenge.

Clinical and Radiographic Manifestations

Tuberculosis may simulate many other diseases. It may mimic, or occur concurrently, with pneumoconiosis, pneumonia, bronchiectasis, sarcoidosis, lung abscess, neoplasm, and fungal infection. Symptomatic patients with disease can be characterized as having generalized or systemic signs and symptoms, pulmonary signs and symptoms, signs and symptoms related to other organs, or a combination of these features.

Systemic Signs and Symptoms

Tuberculosis usually causes symptoms. However, many patients, even some with extensive disease, have insidious symptoms that are commonly ignored. Other patients may be truly asymptomatic. Asymptomatic patients and persons who do not recognize insidious or even frank symptoms can be identified only through a history of exposure, an abnormal chest radiograph, a positive reaction to a tuberculin skin test, and cultures positive for tubercle bacilli.
Many patients may first be aware of fatigue, anorexia, weight loss, irregular menses, or low-grade fevers that persist over weeks to months. These signs and symptoms are often attributed to overwork or emotional stress. Other patients present with acute febrile illness, chills, and generalized influenza-like illness, and medical attention is not sought until the symptoms fail to resolve. Acute symptoms may be superimposed on a more chronic pattern. Erythema nodosum may occur with the acute onset of tuberculosis.

At times, nontuberculous systemic symptoms associated with fever of unknown origin may be the only manifestations of tuberculosis. This syndrome can defy intensive diagnostic evaluation, and may be resolved only through a systematic escalation of diagnostic studies— for example, repeated chest radiographs, biopsies, and cultures of specimens from such organs as lung, pleura, pericardium, liver, and peritoneum. Often identified as abnormal; biopsies and cultures of bone marrow; or even an exploratory laparotomy.

Hematogenous dissemination (miliary tuberculosis) can occur at any age. Patients may be acutely ill with fever, dyspnea, and cyanosis, or be chronically ill with systemic symptoms. A cryptic presentation—unexplained fever, often accompanied by hematologic abnormalities such as pancytopenia or leukemoid reaction—is particularly common in patients older than 60 years of age. Meningocele involvement commonly coexists. Physical findings may include tubercles seen on examination of the optic fiscus, hepatomegaly, splenomegaly, and generalized lymphadenopathy.

### Signs and Symptoms of Pulmonary and Pleural Tuberculosis

Characteristically, in pulmonary tuberculosis, there is the almost imperceptible onset of a cough. This slowly progresses over weeks or months to become more frequent and associated with the production of mucoid or mucopurulent sputum. Hemoptysis also may occur. Occasionally, there is recurring dull, aching pain in the chest. Dyspnea is uncommon and usually indicates extensive parenchymal involvement, massive pleural effusion, or other underlying cardiopulmonary disease. Some patients present with the acute onset of productive cough, fever, chills, myalgia, and sweating similar to the signs and symptoms of influenza, acute bronchitis, or pneumonia. Physical findings may include rales or signs of lung consolidation.

Tuberculous pleuritis is generally unilateral. It may be associated with acute or recurrent pleuritic pain. Most patients with pleuritis have low-grade systemic symptoms, but it is not unusual for a patient to have an acute febrile toxic illness, and some patients are asymptomatic. Tuberculous empyema, with or without a bronchopleural fistula, may complicate extensive parenchymal disease. The physical findings of pleural effusion may or may not be present.

### Radiographic Examination of the Chest

In patients who have signs and/or symptoms suggesting pulmonary or pleural tuberculosis, standard posterior-anterior and lateral radiographs of the chest should be obtained. Apical-lobar or oblique views may aid in visualizing lesions obscured by bony structures of the heart. Special imaging techniques such as computed topography and magnetic resonance imaging may be of particular value in defining nodules, cavities, cysts, calcifications, contours of large bronchi, and vascular details in lung parenchyma. Bronchography may be useful in the definition of bronchial stenosis or bronchiectasis. Fluoroscopy should be reserved for the demonstration of the mobility of thoracic structures and for the visualization of localized lesions to guide diagnostic procedures.

The initial radiographic manifestation of initial infection in the lung, whether in a child or an adult, is usually parenchymal infiltration accompanied by ipsilateral lymph node enlargement. The parenchymal lesion may be detected at any stage of development and in any portion of the lung, or it may be too small to be seen on the radiograph. Tuberculous involvement of the hilar or mediastinal nodes is usually unilateral. Lymph node changes tend to persist longer than the parenchymal shadows. Calcification of the lung lesions and the lymph nodes may occur several years after infection. Tuberculosis in HIV-infected patients may have the radiographic characteristics of primary disease.

More commonly, in adults, lesions are seen in the apical and posterior segments of the upper lobes, or in the superior segments of the lower lobes. However, lesions may appear in any segment; nodular infiltrates of varying size are perhaps most common. Lesions may be dense and homogeneous, with lobar, segmental, or subsegmental distribution. Cavititation is common, except in immunocompromised patients. One may also find lower lobe nodular infiltrates without cavitation upon radiograph observation of tuberculosis in the elderly. Other findings include atelectasis and fibrotic scarring with retraction of the hilus, and deviation of the trachea. Hematogenous tuberculosis is characterized by diffuse, finely nodular, uniformly distributed lesions on the chest radiograph; fever and systemic symptoms and signs may antedate this finding. The term “miliary” is applied to this appearance because the nodules are about the size of millet seeds (approximately 2 mm in diameter). Unilateral or, rarely, bilateral pleural effusion is usually the only radiographic abnormality evident with pleural tuberculosis. Although these are the more common radiographic patterns, tuberculosis may produce almost any form of pulmonary radiographic abnormality. Rarely, patients may present with normal radiographs, particularly patients with HIV infection and endobronchial tuberculosis.

A single chest radiograph should not be used as a guide to stability or the nature of the underlying disease. The use of words such as “old” or “fibrotic” should be avoided when interpreting a single radiograph because this often will be misleading. However, chest radiographs that show no change during a 3-month period generally indicate confirmed tuberculosis or another disease. Any persistent infiltrate in an older person must include tuberculosis among the diagnostic possibilities. Most of these forms of tuberculosis are missed, especially among nursing-home patients.

