

# Chapter 4

## Diagnosis of Tuberculosis Disease

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### Chapter Objectives

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After working through this chapter, you should be able to

- Describe the five components of a TB medical evaluation;
- Identify the major components of TB diagnostic microbiology;
- List at least five symptoms of pulmonary TB disease;
- Explain the purpose and significance of acid-fast bacilli (AFB);
- Explain the purpose and significance of the culture; and
- Explain the purpose and significance of genotyping.



## Introduction

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Tuberculosis (TB) is not as common as it was many years ago in the United States; consequently, clinicians do not always consider the possibility of TB disease when evaluating patients who have symptoms. As a result, the diagnosis of TB disease may be delayed or even overlooked, and the patient may remain ill and possibly infectious for a prolonged period.

Not all persons with TB disease have symptoms; however, most persons with TB disease have one or more symptoms that lead them to seek medical care. All persons with symptoms of TB disease, or either a positive tuberculin skin test (TST) or an interferon-gamma release assay (IGRA) indicative of *M. tuberculosis* infection, should be medically evaluated to exclude TB disease.

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**All persons with symptoms of TB disease, or either a positive TST or IGRA indicative of *M. tuberculosis* infection, should be medically evaluated to exclude TB disease.**

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## Study Question

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**4.1 All persons with symptoms of TB disease, or a positive TST or IGRA result indicating *M. tuberculosis* infection, should be medically evaluated to exclude TB disease.**

(choose the one best answer)

- C. True
- D. False

## Medical Evaluation

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A complete medical evaluation for TB disease includes the following five components:

1. Medical history
2. Physical examination
3. Test for *M. tuberculosis* infection
4. Chest radiograph
5. Bacteriologic examination of clinical specimens.

### 1. Medical History

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When conducting a medical history, the clinician should ask if any symptoms of TB disease are present; if so, for how long, and if there has been known exposure to a person with infectious TB disease. Equally important is obtaining information on whether or not the person has been diagnosed in the past with latent tuberculosis infection (LTBI) or TB disease. Clinicians may also contact the local health department for information on whether a patient has a past history of TB infection or disease. If the previous treatment regimen for TB disease was inadequate or if the patient did **not** adhere to therapy, TB disease may recur and possibly be drug-resistant. It is important to consider demographic factors (e.g., country of origin, age, ethnicity, occupation, or racial group) that may increase the patient's risk for being exposed to TB infection (see Chapter 2, Transmission and Pathogenesis of Tuberculosis). Clinicians should determine if the patient has underlying medical conditions, especially human immunodeficiency virus (HIV) infection or diabetes, that increase the risk for progression to TB disease in those latently infected with *M. tuberculosis*.

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**Clinicians should determine if the patient has underlying medical conditions, especially HIV infection and diabetes, that increase the risk for progression to TB disease in those latently infected with *M. tuberculosis*.**

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As discussed in Chapter 2, Transmission and Pathogenesis of Tuberculosis, TB disease most commonly affects the lungs and is referred to as pulmonary TB disease. Pulmonary TB disease usually causes one or more of the symptoms indicated in Table 4.1.

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**TB disease most commonly affects the lungs and is referred to as pulmonary TB disease.**

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Extrapulmonary TB disease may cause symptoms related to the part of the body that is affected (Table 4.1). For example, TB of the spine may cause back pain; TB of the kidney may cause blood in the urine; TB meningitis may cause headache or confusion. Extrapulmonary TB disease should be considered in the differential diagnosis of ill persons who have systemic symptoms and who are at high risk for TB disease.

Both pulmonary and extrapulmonary TB disease symptoms can be caused by other diseases; however, they should prompt the clinician to consider TB disease.

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**Both pulmonary and extrapulmonary TB disease symptoms can be caused by other diseases; however, they should prompt the clinician to consider TB disease.**

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**Table 4.1**  
**Symptoms of Pulmonary and Extrapulmonary TB Disease**

<b>Symptoms of Pulmonary TB Disease</b> (TB disease usually causes one or more of the symptoms)	<b>Symptoms of Possible Extrapulmonary TB Disease</b> (Depends on the part of the body that is affected by the disease)
<ul style="list-style-type: none"> <li>• Cough (especially if lasting for 3 weeks or longer) with or without sputum production</li> <li>• Coughing up blood (hemoptysis)</li> <li>• Chest pain</li> <li>• Loss of appetite</li> <li>• Unexplained weight loss</li> <li>• Night sweats</li> <li>• Fever</li> <li>• Fatigue</li> </ul>	<ul style="list-style-type: none"> <li>• TB of the kidney may cause blood in the urine</li> <li>• TB meningitis may cause headache or confusion</li> <li>• TB of the spine may cause back pain</li> <li>• TB of the larynx can cause hoarseness</li> <li>• Loss of appetite</li> <li>• Unexplained weight loss</li> <li>• Night sweats</li> <li>• Fever</li> <li>• Fatigue</li> </ul>

## Study Questions

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**Match the patient symptoms with the type of TB.**

(Choose the one best answer and write the letter for the correct answer on the line next to the question number.)

Patient Symptoms		Type of TB
____ 4.2	Regina has back pain and blood in her urine, unexplained weight loss, fever, fatigue, loss of appetite.	<b>A.</b> Pulmonary TB <b>B.</b> Extrapulmonary TB
____ 4.3	Maria has a cough, loss of appetite, and unexplained weight loss. She has also been coughing up blood.	

## 2. Physical Examination

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A physical examination is an essential part of the evaluation of any patient. It cannot be used to confirm or rule out TB disease, but it can provide valuable information about the patient's overall condition, inform the method of diagnosis, and reveal other factors that may affect TB disease treatment, if diagnosed.

