



Self-Study Modules on Tuberculosis

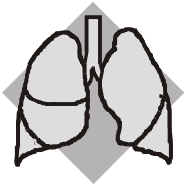
Targeted Testing and the Diagnosis of Latent Tuberculosis Infection and Tuberculosis Disease

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention
Division of Tuberculosis Elimination**



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Targeted Testing and the Diagnosis of Latent Tuberculosis Infection and Tuberculosis Disease

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BACKGROUND

In this module, you will learn about the diagnosis of TB disease and latent TB infection (LTBI), including targeted testing. Targeted testing is a TB control strategy that is used to identify people at high risk for developing TB disease who would benefit by treatment of LTBI, if detected. LTBI is diagnosed with the Mantoux tuberculin skin test (TST) or an interferon-gamma release assay (IGRA) such as the QuantiFERON[®]-TB Gold test (QFT-G). In most cases, TB disease is diagnosed with certain laboratory tests (bacteriologic examination); for patients who may have pulmonary TB disease, a chest x-ray is also useful for diagnosis. It is important to evaluate people who have symptoms of TB disease; if they are found to have TB disease, they need treatment to be cured and to avoid spreading TB to others. For this reason, the diagnosis of TB disease is crucial to controlling the spread of TB in homes and communities.

OBJECTIVES

After working through this module, you will be able to

1. Identify high-risk groups for targeted testing.
2. Describe how to place, read, and interpret a Mantoux tuberculin skin test.
3. Describe how to interpret a QuantiFERON[®]-TB Gold test.
4. Discuss considerations (for example, false-positives, false-negatives and/or limitations) for using either the Mantoux tuberculin skin test or the QuantiFERON[®]-TB Gold test for diagnosing infection with *Mycobacterium tuberculosis*.
5. Describe the components of a medical evaluation for diagnosing TB disease.



NEW TERMS

Look for the following new terms in this module and in the glossary.

acid-fast bacilli (AFB) – mycobacteria that when stained, retain color even after they have been washed in an acid solution; may be detected under a microscope in a stained smear

anergy – the inability to react to a skin test because of a weakened immune system, often caused by HIV infection or severe illness (see **anergy testing**)

anergy testing – giving skin tests using substances other than tuberculin; done in select situations to determine whether a person is anergic

antigen – protein substances that can produce an immune response (such as **CFP-10, ESAT-6, or those in PPD**)

bacteriologic examination – tests done in a mycobacteriology laboratory to diagnose TB disease; includes examining a specimen under a microscope, culturing the specimen, and testing for drug susceptibility

baseline skin test – a tuberculin skin test (TST) given to employees or residents in certain facilities when they start their job or enter the facility (see **TB testing program** and **two-step testing**)

BCG – bacille Calmette-Guérin (BCG), a vaccine for TB disease that is used in many countries but rarely used in the United States; may cause a false-positive reaction to the TST but does not affect QuantiFERON[®]-TB Gold test (QFT-G) results

boosted reaction – a positive reaction to a TST, due to a boosted immune response from a skin test given up to a year earlier; occurs in people who were infected a long time ago and whose ability to react to tuberculin has lessened. Two-step testing is used in TB testing programs to tell the difference between boosted reactions and reactions caused by recent infection (see **booster phenomenon** and **two-step testing**)

booster phenomenon – a phenomenon in which people (especially older adults) who are skin tested many years after becoming infected with *M. tuberculosis* may have a negative reaction to an initial TST, followed by a positive reaction to a TST given up to a year later; this happens because the first TST boosts the immune response. Two-step testing is used in TB testing programs to tell the difference between boosted reactions and reactions caused by recent infection (see **two-step testing**)

bronchoscopy – a procedure used to obtain pulmonary secretions or lung tissue with an instrument called a bronchoscope; used only when patients cannot cough up sputum on their own and an induced specimen cannot be obtained

cavity – a hollow space within the lung, visible on a chest x-ray, that may contain many tubercle bacilli; often occurs in people with severe pulmonary TB disease



CFP-10 – one of the antigens used in the QFT-G that is found in *M. tuberculosis* strains

clinician – a physician, physician’s assistant, or nurse

colonies – groups of mycobacteria that have grown in a culture

control – a standard of comparison for checking or verifying the results of an experiment. In the QFT-G, the substances mitogen and saline are the controls

culture – to grow organisms on media (substances containing nutrients) so that they or the product of this process can be identified; a positive culture for *M. tuberculosis* contains tubercle bacilli, whereas a negative culture contains no detectable tubercle bacilli

drug susceptibility pattern – the list of drugs to which a strain of tubercle bacilli is susceptible and to which it is resistant

erythema – redness around the site of the injection when a TST is done; erythema is not considered when the reaction size is measured, because redness does not indicate that a person has TB infection