### Pulmonary Physiologic Changes

There are no specific changes in pulmonary function related to tuberculosis. With extensive parenchymal involvement, the vital capacity and other lung volumes are decreased. The radiographic changes appear to correlate somewhat better with a decrease in the single-breath carbon monoxide diffusing capacity. Reductions in vital capacity and total lung capacity also may result from extensive pleural involvement. Quite often there is a remarkable preservation of arterial oxygen tension, despite extensive lung destruction, which indicates a decrease in perfusion to match the decreased ventilation to destroyed areas of the lung. With extensive fibrotic residual tuberculosis, cor pulmonale may supervene. Rarely, acute tuberculosis can cause diffuse infiltration of the lungs with resultant respiratory failure in a pattern resembling the adult respiratory distress syndrome.

### Extrapulmonary Tuberculosis

#### Genitourinary

The following findings should prompt the consideration of genitourinary tract tuberculosis: recurrent urinary tract infections with no growth of common bacterial pathogens; pyuria without bacteriuria; unexplained hematuria; recurrent fever without explanation; or an excretory pyelogram of abnormalities in the calices, pelvis, ureters, or bladder, especially when multiple areas are involved. Men may also present with a beaded vas deferens on palpation, a draining scrotal sinus, epididymis (particularly with calcification), or induration of the prostate or seminal vesicles. Women may present with irregular menses, amenorrhea, pelvic inflammatory disease (salpingo-oophoritis or endometritis), or infertility. A diagnosis is generally obtained with repeated cultures of Fist-voided early morning urine specimens or is made on the basis of culture and histologic examination of biopsy material. Some patients with pulmonary tuberculosis (5 to 10%) have urine cultures positive for M. tuberculosis even though there are no signs, symptoms, or laboratory data otherwise to suggest genitourinary involvement (i.e., normal urinalysis, normal intravenous pyelogram).

#### Lymph node

Tuberculosis may involve any lymph node. Hilar or mediastinal lymphadenitis or both are most frequently seen shortly after the initial pulmonary infection. Tuberculosis of the cervical and supraclavicular lymph nodes is common. Attention is often
sought because of a mass in the neck. Spontaneous drainage may be noted. In adults, granulomatous lymphadenitis is almost invariably caused by *M. tuberculosis*; in children, especially those younger than 5 yr of age, nontuberculous mycobacteria are more common. Material for mycobacterial stains and cultures can be obtained by needle aspiration or surgical biopsy, or from draining fluid.

**Bone and joint.** With skeletal tuberculosis there is usually both arthritis and osteomyelitis. Fever and localized pain are common. The lower spine and weight-bearing joints are most often affected; there may be multiple osteolytic lesions (although they may not be noted on radiograph). About half of these patients do not have evidence of pulmonary involvement. Skeletal disease is commonly seen in the elderly.

**Meninges.** Abnormal behavior, headaches, or convulsions often herald tuberculosis meningitis, and, commonly, there is evidence of hemogenous spread. Meningitis is frequent in infants and small children as an early complication of initial infection, but it may be seen in any age group. Usually, the cerebrospinal fluid has a low glucose content (compared with simultaneous blood glucose), increased protein, increased cells (commonly lymphocytes), and no growth of common bacterial pathogens. There may not always be a positive reaction to a tuberculin skin test. Differentiation from a fungal infection requires identification of the organism on slide, by culture, by serologic study, or by a combination of these studies.

**Peritoneum.** Tuberculous peritonitis is characterized by ascites and fever; a “doughy” abdomen or abdominal mass is occasionally noted. Concomitant pleural effusion is common. The majority of patients have no lung parenchymal abnormality visible on chest radiograph. The ascitic fluid usually has a protein percentage of at least 3 g. Histologic identification of a congingent granuloma in tissue can be obtained, usually by means of peritoneal ascites, culdoscopy, or laparotomy; a portion of this tissue should also be stained and cultured for mycobacteria. The diagnosis may also be established by culture of peritoneal fluid; yields are increased by submitting a large volume of fluid.

**Pericardium.** Tuberculous pericarditis is uncommon, but it has a high mortality rate and, therefore, warrants early recognition and treatment. The majority of patients with tuberculous pericarditis have extensive pulmonary involvement and pleural involvement is common. Because the recognition of pericarditis is difficult, signs and symptoms suggestive of pericardial disease should lead to an echocardiogram, pericardiocentesis, and pericardial biopsies.

**Larynx.** Occasionally, patients with tuberculous pericarditis first seek medical attention for hoarseness, a sore throat, or both. Careful questioning usually reveals both respiratory and systemic symptoms because laryngeal involvement with tuberculosis is usually associated with extensive pulmonary involvement and a large number of organisms in the sputum.

**Other organs.** Tuberculosis may involve nearly any organ or structure in the body and produce signs and symptoms related to the specific site of infection. For example, adrenal involvement may result in adrenal insufficiency. Intestinal involvement may cause abdominal pain and diarrhea, etc. Chronic granulomatous skin infections with deep tissue penetration may result from mycobacterial infection.

**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. These studies should be performed only in laboratories that have the work load and continued competence in areas of clinical mycobacteriology are assured. Even as clinical management of tuberculosis has become more successful, the laboratory procedures needed to diagnose and monitor the course of the disease have become more complex. These procedures are time-consuming and employ reagents and special techniques not routinely used 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.

**Levels of Laboratory Services for Mycobacterial Diseases**

With the closing of most tuberculosis sanatoria and the treatment of patients in general hospitals or outpatient clinics, the support of mycobacteriology services has been spread diffusely through many more and, more specialized, laboratories, each processing fewer and fewer specimens. Maintenance of proficiency requires continuing and frequent performance of the required test procedures. When laboratory 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.

Clinical laboratory functions that contribute to the diagnosis and management of tuberculosis have been divided into three major categories of services offered. These are as follows:

**Level I.** Collection and transport of specimens; preparation and examination of smears for acid-fast bacilli.

**Level II.** Procedures of Level I, plus isolation and identification of *M. tuberculosis*. 

**Level III.** All procedures of Level II, plus identification of mycobacteria other than *M. tuberculosis*. The determination of drug susceptibility may be performed at Level II and should be performed at Level III.