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**A physical examination is an essential part of the evaluation of any patient. It cannot be used to confirm or rule out TB disease, but it can provide valuable information about the patient's overall condition, inform the method of diagnosis, and reveal other factors that may affect TB disease treatment, if diagnosed.**

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## Study Question

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**4.4 A physical examination can be used to confirm and rule out TB disease.**

(circle the one best answer)

- A.** True
- B.** False

### 3. Test for *M. tuberculosis* Infection

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Selection of the most suitable tests for detection of *M. tuberculosis* infection should be based on the reasons and the context for testing, test availability, and overall cost effectiveness of testing. Currently, there are two methods available for the detection of *M. tuberculosis* infection in the United States. The tests are:

- Mantoux tuberculin skin test (TST) (Figure 4.1); and
- Interferon-gamma release assays (IGRAs)\*
  - » QuantiFERON-TB Gold In-Tube test (QFT-GIT) (Figure 4.2);
  - » T-SPOT®.TB test (Figure 4.3).

\*See Chapter 3, Testing for Tuberculosis Infection and Control

**Figure 4.1**  
Mantoux Tuberculin  
Skin Test



**Figure 4.2**  
QuantiFERON-TB Gold  
In-Tube Test (QFT-GIT)



**Figure 4.3**  
T-SPOT®.TB Test



These tests help clinicians differentiate people infected with *M. tuberculosis* from those uninfected. However, a negative reaction to any of the tests does **not** exclude the diagnosis of TB disease or LTBI (see Chapter 3, Testing for Tuberculosis Infection and Disease).

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**TST and QFT tests help clinicians differentiate people infected with *M. tuberculosis* from those uninfected. However, a negative reaction to any of the tests does NOT exclude the diagnosis of TB disease or LTBI.**

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### Study Question

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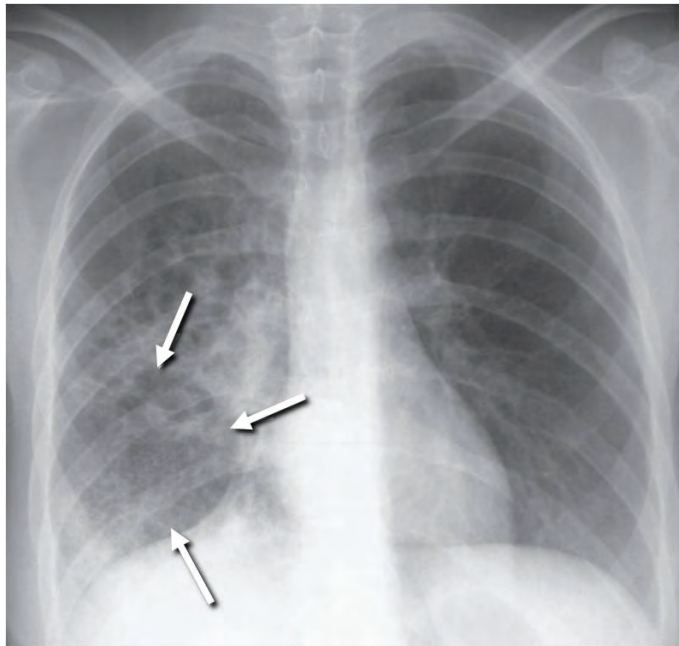
- 4.5** A negative reaction for a TST or IGRA test excludes a person from having TB disease. (choose the one best answer)
- A.** True
  - B.** False

## 4. Chest Radiograph

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With pulmonary TB being the most common form of disease, the chest radiograph is useful for diagnosis of TB disease. Chest abnormalities can suggest pulmonary TB disease (Figure 4.4). A posterior-anterior radiograph of the chest is the standard view used for the detection of TB-related chest abnormalities. In some cases, especially in children, a lateral view may be helpful.

**Figure 4.4**  
**Chest Radiograph with Lower Lobe Cavity**



In some instances, a computerized tomography (CT) scan may provide additional information. A CT scan provides more detailed images of parts of the body that cannot easily be seen on a standard chest radiograph; however, CT scans can be substantially more expensive.

In pulmonary TB disease, radiographic abnormalities are often seen in the apical and posterior segments of the upper lobe or in the superior segments of the lower lobe. However, lesions may appear anywhere in the lungs and may differ in size, shape, density, and cavitation, especially in HIV-infected and other immunosuppressed persons. Radiographic abnormalities in children tend to be minimal with a greater likelihood of lymphadenopathy, more easily diagnosed on the lateral film.

Mixed nodular and fibrotic lesions may contain slowly multiplying tubercle bacilli and have the potential for progression to TB disease. Persons who have lesions consistent with findings of “old” TB disease on a chest radiograph and have a positive TST reaction or positive IGRA result should be considered high-priority candidates for treatment of LTBI (see Chapter 5, Treatment for Latent Tuberculosis Infection), but only after TB disease is excluded by obtaining three specimens for AFB smear and culture because “old” TB cannot be differentiated from active TB disease based on radiographic appearance alone. Conversely, fully calcified, discrete, nodular lesions without fibrosis likely represent granulomas and pose a lower risk for future progression to TB disease.



In HIV-infected persons, pulmonary TB disease may present with atypical findings or with no lesions seen on the chest radiograph. The radiographic appearance of pulmonary TB disease in persons infected with HIV might be typical; however, cavitory disease is less common among such patients. More common chest radiograph findings for HIV-infected persons include infiltrates in any lung zone, mediastinal or hilar adenopathy, or, occasionally, a normal chest radiograph. Typical cavitory lesions are usually observed in patients with higher CD4 counts, and more atypical patterns are observed in patients with lower CD4 counts because cavitation is thought to occur as a result of the immune response to TB organisms. In HIV-infected persons, almost any abnormality on a chest radiograph may be indicative of TB disease. In patients with symptoms and signs of TB disease, a negative chest radiograph result does **not** exclude TB disease.

Abnormalities seen on chest radiographs may be suggestive of, but are never diagnostic of, TB disease. Chest radiographs may be used to exclude pulmonary TB disease in an HIV-negative person who has a positive TST reaction or IGRA and who has no symptoms or signs of TB disease.