ESAT-6 – one of the antigens used in the QFT-G that is found in *M. tuberculosis* strains

exposure to TB – time spent with or near someone who has infectious TB disease

false-negative reaction – a negative reaction to the TST in a person who has TB infection; may be caused by HIV or other immunocompromising diseases, immunosuppressive drugs, a live-virus vaccination, recent infection (within the past 10 weeks), or very young age (younger than 6 months old)

false-positive reaction – a positive reaction to the TST in a person who does not have TB infection; may be caused by infection with nontuberculous mycobacteria or by vaccination with BCG

gastric washing – a procedure done by inserting a tube through the patient’s nose and passing it into the stomach; may be useful for obtaining a specimen for culture from children, who produce little or no sputum when they cough

heparin – an anti-clotting agent added to patient’s blood samples for conducting a QFT-G

induced sputum – sputum that is obtained by having the patient inhale a saline (salt water) mist, causing the patient to cough deeply; this procedure is used to help patients cough up sputum if they cannot do so on their own

induration – swelling that can be felt around the site of injection after a TST is done; the reaction size is the diameter of the swollen area, measured across the forearm

infiltrate – a collection of fluid and cells in the tissues of the lung; visible on a chest x-ray in people with pulmonary TB disease

interferon-gamma (IFN- γ) – protein that is normally produced by the body in response to infection; the QFT-G results are based on how much IFN- γ is released in response to antigens mixed with a patient’s blood sample

interferon-gamma release assay (IGRA) – a type of blood test that measures a person’s immune reactivity to *M. tuberculosis*. In the U.S., QuantiFERON[®]-TB Gold and the QuantiFERON[®]-TB Gold In-Tube are currently available IGRAs



isolate – a group of organisms isolated or separated from a specimen; in an *M. tuberculosis* isolate, the organisms have been grown in culture and identified as *M. tuberculosis*

malaise – a feeling of general discomfort or illness

Mantoux tuberculin skin test (TST) – a method of testing for TB infection; done by using a needle and syringe to inject 0.1 ml of 5 tuberculin units of liquid tuberculin between the layers of the skin (intradermally), usually on the forearm; the reaction to this test, usually a small swollen area (induration), is measured 48 to 72 hours after the injection and is interpreted as positive or negative depending on the size of the reaction and the patient's risk factors for TB

media – substances containing special nutrients and used for growing cultures of bacteria found in specimens

medical history – the part of a patient's life history that is important for diagnosing and treating TB infection or disease, including history of exposure, symptoms, previous diagnosis of TB infection or disease, and risk factors for TB disease

multiple-puncture test – a type of tuberculin skin test done by puncturing the skin of the forearm with a set of short prongs or tines to inject tuberculin (for example, Tine test); although easy to give and convenient, these tests are not accurate and should not be used to determine whether a person has TB infection

mycobacteriology laboratory – a laboratory that deals specifically with *M. tuberculosis* and other mycobacteria

nucleic acid amplification (NAA) – a technique that amplifies (copies) DNA and RNA segments, in order to directly identify microorganisms in sputum specimens

polymerase chain reaction (PCR) – a technique used to copy small segments of DNA

PPD (purified protein derivative) – antigens such as the type of tuberculin used in the TST (see **antigen**)

PPD skin test – a tuberculin skin test that uses PPD tuberculin

QuantiFERON[®]-TB Gold (QFT-G) – a blood test used for diagnosing infection with *M. tuberculosis*. The QFT-G measures a patient's immune reactivity to *M. tuberculosis*

resistant – an organism's ability to grow despite the presence of a particular drug

skin test conversion – a change in a skin test reaction from negative to positive between testing intervals

smear – a specimen that has been smeared onto a glass slide, stained, washed in an acid solution, and then placed under the microscope for examination; used to detect acid-fast bacilli in a specimen

sputum – phlegm from deep in the lungs, collected in a sterile container for processing and examination

susceptible – an organism's ability to be killed by a particular drug



symptoms of TB disease – noticeable conditions caused by TB disease. The symptoms of pulmonary TB disease include coughing, pain in the chest when breathing or coughing, and coughing up sputum or blood. The general symptoms of TB disease (pulmonary or extrapulmonary) include weight loss, fatigue, malaise, fever, and night sweats. The symptoms of extrapulmonary TB disease depend on the part of the body that is affected by the disease

targeted testing – a TB control strategy to identify persons at high risk for latent TB infection and persons at high risk for developing TB disease who would benefit from treatment

TB testing program – a program in which employees and residents of a facility are periodically tested for TB; done to identify people who have TB infection and possibly TB disease and to determine whether TB is being transmitted in the facility