The laboratory and the clinicians requesting service must be confident of the results it provides. A laboratory may choose to develop or maintain the skills defined under one of these levels, depending on the frequency with which specimens are received for isolation of mycobacteria, the need of the clinical community being served, and the availability of a specialized referral service. All laboratories doing clinical mycobacteriology should participate in recognized proficiency testing programs, 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 only a few laboratories in a given community or region where professional expertise and complete and safe facilities are available.

**Identification of Mycobacteria**

A multiplicity of mycobacterial species, both saprophytes and potential pathogens, may be isolated from humans. The Level II or Level III diagnostic laboratory, through the use of a few reliable *in vitro* tests, should be able to provide a precise species identification of most acid-fast bacilli isolated from patients. Through the cooperative efforts of clinicians and bacteriologists over the past decade, it has been possible to determine statistically the potential clinical significance of most species of mycobacteria. A clear-cut separation of pathogen from saprophyte is not always possible for the individual isolate except when *M. tuberculosis* is isolated. The isolation of a nontuberculous organism of potential clinical significance is not usually of importance unless the patient is diseased, nor, for that matter, should all isolates, which are usually clinically insignificant, be disregarded as saprophytes. Each mycobacterial isolate, as each patient, must be evaluated individually.

**Mycobacterium tuberculosis,** the causative agent of tuberculosis, is usually identified by its rough, nonpigmented, corded colonies on oleic-acid-albumin agar; a positive niacin test; generally weak catalase activity; and, although not necessarily in humans. Preliminary screening of all strains of *M. tuberculosis* for catalase activity may provide the clinician with valuable information relative to isoniazid resistance even before susceptibility tests are performed.

*M. bovis* is indistinguishable from *M. tuber-
coccus except by culture followed by in vitro tests. If an isolate proves to be other than *M. tuberculosis*, its precise identification should be established. This may require that the culture be referred to a large reference laboratory such as a state health department laboratory, a Veterans Administration reference laboratory, or the Centers for Disease Control (CDC) in Atlanta for definitive identification.

**Collection of Specimens for Demonstration of Tubercle Bacilli**

Because the identification of organisms is so critical in diagnosing tuberculosis, it is of the 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 their collection. For optimal results, specimens should be transported to the laboratory and processed as soon as possible after collection. In some circumstances, specimens may have to be sent through the mails. Ideally, such specimens should be: (1) confined to single collections of 10 ml or less; (2) shipped according to directions obtained from the laboratory that will provide the requested diagnostic service; (3) mailed each day of the week; and (4) refrigerated or if immediate mailing is not possible. To minimize transit time, the use of overnight delivery should be considered.

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 more common specimens, such as sputum (natural or induced) and gastric aspirate, others include urine, cerebrospinal fluid, pleural fluid, bronchial washings, pus, endometrial scrapings, bone marrow biopsy, and other biopsies or resected tissue. The methods for collecting specimens are briefly outlined below.

**Sputum.** It is impossible for attending personnel to insure the proper collection of sputum at all times; therefore, the patient must be instructed how to obtain the most useful specimen. The patient should 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. Specimens should be collected in approved containers and clearly labeled with patient-identifying information and the date of collection. To prevent the exterior of the container from becoming contaminated during the collection, and to protect personnel while the specimen is in transit, the container should be placed in a disposable watertight plastic bag before being transported to the laboratory. A series of at least three single specimens on different days should be collected from sputum-producing patients.

For patients who have difficulty producing sputum, there are several methods of obtaining a specimen. Inhalation of an aerosol of hypertonic saline can be used to stimulate the production of sputum. Even though aerosol-induced specimens may appear thin and watery, they should be processed. Because the cough induced by this method may be violent and uncontrolled, special methods of air control, such as exhaust-fan-equipped sputum induction cubicles, or portable hoods with air filters, should be used.

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. For these reasons, gastric aspiration is best performed in hospitalized patients.

For patients in whom a diagnosis of tuberculosis has not been established from spontaneously produced sputum or by sputum induction, fiberoptic bronchoscopy may be necessary with bronchial washing, bronchial alveolar lavage, and/or transbronchial biopsy. Sputum produced after bronchoscopy 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. They may reveal tubercle bacilli even though none were seen in the specimen obtained on the day of the bronchoscopy.

Occasionally, a pooled specimen, collected over a period of 12 to 24 h, may be helpful when the methods described above are not effective or appropriate. Because such specimens are more subject to contamination, best results are obtained when they can be processed in the same institution where collection occurred. If pooled specimens must be sent through the mails, the use of cetylpyridium chloride as a digestant/decontaminant in transit should be considered.

**Urine.** The first morning-voided midstream specimen is preferred. Multiple specimens are advisable because of the presence of mycobacteria. 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.

**Tissue and other body fluids.** Under a variety of circumstances, when invasive 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 with no 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, cerebrospinal, peritoneal, and pericardial fluids should be analyzed for protein and glucose (compared with simultaneous blood 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. For pleural tuberculosis the diagnostic yield can be increased by obtaining pleural tissue for histologic study and culture by needle biopsy at the time of diagnostic thoracentesis. Peritoneal biopsies are best obtained via laparoscopy.

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 permit 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 such as miliary tuberculosis—bone marrow biopsy, lung biopsy, and liver biopsy for histologic examination and culture must be considered.

**Digestion and Decontamination of Specimens**

Most clinical specimens contain an abundance of nontuberculous mycobacterial contaminants. 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) 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 other microorganisms, chemical digestion decontamination procedures have been successfully applied to insure the recovery of acid-fast bacteria from clinical materials.

**Staining and Microscopic Examination**

The detection of acid-fast bacilli 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.

It is estimated that the lowest concentration of organisms that can be detected by microscopic examination is 10/ml of sputum. Various studies have indicated that 50 to 80% of patients with pulmonary tuberculosis will have positive sputum smears.
In reading smears, the microscopist should provide the clinician with a rough estimate of the number of acid-fast bacilli detected. Methods for selective staining of mycobacteria are the conventional acid-fast stain (of which the Ziehl-Neelsen is an example) and the fluorescence procedure, which uses such stains as auramine and rhodamine.