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**Abnormalities seen on chest radiographs may be suggestive of, but are never diagnostic of, TB disease. Chest radiographs may be used to exclude pulmonary TB disease in a person with a normal immune system who has a positive TST reaction or IGRA and who has no symptoms or signs of TB disease.**

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## Study Question

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**4.6 Chest radiographs may be used to exclude pulmonary TB disease in an HIV-negative person who has a positive TST reaction or IGRA and who has no symptoms or signs of TB disease.** (choose the one best answer)

- A. True
- B. False

## 5. Bacteriologic Examination of Clinical Specimens

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Examinations of clinical specimens (e.g., sputum, urine, or cerebrospinal fluid) are of critical diagnostic importance. The specimens should be examined and cultured in a laboratory that specializes in testing for *M. tuberculosis*. The bacteriologic examination has five parts:

- Specimen collection, processing, and review
- AFB smear classification and results
- Direct detection of *M. tuberculosis* in clinical specimen using nucleic acid amplification (NAA)
- Specimen culturing and identification
- Drug-susceptibility testing

## Specimen Collection, Processing, and Review

For diagnostic purposes, all persons suspected of having TB disease at any site should have sputum specimens collected for an AFB smear and culture, even those without respiratory symptoms. At least three consecutive sputum specimens are needed, each collected in 8- to 24-hour intervals, with at least one being an early morning specimen. If possible, specimens should be obtained in an airborne infection isolation (AII) room or other isolated, well-ventilated area (e.g., outdoors) (Figure 4.5).

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**Figure 4.5**  
**TB Patient Coughing Up Sputum**



A TB patient has coughed up sputum and is spitting it into a sterile container. The patient is sitting in a special sputum collection booth that, if properly ventilated, prevents the spread of tubercle bacilli.

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**For diagnostic purposes, all persons suspected of having TB disease should have sputum collected for AFB smear and culture.**

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During specimen collection, patients produce an aerosol that may be hazardous to health-care workers or other patients in close proximity. For this reason, precautionary measures for infection control must be followed during sputum induction, bronchoscopy, and other common diagnostic procedures (see Chapter 7, TB Infection Control).

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**During specimen collection, patients produce an aerosol that may be hazardous to health-care workers or other patients in close proximity.**

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### **Specimen Collection Methods for Pulmonary TB Disease**

There are four specimen collection methods for pulmonary TB disease (Table 4.2):

- Coughing
- Induced sputum
- Bronchoscopy
- Gastric aspiration

**Coughing**—Coughing is the most commonly used method of sputum collection. Coughing should be supervised to ensure that sputum is collected correctly. A health-care worker wearing the recommended personal protective equipment should coach and directly supervise the patient when sputum is collected (Figure 4.6). Patients should be informed that sputum is the material brought up from the lungs, and that mucus from the nose or throat and saliva are **not** good specimens. Unsupervised patients are less likely to provide an adequate specimen, especially the first time.

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**Patients should be informed that sputum is the material brought up from the lungs, and that mucus from the nose or throat and saliva are not good specimens.**

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**Figure 4.6**  
**Patient Coughing Up Sputum**



**Sputum Induction**—For patients unable to cough up sputum, deep sputum-producing coughing may be induced by inhalation of an aerosol of warm, sterile, hypertonic saline (3%–5%). Because induced sputum is very watery and resembles saliva, it should be labeled “induced” to ensure that the laboratory staff workers do **not** discard it.

**Bronchoscopy**—A bronchoscopy is a medical procedure that allows visualization of the inside of a person’s airways. The airways are called the bronchial tubes or bronchi. Bronchoscopy might be needed for specimen collection, especially if previous results have been nondiagnostic and doubt exists as to the diagnosis. At other times, bronchoscopy is considered because TB is among several other diagnoses being considered. If possible, examine three spontaneous or induced sputum to exclude a diagnosis of TB disease before bronchoscopy. If possible, avoid bronchoscopy on patients with suspected or confirmed TB disease or postpone the procedure until the patient is determined to be noninfectious, by confirmation of the three negative AFB sputum smear results (Figure 4.7). Bronchial washings, brushings, and biopsy specimens may be obtained, depending on the bronchoscopy findings. Sputum collected after a bronchoscopy may also be useful for a diagnosis. A bronchoscopy should never be substituted for sputum collection, but rather used as an additional diagnostic procedure.

**Figure 4.7**  
**Performing a Bronchoscopy**



Whenever feasible, bronchoscopy should be performed in a room that meets the ventilation requirements for an airborne infection isolation (AII) room. Health-care workers should wear N95 respirators while present during a bronchoscopy procedure on a patient with suspected or confirmed infectious TB disease (see Chapter 7, TB Infection Control).

**Gastric Aspiration**—Gastric aspiration is a procedure sometimes used to obtain a specimen for culture when a patient cannot cough up adequate sputum. A tube is inserted through the mouth or nose and into the stomach to recover sputum that was coughed into the throat and then swallowed. This procedure is particularly useful for diagnosis in children, who are often unable to cough up sputum (Figure 4.8). Gastric aspiration often requires hospitalization and should be done in the morning before the patient gets out of bed or eats, as it is the optimal time to collect swallowed respiratory secretions from the stomach. Specimens obtained by gastric aspiration should be transported to the lab immediately for neutralization or neutralized immediately at the site of collection.