TST (tuberculin skin test) – see Mantoux tuberculin skin test

tuberculin – a substance made from tubercle bacilli that have been killed by heating; used to determine whether a person has TB infection. Tuberculin is not a vaccine

tuberculin unit – a standard strength of tuberculin used in the United States and Canada; a strength of 5 tuberculin units is used for the Mantoux TST

two-step testing – a strategy used in TB testing programs to distinguish a boosted reaction (caused by TB infection that occurred many years before the skin test) from a reaction caused by recent infection. If a person has a negative reaction to an initial skin test, a second test is given 1 to 3 weeks later; a positive reaction to the second test probably represents a boosted reaction, not recent infection. Two-step testing is used in many TB testing programs for skin testing employees when they start their job



READING MATERIAL

Targeted Testing

Targeted testing is the TB control strategy that is used to identify and treat persons who are at high risk for LTBI or at high risk for developing TB disease once infected with *M. tuberculosis*. Identifying persons with LTBI is important to the goal of TB elimination because LTBI treatment can prevent these persons from developing TB disease and thereby stop the further spread of TB to others.

.....
A decision to test is a
decision to treat.
.....

Thus, during routine patient evaluations, health care providers should identify and test persons who are at high risk for TB. However, TB testing activities should be done only when there is a plan for follow-up care to evaluate and treat all individuals diagnosed with LTBI or TB disease. Healthcare agencies or other facilities should consult with the local health department before starting a TB testing program to make sure that any person whose test result is positive will have access to follow-up care.

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People who are not at high
risk for LTBI generally
should not be tested.
.....

People who are not at high risk for LTBI generally should not be tested. Testing in low-risk populations can take resources away from other important activities. Also, positive test results in low-risk populations are sometimes inaccurate. There, however, may be instances in which health care providers are asked to test individuals who are not necessarily regarded as high risk (for example, daycare center workers, teachers, and college students).

Identifying High-Risk Groups for TB Testing:

High-risk groups can be divided into two categories:

- People who are at high risk for becoming infected with *Mycobacterium tuberculosis*
- People who are at high risk for developing TB disease once infected with *Mycobacterium tuberculosis*



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Flexibility should be used in
defining high-risk groups for
testing.
.....

Flexibility should be used in defining high-risk groups for testing. Since the epidemiology of TB can change, the risk of LTBI or TB disease among groups may change over time. Groups that are currently identified as being at low-risk may later be considered high priority. Moreover, because of the differences in populations from one community to another, definitions of high risk should be made at the local (city, county, or state) level according to local demographics and TB epidemiology.

In general, however, high-risk groups listed in both categories of Table 3.1 should be tested.

Table 3.1
Groups at High Risk for LTBI and TB Disease

<p>People at High Risk for Becoming Infected with <i>M. tuberculosis</i></p>	<p>People at High Risk for TB Disease after <i>M. tuberculosis</i> Infection</p>
<ul style="list-style-type: none"> ▪ Close contacts of people known or suspected to have TB ▪ People, including children, who have come to the United States (within the last 5 years) from areas of the world where TB is common (for example, Asia, Africa, Russia, Eastern Europe, or Latin America) ▪ Low-income groups with poor access to health care, including homeless people ▪ People who inject illegal drugs ▪ People who live or work in high-risk residential settings (for example, nursing homes, homeless shelters, or correctional facilities) ▪ Health care workers who serve high-risk clients ▪ High-risk racial or ethnic minority populations, as locally defined ▪ Infants, children, and adolescents exposed to adults in high-risk groups 	<ul style="list-style-type: none"> ▪ People living with HIV ▪ People recently infected with <i>M. tuberculosis</i> (within the past 2 years) ▪ People with medical conditions known to increase the risk for TB <ul style="list-style-type: none"> – silicosis – diabetes mellitus – severe kidney disease – certain types of cancer – certain intestinal conditions – organ transplant – immunosuppressive therapy (including prolonged use of oral or intravenous corticosteroids, and tumor necrosis factor-alpha [TNF-α] antagonists) – low body weight ▪ People who inject illegal drugs ▪ Infants and children younger than 4 years



Diagnosis of LTBI

Currently, the available methods of testing for *M. tuberculosis* infection are the **Mantoux tuberculin skin test (TST)** and the **interferon-gamma release assays (IGRA)** such as the **QuantiFERON®-TB Gold test (QFT-G)**.

.....
The TST can be used to determine whether a person has LTBI.
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.....
Tuberculin is not a vaccine.
.....