Cultivation of Mycobacteria

All clinical specimens suspected of containing mycobacteria should be inoculated (after appropriate digestion and decontamination, if required) onto culture media for two reasons: (1) culture is much more sensitive than microscopy, being able to detect as few as 10 bacteria/ml of digested, concentrated material, (2) growth of the organisms is necessary for precise species identification. Numerous mycobacterial culture media are available. Most of them fall into the two general categories: (a) the non-base media and agar-base media. Whenever possible, digested clinical specimens should be inoculated onto both kinds of media. The most popular egg-base media are the Lowenstein-Jensen buffered egg-potato medium and the American Trudeau Society egg yolk-potato flour medium. Among the agar-based media, Middlebrook 7H-10, Middlebrook 7H-11, and Dubos oleic-albumin agar are recommended. Incubation of inoculated media in an atmosphere of 5 to 10% carbon dioxide enhances both the number of positive isolations and the actual number of cultivable colonies. Whatever procedure is used, the time from the laboratory’s receipt of the specimen to the clinician’s receipt of the culture report is usually 3 to 6 wk.

Mycobacterial growth observed on culture media should be quantified in some way. The following is a widely used scale:

- No colonies (negative)
- Fewer than 50 colonies
- 50-100 colonies
- 100-200 colonies
- 200-500 colonies (almost confluence)
- > 500 colonies (confluence)

With the more refined culture methods available today and with the processing of multiple specimens from the same patient, it is not necessary for the clinical laboratory to resort to animal inoculation. In a few rare instances, guinea pig inoculation may be used when: (1) specimens are consistently contaminated on culture; (2) specimens are positive on microscopy but repeatedly negative on culture (untreated specimens should be injected); (3) specimens are aseptically collected where organisms may be few in number, and every attempt is made to establish the diagnosis of tuberculosis; and (4) small numbers of M. tuberculosis are thought to be present in specimens known to contain other mycobacteria.

Drug Susceptibility Testing

The performance and interpretation of drug-susceptibility tests for M. tuberculosis may be helpful to the clinician in choosing the most effective antituberculosis agents and in the appraisal of the patient’s response to chemotherapy. The susceptibility of tubercle bacilli to various antituberculosis drugs may be determined by either the direct or the indirect test.

The direct drug-susceptibility test is performed by using clinical specimens in which acid-fast bacilli have been demonstrated in a smear of the digested, concentrated specimen. The specimen is inoculated directly onto drug-containing culture medium, and growth is compared with growth on non-drug-containing medium.

The indirect test is performed by using a subculture from the primary isolation as the inoculum. Although the direct test is preferred because it is more representative of the actual bacterial population in the patient, the indirect test may be required when: (1) the initial smear is negative, but the culture is positive; (2) growth on the control medium is inadequate for a reliable test; or (3) a reference culture is submitted by another laboratory.

The laboratory should report to the clinician the amount of growth on drug-containing medium as compared with growth on drug-free control medium. In a properly performed drug-susceptibility test, the control will have countable colonies. By counting the colonies on the drug-containing medium and on the control medium, the proportion of resistant cells in the total population can be calculated and expressed as a percentage. Generally, when 1% or more of a bacillary population becomes resistant to the “critical” concentration of a drug, then that agent is not, or soon will not be, useful for continued therapy because the resistant population will soon predominate.

Most populations of tubercle bacilli are either frankly resistant in vitro (i.e., > 50% resistance) or frankly susceptible (i.e., < 1% resistance). Intermediate readings on drug-containing medium are rare, but they may indicate that resistance is developing. Tests showing 1 to 10% resistance should be repeated, or another isolate should be examined if available.

The decision about whether to perform drug-susceptibility tests depends on an assessment of clinical and epidemiologic factors. Given the low prevalence of drug-resistant M. tuberculosis in most parts of the United States, the cost of routine testing of all initial isolates is difficult to justify. Thus, the previously untreated patient with newly diagnosed tuberculosis is generally started on chemotherapy without prior drug-susceptibility testing. If pretreatment susceptibility testing is not performed, an initial isolate should be retained for approximately 6 months for subsequent study by the laboratory if the patient does not respond as expected.

Initial susceptibility testing should be done in persons known to be at high risk for infection with drug-resistant organisms. These include: patients with a history of antituberculosis chemotherapy, foreign-born patients from areas with a high prevalence of drug resistance such as Asia, Africa, and Latin America; contacts of known or suspected resistant cases; and residents of geographic areas in the United States with a documented high prevalence of drug-resistant tuberculosis. Susceptibility testing should also be done in patients with life-threatening forms of tuberculosis such as disseminated disease or tuberculous meningeal to assure that they receive an appropriate chemotherapeutic regimen.

Susceptibility testing should be requested on subsequent isolates when a regimen appears to be failing. The manifestations of a failing regimen are: lack of conversion of smear and culture to negative within 3 months for persons receiving regimens containing both isoniazid and rifampin; lack of conversion of smear and culture to negative after 5 months for those receiving other regimens (without both isoniazid and rifampin); smears and cultures showing a decrease in number of organisms or colonies followed by a persistent increase in numbers (“fall and rise”).

Although it is expected that susceptibility tests will be requested when indicated by responsible physicians, there are some instances in which a laboratory should perform the tests even in the absence of a physician request. These are: culture-positive or smear-positive cerebrospinal fluid; positive cultures occurring 5 months beyond the first culture, even though it is recognized that a few specimens occasionally yield a small number of colonies during the period of patient recovery, and culture from retreatment cases.

Newer Technologies

For several decades, the field of clinical mycobacteriology remained rather stable. There were improvements in microscopy, refinements in digestion and decontamination methods, development of new tools for differential species identification, and application of numerical taxonomic methods. These resulted in sharpened capabilities to isolate mycobacteria from clinical specimens and to define the more than 50 species in the genus Mycobacterium. However, the long generation time of the mycobacteria and the multiplicity of time-consuming differential tests needed for species identification continued, until recently, to hamper the microbiologist.

In contrast, during the 1980s, several new technologies were introduced and several older, established techniques were recalled to service, which have combined to revolutionize mycobacteriology. The application of some of these technologies is still in its infancy, but there are several that should make a dramatic impact on the speed and precision of clinical mycobacteriology in the coming years.