**Figure 4.8**  
**Performing a Gastric Aspiration**



**Table 4.2**  
**Methods of Obtaining a Sputum Specimen**

<b>Method</b>	<b>Description</b>	<b>Advantage</b>	<b>Disadvantage</b>
<b>Spontaneous sputum sample</b>	Patient coughs up sputum into a sterile container	<ul style="list-style-type: none"> <li>• Inexpensive</li> <li>• Easy to do</li> </ul>	<ul style="list-style-type: none"> <li>• Patient may <b>not</b> be able to cough up sputum without assistance or may spit up saliva instead of sputum</li> <li>• Health-care worker has to coach and supervise the patient when collecting sputum</li> </ul>
<b>Sputum induction</b>	Patient inhales a saline mist which can cause a deep cough	<ul style="list-style-type: none"> <li>• Easy to do</li> <li>• Use to obtain sputum when coughing sputum is not productive</li> </ul>	<ul style="list-style-type: none"> <li>• Specimens may be watery and may be confused with saliva (should be labeled "induced specimen")</li> <li>• Requires special equipment</li> <li>• May cause bronchospasm</li> </ul>
<b>Bronchoscopy</b>	Bronchoscope is passed through the mouth or nose directly into the diseased portion of the lung, and sputum or lung tissue is removed	Use to obtain sputum when coughing or inducing sputum is not productive or other diagnoses are being considered	<ul style="list-style-type: none"> <li>• Most expensive and invasive procedure</li> <li>• Requires special equipment</li> <li>• Must be done by a specialist in a hospital or clinic</li> <li>• Requires anesthesia</li> </ul>
<b>Gastric washing</b>	Tube is inserted through the patient's mouth or nose and passed into the stomach to get a sample of gastric secretions that contain sputum that has been coughed into the throat and then swallowed	Use to obtain samples in children, who do <b>not</b> produce sputum when they cough	<ul style="list-style-type: none"> <li>• Must be done as soon as patient wakes up in the morning; patient may be required to stay in hospital</li> <li>• Can be uncomfortable for the patient</li> </ul>

## Specimen Collection Methods for Extrapulmonary TB

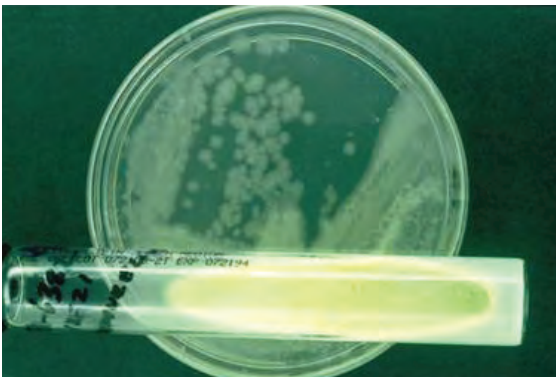
TB disease can occur in almost any anatomical site; thus, a variety of clinical specimens other than sputum (e.g., urine, cerebrospinal fluid, pleural fluid, pus, or biopsy specimens) may be submitted for examination when extrapulmonary TB disease is suspected (Figures 4.9 and 4.10). Procedures for the expeditious and recommended handling of the specimen must be in place or assured before the specialist performs an invasive procedure to obtain the specimen. Especially important is rapid transportation to the laboratory according to the laboratory's instructions. It is important to note that the portion of the specimen placed in formalin for histologic examination cannot be used for culture.

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**Figure 4.9**  
**Clinical Specimen Used for Examination When Extrapulmonary TB Disease Is Suspected**



**Figure 4.10**  
**Collection Bottles Used for Collecting Specimens When Extrapulmonary TB Disease Is Suspected**



## AFB Smear Classification and Results

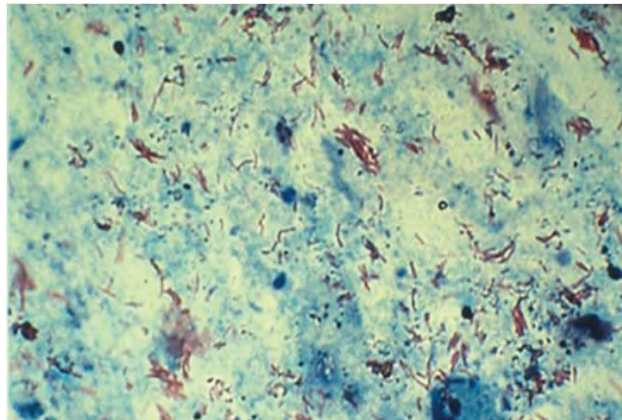
Detection of acid-fast bacilli in stained and acid-washed smears examined microscopically may provide the initial bacteriologic evidence of the presence of mycobacteria in a clinical specimen (Figure 4.11). Smear microscopy is the quickest and easiest procedure that can be performed.

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**Detection of acid-fast bacilli in stained and acid-washed smears examined microscopically may provide the first bacteriologic evidence of the presence of mycobacteria in a clinical specimen.**

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**Figure 4.11**  
**Acid-Fast Bacilli Stained in Smear**  
Tubercle bacilli are shown in red



There are two procedures commonly used for acid-fast staining:

- Carbofuchsin methods which include the Ziehl-Neelsen and Kinyoun methods (direct microscopy)
- Fluorochrome procedure using auramine-O or auramine-rhodamine dyes (fluorescent microscopy).

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. In contrast, 10 to 100 bacilli are needed for a positive culture. Smear examination is a quick procedure; results should be available within 24 hours of specimen collection when specimens are delivered to the laboratory promptly. However, smear examination permits only the presumptive diagnosis of TB disease because the acid-fast bacilli in a smear may be acid-fast organisms other than *M. tuberculosis*. Also, many TB patients have negative AFB smears with a subsequent positive culture. Negative smears do **not** exclude TB disease (Table 4.3).

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**Many TB patients have negative AFB smears with a subsequent positive culture. Negative smears do not exclude TB disease.**

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When acid-fast bacilli are seen in a smear, they are counted. There is a system for reporting the number of acid-fast bacilli that are seen at a certain magnification. According to the number of acid-fast bacilli seen, the smears are classified as 4+, 3+, 2+, or 1+. The greater the number, the more infectious the patient (Table 4.3).