Radiometric Technology

Perhaps the most widely used radiometric method to detect early growth of mycobac-
teria in culture is the BACTEC system, which employs a Y-labeled substrate medium that is almost specific for mycobacteria. Since its introduction, the BACTEC method has provided more rapid growth (average, 9 days), specific identification of M. tuberculosis (5 days), and rapid drug susceptibility testing (6 days). Although radiometric technology cannot replace completely the classic mycobacteriologic methods, and may under estimate drug resistance, this is a valuable new tool. Interfacing BACTEC (for more rapid growth) with techniques for rapid identification (e.g., genetic probes, high-pressure liquid chromatography, monoclonal antibodies) offers intriguing possibilities for future improvements in diagnosis.

**Genetic Probes**

The use of genetic probe technologies offers tremendous promise in providing microbial identification at a variety of levels-family, genus, species, or subspecies. The most common probe technology is the single-stranded, radiolabeled DNA probe, now available commercially. Probes specific for the genus Mycobacterium, the M. tuberculosis complex (including M. tuberculosis, M. bovis, M. bovis BCG, M. africanum, and M. microti), and the two species M. avium and M. intracel lular, are now available. Currently, all four probes may be used to identify the indicated mycobacteria grown in pure culture. A new technology for identification of both the genus Mycobacterium and of the M. tuberculosis complex directly in sputum is under study and shows promise. These probe identifications commonly are completed within 2 to 8 h, depending on the number of samples tested. The possibility of precise identification of M. tuberculosis directly in sputum within a few hours is revolutionary.

**Immunassay of Mycobacterial Antigens**

Antigens have been detected in liquid mycobacterial cultures only shortly after radiometric growth indices become positive. Both enzyme-linked immunosorbent assays (ELISA) and radiomunossays (RIA) have been used. Although still in the developmental phase, these assays appear to offer rapid species-specific identification. Monoclonal antibodies may be useful to confer specificity for individual epitopes in these assays. Dot blot immunossays are capable of recognizing species-specific catalases.

Direct antigen detection by immunoblot assay has been successfully accomplished by several laboratories in cerebrospinal fluid, and this approach may prove to be useful for the diagnosis of tuberculous meningitis. Immunassay of mycobacterial antigen in sputum or in other clinical specimens has been found to be technically difficult, and there are only a few reports of success with this approach.

**Serologic Diagnosis of Tuberculosis**

Many research laboratories have demonstrated that ELISA measurement of IgG antibody to mycobacterial antigens can be used for the serologic diagnosis of tuberculosis. Tests using highly purified antigens not widely available have been found to be more specific than those using PPD or other unpurified antigens. Under optimal circumstances, ELISA methodology has diagnostic test characteristics similar to those of sputum smear. As with the sputum smear, serology is most frequently positive in patients with advanced disease. Limited data suggest that ELISA serologic diagnosis may be useful for the diagnosis of extrapulmonary tuberculosis and of pulmonary tuberculosis in children from whom it is difficult to obtain sputum specimens. Other serodiagnostic techniques, including RIA-inhibition of monoclonal antibodies and latex agglutination, have had less extensive study but appear promising.

**Mycobacteriophage Typing**

An old tool brought into more active use, phage typing has been useful in detecting laboratory cross-contamination, investigating epidemics of tuberculosis, and determining whether relapse cases were due to reinfec tion or reactivation.

**Chemical Detection of Biologic Compounds**

Several new techniques have been developed to detect specific components produced either by the mycobacterial cells or by the diseased host in response to mycobacterial infection. Rapid confirmation of tuberculous meningitis has always been difficult for the microbiologist. Recently, adenosine deaminase, a host enzyme produced by activated T cells and easily detected by a colorimetric procedure, was shown to increase in concentration during the active stages of tuberculous meningitis and to decrease to normal levels after effective antituberculosis therapy. A more complicated technology detects the presence of tuberculosis acid in the spinal fluid or serum of patients. The presence of this compound in patients with meningitis supports a tuberculous etiology. Both of these techniques will have critical evaluation with regard to sensitivity, specificity, speed and ease of performance, and cost.

Another valuable tool has been the use of high performance liquid chromatography (HPLC) to detect the species-specific mycolic acids produced by those genera that contain these unique fatty acids. For the genus Mycobacterium, each of the species examined to date has its own unique mycolic acid pattern. When used on primary culture isolates, this technique enables species to be identified within 6 to 18 h rather than the 2 to 6 wk commonly needed for biochemical differentiation. Pilot studies on sputum samples have also revealed that strongly smear-positive specimens contain enough bacilli (>10^7/ml) to enable their direct specific identification by HPLC of sputum. In addition, capillary gas chromatography has been used to study the short-chain fatty acids and cleavage products of mycolic acids from mycobacteria. Combination of this with HPLC may provide a useful method of speciating the mycobacteria.

**Tuberculin Skin Test**

The tuberculin skin test has been the traditional method of demonstrating infection with M. tuberculosis. Although currently available tuberculin skin tests are substantially less than 100% sensitive and specific for detection of infection with M. tuberculosis, no better diagnostic methods have yet been devised. Intelligent interpretation of skin test results 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.

**Tuberculin**

The tuberculin test is based on the fact that infection with M. tuberculosis produces sensitivity to certain antigenic components of the organism that are contained in culture extracts called “tuberculins.” Two preparations of tuberculin are currently licensed for use in the United States: Old Tuberculin (GT) and Purified Protein Derivative (PPD). OT is available only in multiple puncture devices; Tuberculin PPD is available for intracutaneous injection by the Mantoux technique and by multiple puncture devices.

OT is a filtrate prepared from heat-sterilized, concentrated broth cultures of tubercle bacilli. PPD is a precipitate obtained from filtrates of OT. A large batch of PPD produced by Seibert in 1939 (Lot 49608) was designated PPD-S and became the International Standard as well as the U.S. reference standard for all tuberculins.

The standard 5 Tuberculin Unit (TU) dose of PPD-S is defined as the delayed skin test activity contained in 0.1 µg/0.1 ml dose of PPD-S. The standard test dose of a commercial PPD preparation is defined as the dose of the product that is biologically equivalent to that contained in 5 TU of PPD-S (i.e., it elicits reactions of equivalent size ±20%). Products labeled 1 TU and 250 TU do not necessarily contain one-fifth and 50 times the biologic activity of 5 TU of PPD-S. Instead, these preparations contain one-fifth and 50 times the concentration of antigen determined to be biologically equivalent to 5 TU PPD-S. Evaluation of the biologic activity of 1-TU and 250-TU products has not been required. The 250-TU product is sometimes useful in assessing the immunologic status of patients. Both the 1-TU and 250-TU products have limited usefulness in the diagnosis of tuberculous infection.

Tuberculoprotein, 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 manufac-
turer to the diluent for PPD to reduce adsorption. To minimize reduction in potency by adsorption, tuberculin should never be transferred from one container to another, and skin tests should be given soon after the syringe has been filled. 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.

**Immunologic Basis for the Tuberculin Reaction**

The reaction to intracutaneously injected tuberculin is the classic example of a delayed (cellular) hypersensitivity reaction. Characteristic features of the reaction include: (1) its delayed course, reaching a peak more than 24 h after testing; (2) its indurated character, largely because of cell infiltration; (3) its occasional vesiculation and necrosis. A delayed hypersensitivity reaction to tuberculin will indicate previous natural infection with *M. tuberculosis*, infection with a variety of non-tuberculous mycobacteria, or vaccination with BCG, a live attenuated mycobacterial strain derived from *M. bovis*.

Characteristically, delayed hypersensitivity reactions to tuberculin begin at 5 to 6 h, are maximal at 48 to 72 h, and subside over a period of days. In a few persons (those who are elderly or those who are being tested for the first time), reactions may develop slowly and may not peak until after 72 h. Immediately after injection, hypodermic needle, disappear by 24 h, and are not likely to be confused with delayed hypersensitivity reactions.

Unfortunately, not all persons infected with *M. tuberculosis* or *M. bovis* will have a delayed hypersensitivity reaction to a tuberculin skin test. A large number of factors (table 1) have been reported to cause a decreased ability to respond to tuberculin. The existence of one or more of these factors does not mean that testing should not be undertaken because only a fraction of infected persons with these conditions may have falsely nonreactive tests. The presence of reactions in such persons may still identify those in whom infection is highly probable. If the lack of reaction to the test is suspected of being a false response, a repeat tuberculin skin test should be done. If generalized inability to respond is suspected, then it may also be desirable to test delayed hypersensitivity using several other antigens to which the person has very likely been exposed. Those who fail to respond to any of these antigens are more likely to be anergic, a condition suggesting that the immune system is not functioning properly and that the lack of reaction to the tuberculin test may be a false response.

**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. Two techniques of applying the tuberculin test are currently in general use: the intracutaneous Mantoux and the punctate multiple-puncture methods.

**The Intracutaneous (Mantoux) Test**

The intracutaneous administration of a measured amount of tuberculin is the best means of detecting infection with *M. tuberculosis*. One-tenth milliliter of PPD will be injected into either the volar or the 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. 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 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. Reading should be performed in good light, with the forearm slightly flexed at the elbow. 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. The diameter of induration should be measured transversely to the long axis of the forearm and recorded in millimeters.

**The Multiple-Puncture Test**

This 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. Several types of applicators are available for multiple-puncture tests. A multiple-puncture test presently available uses concentrated tuberculin, either OT or PPD. The quantity of tuberculin introduced into the skin using the multiple-puncture technique cannot be precisely controlled. For these reasons, multiple-puncture tests are not intended to be used as diagnostic tests.

Multiple-puncture test reactions are measured at 48 to 72 h as follows. (1) If the reaction is in the form of discrete papules, the diameter of the largest single papule should be measured. (2) If there is a coalescence of papular reactions, the largest diameter of coalescent induration should be measured. (3) If the reaction is vesicular, this is noted on the record. Verification of a reaction to any multiple-puncture test by the standard Mantoux test is recommended unless vesiculation is present, in which case the reaction should be recorded as positive.

For each tuberculin test, a record should be made of the technique of administration (Mantoux or multiple-punctures), the kind and

**TABLE 1**

<table>
<thead>
<tr>
<th>FACTORS CAUSING DECREASED ABILITY TO RESPOND TO TUBERCULIN</th>
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</thead>
<tbody>
<tr>
<td>Factors Related to the Person Being Tested</td>
</tr>
<tr>
<td>Infections</td>
</tr>
<tr>
<td>Viral (measles, mumps, chicken pox)</td>
</tr>
<tr>
<td>Bacterial (typhoid fever, brucellosis, typhus, leprosy, pertussis, overwhelming tuberculosis, tuberculous pleurisy)</td>
</tr>
<tr>
<td>Fungal (South American blastomycosis)</td>
</tr>
<tr>
<td>Live virus vaccinations (measles, mumps, polio)</td>
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<tr>
<td>Metabolic derangements (chronic renal failure)</td>
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<tr>
<td>Nutritional factors (severe protein depletion)</td>
</tr>
<tr>
<td>Diseases affecting lymphoid organs (Hodgkin’s disease, lymphoma, chronic lymphocytic leukemia, sarcoidosis)</td>
</tr>
<tr>
<td>Drugs (corticosteroids and many other immunosuppressive agents)</td>
</tr>
<tr>
<td>Age (newborns, elderly patients with “wanes” sensitivity)</td>
</tr>
<tr>
<td>Recent or overwhelming infection with <em>M. tuberculosis</em></td>
</tr>
<tr>
<td>Stress (surgery, burns, mental illness, graft versus host reactions)</td>
</tr>
<tr>
<td>Factors Related to the Tuberculin Used</td>
</tr>
<tr>
<td>Improper storage (exposure to light and heat)</td>
</tr>
<tr>
<td>Improper dilutions</td>
</tr>
<tr>
<td>Chemical denaturation</td>
</tr>
<tr>
<td>Contamination (partially controlled by adding Tween® 80)</td>
</tr>
<tr>
<td>Factors Related to the Method of Administration</td>
</tr>
<tr>
<td>Injection of too little antigen</td>
</tr>
<tr>
<td>Delayed administration after drawing into syringe</td>
</tr>
<tr>
<td>Injection too close</td>
</tr>
<tr>
<td>Factors Related to Reading the Test and Recording Results</td>
</tr>
<tr>
<td>Inexperienced reader</td>
</tr>
<tr>
<td>Conscious or unconscious bias</td>
</tr>
<tr>
<td>Error in recording</td>
</tr>
</tbody>
</table>

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dose of tuberculin, and the size of reaction in millimeters of induration.