**Table 4.3**  
**Smear Classification Results**

<b>Smear Result</b> (Number of AFB observed at 1000X magnification)	<b>Smear Interpretation</b>	<b>Infectiousness of Patient</b>
<b>4+</b> (>9/field)	Strongly positive	Probably very infectious
<b>3+</b> (1-9/field)	Strongly positive	Probably very infectious
<b>2+</b> (1-9/10 fields)	Moderately positive	Probably infectious
<b>1+</b> (1-9/100 fields)	Moderately positive	Probably infectious
<b>+/-</b> (1-2/300 fields)*	Weakly positive <sup>†</sup>	Probably infectious
No acid-fast bacilli seen	Negative	Probably not infectious**

\* There are variations on labeling for this result, and include listing the number of AFB counted.

<sup>†</sup> Laboratories may report these smear results as “doubtful” or “inconclusive” based on CDC guidelines.

\*\* The criteria for determining whether a patient may be considered noninfectious are discussed in Chapter 7 on TB Infection Control.

### **Direct Detection of *M. tuberculosis* in Clinical Specimen Using Nucleic Acid Amplification (NAA)**

NAA tests are used to amplify DNA and RNA segments to rapidly identify the microorganisms in a specimen. NAA testing can reliably detect *M. tuberculosis* bacteria in specimens in hours as compared to 1 week or more for culture (Figure 4.12). Possible benefits of using NAA tests include

- Earlier laboratory confirmation of TB disease;
- Earlier treatment initiation;
- Improved patient outcomes;
- Interruption of transmission by early diagnosis, respiratory isolation and appropriate treatment;
- Earlier, more efficient use of respiratory isolation;
- Earlier initiation of contact investigation; and
- More effective public health interventions.

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**NAA tests are used to amplify DNA and RNA segments to rapidly identify the microorganisms in a specimen.**

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CDC recommends that NAA testing be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities, such as contact investigations.

**Figure 4.12**  
**Nucleic Acid Amplification (NAA) Test**



Clinicians should interpret all laboratory results in the context of the clinical situation. A single negative NAA test result should not be used as a definitive result to exclude TB disease, especially when the clinical suspicion of TB disease is moderate to high. Rather, the negative NAA test result should be used as additional information in making clinical decisions, to expedite testing for an alternative diagnosis, or to prevent unnecessary TB disease treatment.

Culture remains the gold standard for laboratory confirmation of TB disease, and growing bacteria are required to perform drug-susceptibility testing and genotyping. In accordance with current recommendations, sufficient numbers and portions of specimens should always be reserved for culture. Nonetheless, NAA testing should become standard practice for patients suspected of having TB, and all clinicians and public health TB programs should have access to NAA testing for TB to shorten the time to diagnosis.

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**Culture remains the gold standard for laboratory confirmation of TB disease, and growing bacteria are required to perform drug-susceptibility testing and genotyping.**

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## Specimen Culture and Identification

Positive cultures for *M. tuberculosis* confirm the diagnosis of TB disease; however, in the absence of a positive culture, TB disease may also be diagnosed on the basis of clinical signs and symptoms alone. Culture examinations should be done on all diagnostic specimens, regardless of AFB smear or NAA results. The commercially available broth culture systems (e.g., BACTEC, MGIT, VersaTREK, MBBACT) allow detection of most mycobacterial growth in 4 to 14 days compared to 3 to 6 weeks for solid media (Figure 4.13). Laboratories performing TB cultures should routinely use a broth-based system (Table 4.4).

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**Positive cultures for *M. tuberculosis* confirm the diagnosis of TB disease; however, in the absence of a positive culture, TB disease may also be diagnosed on the basis of clinical signs and symptoms alone.**

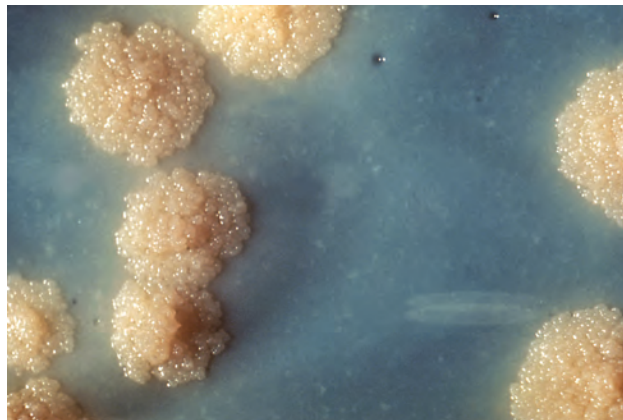
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**Culture examinations should be done on all diagnostic specimens, regardless of AFB smear or NAA results.**

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**Figure 4.13**  
**Colonies of *M. tuberculosis* Grown in Culture**



**Table 4.4**  
**Differences Between Sputum Smears and Cultures**

Feature	Smears	Cultures
Equipment needed	<ul style="list-style-type: none"> <li>• Microscope</li> <li>• Glass slides</li> <li>• Special dyes</li> </ul>	<ul style="list-style-type: none"> <li>• Incubators</li> <li>• Safety cabinet</li> <li>• Culture plates or tubes</li> <li>• Culture media, biochemicals for tests</li> </ul>
Time needed to make report	<ul style="list-style-type: none"> <li>• 1 day</li> </ul>	<ul style="list-style-type: none"> <li>• 4 days to 12 weeks (depending on method used and how quickly the organism grows)</li> </ul>
Basis of procedure	<ul style="list-style-type: none"> <li>• Looking for acid-fast bacilli on slide under microscope</li> </ul>	<ul style="list-style-type: none"> <li>• Growth and identification of tubercle bacilli or other mycobacteria on culture media in incubator</li> </ul>
Significance of a negative report	<ul style="list-style-type: none"> <li>• Patient is probably <b>not</b> infectious</li> <li>• Does <b>not</b> rule out TB disease (culture may be positive)</li> </ul>	<ul style="list-style-type: none"> <li>• No live tubercle bacilli found in specimen</li> <li>• Does <b>not</b> rule out TB disease (live tubercle bacilli may be in other specimens and/or body sites)</li> </ul>
Significance of a positive report	<ul style="list-style-type: none"> <li>• Patient is more likely to be infectious (if acid-fast bacilli are tubercle bacilli)</li> <li>• Acid-fast bacilli could be nontuberculous mycobacteria</li> </ul>	<ul style="list-style-type: none"> <li>• Confirms diagnosis of TB disease</li> </ul>

### **Follow-up Bacteriologic Examination**

Follow-up bacteriologic examinations are important for assessing the patient's infectiousness and response to therapy. Specimens should be obtained at monthly intervals until two consecutive specimens sent for culture are reported as negative. Culture conversion is the most important objective measure of response to treatment. Conversion is documented by the first negative culture in a series of previously positive cultures. In addition, all subsequent culture results must remain negative.