Interpretation of Skin Test Reactions

Persons with sensitivity to tuberculin are known as reactors. The definition of a tuberculin reaction size that is indicative of infection with M. tuberculosis is influenced by the dose, dilution, and nature of the tuberculin preparation being used, immunologic factors and the relative prevalence of tuberculin sensitivity resulting from infection with M. tuberculosis and that resulting from other mycobacteria in the population being studied. Reactions caused by infection with mycobacteria other than M. tuberculosis (cross-reactions) commonly occur in many parts of the world. The distinction between reactions that represent tuberculous infection and cross-reactions is not precise, but in general, the larger the reaction, the greater the probability that the reaction represents infection with M. tuberculosis.

The interpretation of a tuberculin reaction should be influenced by the purpose for which the test was given and by the consequences of false classification. Errors in classification cannot be avoided, but they can be minimized by establishing an appropriate definition of a positive reaction.

Separating Tuberculous Infection from Reactions Created by Other Causes

In persons with reactive tuberculin tests, the major confounding factor is infection with and hypersensitivity to mycobacteria other than M. tuberculosis. These reactions (cross-reactions) tend to be smaller than reactions caused by tuberculous infection. 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. However, persons who are immunosuppressed because of disease (e.g., HIV infection) or drugs (e.g., corticosteroids) may have a limited ability to respond to tuberculin even if they have been infected with M. tuberculosis. Therefore, using a lower cutting point (e.g., 5 mm) for separating positive from negative reactions is appropriate in these groups. This will ensure that very few persons infected with M. tuberculosis will be classified as having negative reactions, and few persons not infected with tubercle bacilli will be classified as having positive reactions.

Among persons who have not had recent close contact with tuberculosis or do not have abnormal chest radiographs consistent with tuberculosis, or are not immunosuppressed but have other risk factors for tuberculosis, a higher cutting point is appropriate.

In the remainder of the population having very low probability of exposure to tuberculosis and in which cross-reactions are likely to be more common than reactions caused by tuberculous infections, a still higher cutting point should be selected.

Factors That Cause Persons to Be Falsely Classified as Uninfected

When the tuberculin test is used in the evaluation of persons with a process that might have a tuberculous source, the major concern is with reactions that cause the person to be falsely classified as uninfected. It is important to recognize that a small or no reaction to a tuberculin skin test alone does not exclude the diagnosis of tuberculosis from further consideration. The generation and maintenance of a tuberculin reaction in persons infected with the tubercle bacillus requires a complex interaction of immunologic responses. Failure of single components or of their interaction may result in a nonreactive test in a person infected with M. tuberculosis. This failure to respond may be mild and short-lived or profound and permanent. There are many conditions that have been reported to impair delayed hypersensitivity and thus cause such false negative reactions. Some of these have been well studied, whereas others are reported only in anecdotal fashion. These causes are summarized in table 1.

General Classification of Reactions

The interpretation of a tuberculin test should be influenced by the purpose for which the test was given and by the consequences of false classification. Errors in classification cannot be avoided, but they can be minimized by establishing an appropriate definition of a positive reaction.

Zntracutaneous Mantoux test with a negative reaction. For any of the above categories, reactions below the cutting point are considered negative.

Multiple-puncture tests. Such tests are intended not for diagnostic use but for surveys or screening among groups of asymptomatic persons not exposed to a case of tuberculosis in whom only a small proportion are expected to have tuberculous infection. In general, persons who have no reaction to a multiple-puncture test will also have none if tested with the Mantoux test.

With the exception of persons with a vesicular reaction, which may be interpreted as positive, those with any other reaction must be given a Mantoux test for diagnostic evaluation. Decisions concerning management should be based on the reaction to the Mantoux test.

Previous Vaccination with BCG

There is no reliable method of distinguishing tuberculin reactions caused by vaccination with BCG from those caused by natural mycobacterial infections. Therefore, it is usually prudent to consider large 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 vaccinees is often <5 mm, and (3) tuberculin sensitivity tends to wane after vaccination. Because many BCG-vaccinated persons tend to come from areas of the world where transmission frequently occurs, it is important that previously vaccinated persons with significant reactions to a tuberculin skin test be evaluated for presence of disease and managed accordingly.

Use of the Tuberculin Test

The Mantoux test with 5 TU PPD may be used as a diagnostic aid to detect tuberculous infection and to determine the prevalence of infection in groups of people. Furthermore, patterns of reactions to the test are useful in establishing priorities for follow-up and preventive therapy with isoniazid. Studies from many parts of the world have shown that there is a tendency for persons who have larger tuberculin reactions to be at greater risk of developing tuberculosis, probably because large reactions almost always represent infection with M. tuberculosis, whereas small reactions represent a mixture of tuberculous infections and other mycobacterial infections.

Persons for whom tuberculin testing is routinely indicated are listed in table 2.
TABLE 2
PERSONS IN WHOM TUBERCULIN TESTING IS INDICATED

1. Persons with signs (e.g., radiographic abnormality) and/or symptoms (cough, hemoptysis, weight loss, etc.) suggestive of current tuberculosis disease.
2. Recent contacts with known tuberculosis cases or persons suspected of having tuberculosis.
3. Persons with abnormal chest roentgenograms compatible with past tuberculosis.
4. Persons with medical conditions that increase the risk of tuberculosis (sarcoidosis, gastronomy, diabetes, immunosuppressive therapy, lymphomas, etc.).
5. Persons with HIV infection.
6. Groups at high risk of recent infection with M. tuberculosis, such as immigrants from Asia, Africa, Latin America, and Oceania; some inner-city and skid row populations; personnel and long-term residents in some hospitals, nursing homes, mental institutions, and prisons.

Diagnostic Aid
A positive reaction to a Mantoux test with 5 TU PPD demonstrates that hypersensitivity to mycobacteria has developed. The larger the reaction, the greater is the probability that the responsible organism is M. tuberculosis. A positive reaction to the skin test does not necessarily signify the presence of disease. Sensitivity develops in 2 to 10 wk after initial infection with M. tuberculosis. Once acquired, sensitivity to tuberculin tends to persist, although it may wane with time.