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**Specimens should be obtained at monthly intervals until two consecutive specimens sent for culture are reported as negative.**

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## Reporting Results

Laboratories should report initial positive smears, positive *M. tuberculosis* cultures, and positive NAA results within 24 hours by telephone or fax to the primary health-care provider and health department. Out-of-state laboratories who receive referral specimens must contact the health-care provider and health department in the patient's state of origin. Follow-up results may be reported by mail. It is the responsibility of the primary health-care provider to report all suspected or confirmed cases of TB disease promptly to the state or local health department unless state laws indicate otherwise. Prompt reporting to health authorities ensures that the person with TB disease can be adequately treated, interrupting the potential for ongoing transmission. It also ensures that contact investigations can be initiated quickly to find contacts of the patient who may have LTBI or TB disease.

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**Laboratories should report initial positive smears, positive *M. tuberculosis* cultures, and positive NAA results within 24 hours by telephone or fax to the primary health-care provider and health department.**

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## Drug-Susceptibility Testing

For all patients, the initial *M. tuberculosis* isolate should be tested for resistance to the first-line anti-TB drugs: isoniazid, rifampin, ethambutol, and pyrazinamide (Figure 4.14). The results of drug-susceptibility tests should direct clinicians to choose the appropriate drugs for treating each patient. Patients with TB disease who are treated with drugs to which their strain of TB is resistant may **not** be successfully cured. In fact, their strain of TB may become resistant to additional drugs.

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**For all patients, the initial *M. tuberculosis* isolate should be tested for resistance to the first-line anti-TB drugs: isoniazid, rifampin, ethambutol, and pyrazinamide.**

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**Figure 4.14**  
**Drug-Susceptibility Testing**



Rapid, broth-based systems should be used to identify drug resistance as early as possible in order to ensure appropriate treatment. Susceptibility results from laboratories should be promptly forwarded to the physician and health department. Drug-susceptibility tests should be repeated for patients who do **not** respond as expected or who have positive culture results despite 3 months of adequate treatment.

Second-line drug susceptibility testing should be done only in reference laboratories and generally be limited to specimens from patients who have the following characteristics:

- Prior TB disease treatment;
- Contact with a patient with known anti-TB drug resistance;
- Demonstrated resistance to first-line anti-TB drugs; or
- Positive cultures after more than 3 months of treatment.

A patient is diagnosed with multidrug-resistant TB (MDR TB) disease if the organisms are resistant to at least isoniazid and rifampin, the two most potent first-line anti-TB drugs. A patient is diagnosed with extensively drug-resistant TB (XDR TB) disease if the TB isolate is resistant to isoniazid and rifampin, any fluoroquinolone, and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin).

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### **Molecular Detection of Drug Resistance**

The drug resistance of clinical isolates as determined by conventional methods (e.g, broth-based and agar proportion) is due to the presence of mutations in specific *M. tuberculosis* genes. These mutations often are single base pair changes in the DNA sequence of the bacteria. There are a variety of commercial assays and laboratory developed tests that can detect mutations associated with drug resistance. The assays are done on patient specimens or isolates from patient specimens.

- Line-probe assays use polymerase chain reaction (PCR) to amplify the region of a gene known to be associated with resistance. The amplified product is labeled and specifically joins to probes on a nitrocellulose strip. Mutations are detected by the lack of binding to probes with the normal sequence or by binding to probes specific for commonly occurring mutations.
- PCR amplification of genes known to be associated with drug resistance can be followed by DNA sequencing that can detect mutations.
- Real-time PCR with fluorescing probes that specifically join to the target can do so in one step, a technique sometimes called “molecular beacons.”

All of these assays allow rapid detection of drug resistance through the identification of genetic mutations associated with resistance and provide **preliminary** guidance on effective therapy. Molecular detection of drug resistance should be considered for patients with the following characteristics:

- High risk of rifampin resistance, including MDR TB (e.g., previously treated TB, contact with someone with MDR TB, or being foreign born from a high-risk country);
- First-line drug susceptibility results are available and show resistance to rifampin;
- Infectiousness poses a risk to vulnerable contacts (e.g., daycare workers, nurses, and infants); and
- Contraindications to essential first-line medications (e.g., rifampin allergy).

A limitation of molecular testing for drug resistance is that the clinical relevance of some mutations remains unknown. Further, not all biological mechanisms of resistance are known. As a result, if no mutations are detected by the molecular assay, resistance cannot be ruled out. **Therefore, it is essential that conventional growth-based drug-susceptibility tests are done and used in conjunction with molecular results.**

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**It is essential that conventional growth-based drug-susceptibility tests are done and used in conjunction with molecular results.**

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## Study Questions

Indicate which of the following activities is a component of a complete medical evaluation for TB. (Choose the one best answer and write the letter for the correct answer on the line next to the question number.)