Tuberculin testing is useful in the evaluation of patients clinically suspected of having tuberculosis, a positive reaction supporting the diagnosis. A negative reaction makes tuberculosis less likely, although caution must be exercised in the presence of possible anergy, especially in the presence of severe clinical illness or disseminated tuberculosis. In elderly patients, it may be useful to repeat the Mantoux test with 5 TU after 1 wk, thus capitalizing on the booster phenomenon to detect reactivity that has waned with time.

Detection of Previously Infected Persons
The Mantoux test with 5 TU PPD is used to identify infected persons who might benefit from preventive therapy. It may also be used as the initial step in screening apparently well persons for tuberculosis disease. Its value for this purpose increases as the prevalence of tuberculin sensitivity in the screened population decreases. When only a few are infected (as is the situation in most of the United States), initial tuberculin testing used in place of radiographic screening can lead to important reductions in expense and radiation exposure.

Detection of Newly Infected Persons
The tuberculin test can be especially valuable when repeated periodically in surveillance of tuberculin-negative persons likely to be exposed to tuberculosis. However, there are special problems in identifying newly infected persons.

First, there are unavoidable errors in even the most carefully performed tests. For this reason, small increases in reaction size may not be meaningful. For all persons younger than 35 yr of age whose previous reaction was negative, an increase in reaction size of 10 mm or more in diameter within a period of 2 yr would be considered a skin test conversion. For those older than 35 yr of age, an increase of 15 mm or more is considered a positive conversion, and they should be considered newly infected with M. tuberculosis and strongly considered for preventive therapy. A second problem in identifying newly infected persons is the so-called booster phenomenon. Repeated testing of uninfected persons does not sensitize them to tuberculin. However, delayed hypersensitivity to tuberculin, once it has been established by infection with any species of mycobacteria or by BCG vaccination, may gradually wane over the years, resulting in reactions that are negative. The stimulus of this test may recall the hypersensitivity, which results in an increase in the size of the reaction to a subsequent test, sometimes causing an apparent conversion that is interpreted as indicating new infection.

Although the booster phenomenon may occur at any age, boosting increases with age and is most frequently encountered among persons older than 55 yr of age. The booster effect can be seen on a second test done as soon as a week after the initial stimulating test and can persist for a year and perhaps longer.

When tuberculin skin testing of adults is to be repeated periodically, the initial use of a two-step testing procedure can reduce the likelihood of interpreting a boosted reaction as representing recent infection. If the reaction to the first test is classified as negative, a second test should be given a week later. If the second test result remains below the cutting point, the person is classified as being uninfected. A positive reaction to a third test (with an increase of more than 10 mm) in such a person, within the next few years, is likely to represent the occurrence of infection with M. tuberculosis in the interval. If the reaction to the second of the initial two tests is positive, this probably represents a boosted reaction. On the basis of this second test result, the person should be classified as being infected and managed accordingly.

Because detection of newly infected persons requires accurate testing and reading, multiple-puncture devices should not be used in tuberculin testing surveillance programs designed to detect newly infected persons (such as in periodic testing programs for employees of hospitals and other institutions or in evaluation of contacts).

Untoward Reactions to PPD
Untoward reactions to PPD are very uncommon and usually represent a high degree of sensitivity to the tuberculin. Allergic reactions to the phosphate-buffered saline diluent, the phenol included as a preservative, and Tween® 80 have not been documented despite the millions of doses used. The PPD in doses used for tuberculin testing does not induce delayed hypersensitivity. Immediate skin reactions to tuberculin tests have no clinical or epidemiologic importance and do not indicate tuberculosis infection.

A few exquisitely sensitive persons may respond to skin testing with vesicular or ulcerating local reaction, lymphangitis, regional adenopathy, and fever. As much as one would like to avoid such reactions, their occasional occurrence should not suggest the preliminary testing of patients with suspected tuberculosis infection with 1 TU. The 1 TU test has not been adequately standardized to allow accurate interpretation.

Classification of Persons Exposed to and/or Infected with M. tuberculosis
This classification is based on the broad post-parasite relationships as described by exposure history, infection, and disease. It is intended mainly as an operational framework for public health programs.

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

1. Tuberculosis exposure, no evidence of infection. Persons in this class 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. If there has been close exposure within 3 mo, follow-up is required, and preventive therapy should be considered.

2. Tuberculous infection, no disease. Persons in this class have a positive reaction to the tuberculin skin test, negative bacteriologic studies (if done), and no clinical or radiographic evidence of tuberculosis. Preventive chemotherapy may be indicated in some persons in this group.

Chemotherapy Status
None
Receiving chemotherapy since (date)
Chemotherapy terminated (date)
Complete (prescribed course of therapy)
Incomplete

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 and/or radiographic evidence of current tuberculosis. This is established most definitively by isolation of M.
tuberculosis. In the absence of a positive culture for *M. tuberculosis*, persons in this class must have a positive reaction to the tuberculin test. A person who had past tuberculosis and who 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**
- Positive by:
  - Microscopy only (date)
  - Culture only (date)
  - Microscopy and culture (date)
  - Negative (date)
  - Not done

**Chemotherapy Status**
- Receiving chemotherapy since (date)
- Complete
- Incomplete (present episode)

**Chest Radiograph Findings**
- Normal
- Abnormal
  - Cavitary or noncavitary
  - Stable or worsening or improving

**Tuberculin Skin Test Reaction**
- Positive
- Negative

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, 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 prevention chemotherapy, 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.

**Chemotherapy Status**
- None
- Receiving Chemotherapy since (date)
- Complete
- Incomplete (present episode)

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 months. When diagnostic procedures have been completed, the person should be placed in one of the preceding classes.

**Chemotherapy Status**
- None
- Receiving chemotherapy since (date)

**Reporting of Tuberculosis**

By law and regulation, a case of tuberculosis must be reported to the local health department. The reporting of the characteristics of the cases is essential to the conduct of the tuberculosis control program at local, state, and national levels, and to the evaluation 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. Health department laboratory and radiographic services and consultation are generally available to assist the physician in carrying out responsibilities in the treatment of tuberculosis.

This statement was prepared by a subcommittee of the Scientific Assembly on Microbiology, Tuberculosis and Pulmonary Infections. Members of the committee are:

**John B. Hass, Jr., M.D., Chairman**

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