Activity	Yes or No
____ <b>4.7</b> Medical history	<b>A.</b> Yes, is a component of a complete medical evaluation for TB  <b>B.</b> No, is <b>not</b> a component of a complete medical evaluation for TB
____ <b>4.8</b> Physical examination	
____ <b>4.9</b> Test for <i>M. tuberculosis</i> infection	
____ <b>4.10</b> Chest radiograph	
____ <b>4.11</b> Bone density testing	
____ <b>4.12</b> Bacteriological examination of clinical specimens	

Match the specimen collection method with how to use it.

(Choose the one best answer and write the letter for the correct answer on the line next to the question number.)

Use of Specimen Collection Method	Method
____ <b>4.13</b> Use only if there is a suspicion of TB disease and there are three negative sputum smears or induced sputum AFB results.	<b>A.</b> Coughing <b>B.</b> Sputum induction <b>C.</b> Bronchoscopy <b>D.</b> Gastric aspiration <b>E.</b> Biopsy
____ <b>4.14</b> Best way to obtain specimens from children who cannot produce sputum.	
____ <b>4.15</b> Use for extrapulmonary TB disease.	
____ <b>4.16</b> Use for patients unable to cough up sputum to encourage deep coughing.	
____ <b>4.17</b> Most common method for collecting sputum.	



**4.18 What do laboratory personnel look for in a sputum smear?**

(choose the one best answer)

- A. White-blood cells
- B. Fast-moving bacilli
- C. Drug-resistant bacilli
- D. Acid-fast bacilli

**Case Study– Chin**

**Chin has symptoms of TB disease and a cavity on his chest radiograph, but all of his sputum smears are negative for acid-fast bacilli.**

**4.19 Does this rule out the diagnosis of pulmonary TB disease for Chin?**

(circle the one best answer)

- A. **Yes**, because his sputum smears are negative for acid-fast bacilli. Even though he has symptoms of TB disease and a cavity on his chest radiograph, his sputum smears have to be positive for acid-fast bacilli to indicate a diagnosis of pulmonary TB disease.
- B. **No**, because he has symptoms of TB disease and his abnormal chest x-ray suggest that he does have pulmonary TB disease. Also, *M. tuberculosis* may grow in the cultures even though there are no acid-fast bacilli on the smear.

**Which of the following statements about nucleic acid amplification (NAA) tests and cultures are true or false?** (Choose the one best answer and write the letter for the correct answer on the line next to the question number.)

Statements about NAA and Cultures	True or False
_____ <b>4.20</b> NAA tests are used to amplify DNA and RNA segments to rapidly identify the microorganisms in a specimen.	<b>A.</b> True <b>B.</b> False
_____ <b>4.21</b> Culture is required for growing bacteria for drug-susceptibility testing and genotyping.	
_____ <b>4.22</b> Culture is the gold standard for laboratory confirmation of TB disease.	
_____ <b>4.23</b> A single negative NAA test result should be used as a definitive result to exclude TB disease.	
_____ <b>4.24</b> Cultures should be done on all diagnostic specimens, regardless of AFB smear or NAA results.	

**4.25 During patient follow-up, how often should specimens be obtained?**

(circle the one best answer)

- A.** At monthly intervals until one specimen sent for culture is reported as negative.
- B.** At monthly intervals until three consecutive specimens sent for culture are reported as negative.
- C.** At monthly intervals until two consecutive specimens sent for culture are reported as negative.

**Are the following statements about drug-susceptibility testing true or false?**

(Choose the one best answer and write the letter for the correct answer on the line next to the question number.)

Statements about Drug-Susceptibility Testing	True or False
____ <b>4.26</b> For all patients, the initial <i>M. tuberculosis</i> isolate should be tested for resistance to the first-line anti-TB drugs.	<b>A.</b> True <b>B.</b> False
____ <b>4.27</b> For all patients, the initial <i>M. tuberculosis</i> isolate should be tested for resistance to the second-line anti-TB drugs.	
____ <b>4.28</b> Drug-susceptibility tests should be repeated for patients who do not respond as expected.	
____ <b>4.29</b> Drug-susceptibility tests should be repeated for patients who have positive culture results despite 3 months of adequate therapy.	

**Case Study–Lea**

**Lea gave three sputum specimens, which were sent to the laboratory for smear examination and culture. The smear results were reported as 4+, 3+, and 4+.**

**4.30 What do these results tell you about Lea’s diagnosis and her infectiousness?**

(circle the one best answer)

- A.** Because the smears are positive, clinicians should suspect that Lea has TB disease.
- B.** She is probably very infectious.
- C.** It is possible that the acid-fast bacilli are mycobacteria other than tubercle bacilli. Therefore, diagnosis of TB disease cannot be proven until further results are available.
- D.** A, B, and C are all correct.
- E.** Only A and B are correct.

### Case Study– Francis

**In the health clinic you see Francis, a new patient. She complains of weight loss, fever, and a cough of 4 weeks' duration. When questioned, she reports that she has been treated for TB disease in the past and that she occasionally injects heroin.**

#### **4.31 What parts of Francis' medical history lead you to suspect TB disease?**

(circle the one best answer)

- A.** Her symptoms of TB disease (weight loss, fever, and a persistent cough).
- B.** The fact that she has been treated for TB disease in the past.
- C.** Her history of injecting illegal drugs (heroin).
- D.** A, B, and C are all correct.
- E.** Only A and C are correct.

#### **4.32 What diagnostic tests should be done on Francis?**

(circle the one best answer)

- A.** Sputum specimen collection for smear and culture.
- B.** Drug-susceptibility testing if culture is positive.
- C.** Chest radiograph because she has symptoms of pulmonary TB disease.
- D.** A, B, and C are all correct.
- E.** Only A and B are correct.

### **Genotyping**

TB genotyping is a laboratory-based approach used to analyze the genetic material (i.e., DNA) of *M. tuberculosis*. The total genetic content is referred to as the genome. Specific sections of the *M. tuberculosis* genome form distinct genetic patterns that help distinguish different strains of *M. tuberculosis*. *M. tuberculosis* genotyping is based on polymorphisms in the number and genomic location of mycobacterial repetitive elements. *M. tuberculosis* isolates with identical genotypes suggest that there may have been recent TB transmission among the persons from whom they were isolated. The main purpose of genotyping is to add to TB controllers' understanding of TB transmission in their community.

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**TB genotyping is a laboratory-based approach used to analyze the genetic material (i.e., DNA) of *M. tuberculosis*.**

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***M. tuberculosis* isolates with identical genotypes are often indicative of recent TB transmission among the persons from whom they were isolated.**

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When coupled with traditional epidemiologic investigations, analyses of the genotype of *M. tuberculosis* strains have confirmed suspected transmission and detected unsuspected transmission of *M. tuberculosis*. These analyses have also identified risk factors for recent infection with rapid progression to disease, demonstrated re-infection with different strains, identified weaknesses in conventional contact investigations, documented the existence of laboratory cross-contamination, and identified outbreaks of TB that were not previously recognized (Table 4.5). Genotyping has become an increasingly useful tool for studying the pathogenesis, epidemiology, and transmission of TB infection and disease.

**Table 4.5**  
**Use of Genotyping Analyses**

<b>How Genotyping Has Been Used</b>
<ul style="list-style-type: none"><li>• Confirmed suspected transmission and detected unsuspected transmission of <i>M. tuberculosis</i>, when coupled with traditional epidemiologic investigations</li><li>• Identified risk factors for recent infection with rapid progression to disease</li><li>• Demonstrated re-infection with different strains</li><li>• Identified weaknesses in conventional contact investigations</li><li>• Documented the existence of laboratory cross-contamination</li><li>• Identified outbreaks of TB that were not previously recognized</li></ul>

### Study Question

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**4.33 What is the main purpose of genotyping?**

(choose the one best answer)

- A.** To amplify DNA and RNA segments to rapidly identify the microorganisms in a specimen.
- B.** To add to TB controllers' understanding of TB transmission in their community.
- C.** To determine drug susceptibility of TB strains.
- D.** A, B, and C are all correct.
- E.** Only A and B are correct.

## Chapter Summary

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TB is **not** as common as it was many years ago in the United States; consequently, clinicians do **not** always consider the possibility of TB disease when evaluating patients who have symptoms. As a result, the diagnosis of TB disease may be delayed or even overlooked, and the patient may remain ill and possibly infectious for a prolonged period.

Not all persons with TB disease have symptoms; however, most persons with TB disease have one or more symptoms that lead them to seek medical care. All persons with symptoms of TB disease, and either a positive TST or IGRA indicative of *M. tuberculosis* infection, should be medically evaluated.

A complete medical evaluation for TB disease includes the following five components:

1. Medical history
2. Physical examination
3. Test for *M. tuberculosis* infection
4. Chest radiograph
5. Bacteriologic examination of clinical specimens

### 1. Medical History

When conducting a medical history, the clinician should ask if any symptoms of TB disease are present; if so, for how long, and if there has been known exposure to a person with infectious TB disease. Equally important is obtaining information on whether or not the person has been diagnosed in the past with LTBI or TB disease.

TB disease most commonly affects the lungs and is referred to as pulmonary TB disease. Symptoms include:

- Cough (especially if lasting for 3 weeks or longer) with or without sputum collection
- Coughing up blood (hemoptysis)
- Chest pain
- Loss of appetite
- Unexplained weight loss
- Night sweats
- Fever
- Fatigue

Extrapulmonary TB disease may cause symptoms related to the part of the body that is affected. For example, TB of the spine may cause back pain; TB of the kidney may cause blood in the urine; TB meningitis may cause headache or confusion. Extrapulmonary TB disease should be considered in the differential diagnosis of ill persons who have systemic symptoms and who are at high risk for TB disease.

Both pulmonary and extrapulmonary TB disease symptoms can be caused by other diseases; however, they should prompt the clinician to consider TB disease.

## 2. Physical Examination

A physical examination is an essential part of the evaluation of any patient. It **cannot** be used to confirm or rule out TB disease, but it can provide valuable information about the patient's overall condition, inform the method of diagnosis, and reveal other factors that may affect TB disease treatment, if diagnosed.

## 3. Test for *M. tuberculosis* Infection

Selection of the most suitable tests for detection of *M. tuberculosis* infection should be based on the reasons and the context for testing, test availability, and overall cost effectiveness of testing. Currently, there are two methods available for the detection of *M. tuberculosis* infection in the United States. The tests are:

- Mantoux tuberculin skin test (TST); and
- Interferon-gamma release assays (IGRAs)
  - » QuantiFERON-TB Gold In-Tube test (QFT-GIT)
  - » T-SPOT®.TB test.

These tests may help clinicians differentiate people infected with *M. tuberculosis* from those uninfected. However, a negative reaction to any of the tests does **not** exclude the diagnosis of TB disease or LTBI.

## 4. Chest Radiograph

With pulmonary TB being the most common form of disease, the chest radiograph is useful for diagnosis of TB disease. Chest abnormalities can suggest pulmonary TB disease. A posterior-anterior radiograph of the chest is the standard view used for the detection of chest abnormalities. In some cases, especially in children, a lateral view may be helpful.

Abnormalities seen on chest radiographs may be suggestive of, but are never diagnostic of, TB disease. Chest radiographs may be used to exclude TB disease in an HIV-negative person who has a positive TST reaction or IGRA and who has no symptoms or signs of TB disease.

## 5. Bacteriologic Examination of Clinical Specimens

Examinations of clinical specimens (for example, sputum or urine) are of critical diagnostic importance. The specimens should be examined and cultured in a laboratory that specializes in testing for *M. tuberculosis*. The bacteriologic examination has five parts:

- Specimen collection, processing, and review;
- AFB smear classification and results;
- Direct detection of *M. tuberculosis* in clinical specimen (NAA);
- Specimen culturing and identification; and
- Drug-susceptibility testing.

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