The Mantoux Tuberculin Skin Test (TST)

The TST is used to determine if a person is infected with *M. tuberculosis*. In this test, a substance called **tuberculin** is injected into the skin. Tuberculin contains **antigens** used for diagnosing TB infection; **it is not a vaccine**. An antigen is a protein substance that can produce an immune response. Tuberculin is made from proteins derived from tubercle bacilli that have been killed by heating. In most people who have TB infection, the immune system will recognize the tuberculin because it is similar to the tubercle bacilli that caused infection. This will cause a reaction to the tuberculin at the site of the injection. Tuberculin used for the skin test is also known as **purified protein derivative**, or **PPD**. For this reason, the TST is sometimes called a **PPD skin test**.

Administering the TST

The TST is given by using a single dose disposable tuberculin syringe to inject 0.1 ml of **5 tuberculin units** of liquid tuberculin between the layers of the skin (intradermally), on the forearm (Figure 3.1). A tuberculin unit is a standard strength of tuberculin. When giving the TST, institutional guidelines for infection control should be followed.



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Most people with TB
infection have a positive
reaction to the tuberculin.
.....

.....
The reaction is an area of
induration, or swelling,
around the site of the
injection.
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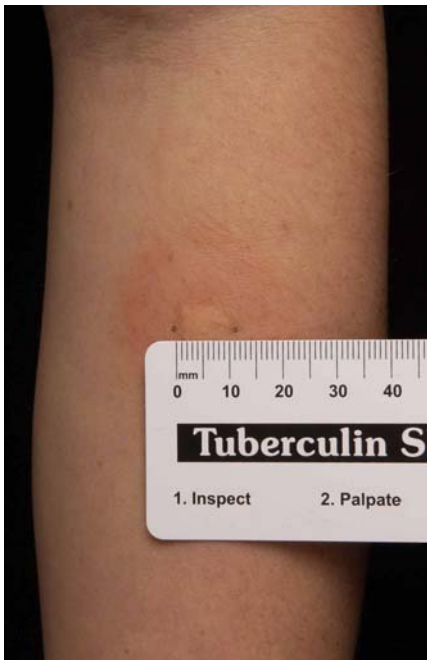
.....
The Mantoux TST is the
preferred type of TB skin
test.
.....

A patient’s forearm should be examined by a trained health care worker 48 to 72 hours after the tuberculin is injected. Health care workers should not ask patients to read their own skin test results. Most people with TB infection will have a positive reaction to the tuberculin. The reaction is an area of **induration** (swelling that can be felt) around the site of the injection. The diameter of the indurated area is measured in millimeters across the forearm (Figure 3.2); **erythema** (redness) around the indurated area is not measured, because the presence of erythema does not indicate that a person has TB infection (Figure 3.3).

In the past, multiple-puncture tests (for example, the Tine test) were a popular skin testing method for TB; however, the multiple-puncture test is no longer recommended. The multiple-puncture tests are done by puncturing the skin of the forearm with a device having a set of short prongs or tines coated with tuberculin. However, since the amount of tuberculin that actually enters the skin cannot be measured, multiple-puncture tests are not reliable and should not be used. Because the amount of tuberculin can always be measured during a Mantoux TST, this type of test is more accurate, and is the preferred skin testing method.



Figure 3.1 Giving the Mantoux TST.



*Figure 3.2 Only the induration is being measured. This is **CORRECT**.*



*Figure 3.3 The erythema is being measured. This is **INCORRECT**.*



Study Questions 3.1 – 3.2

3.1 What is the TST used for?

3.2 How is the Mantoux TST given?

Answers to study questions are on pages 71 – 77.



Study Questions 3.3 – 3.5

3.3 With the TST, when is the patient's arm examined?

3.4 How is the induration measured?

3.5 Why is the Mantoux TST preferable to multiple-puncture tests?

Answers to study questions are on pages 71 – 77.



.....
Interpretation of a reaction to
a TST depends on the size of
the induration and on the
person's risk factors for TB.
.....

Interpreting the Reaction

Interpreting a TST reaction depends on the size of the induration and the person's risk factors for TB (Table 3.2).

An induration of **5 or more millimeters** is considered a positive reaction for the following people:

- People living with HIV
- Recent close contacts of people with infectious TB
- People with chest x-ray findings suggestive of previous TB disease
- People with organ transplants
- Other immunosuppressed patients (for example, patients on prolonged therapy with corticosteroids, equivalent to 15mg per day or more of prednisone for at least 1 month, or those taking TNF- α antagonists)

An induration of **10 or more millimeters** is considered a positive reaction for the following people:

- People who have recently come to the United States (within the last 5 years) from areas of the world where TB is common (for example, Asia, Africa, Russia, Eastern Europe, or Latin America)
- People who inject illegal drugs
- People who live or work in high-risk congregate settings (for example, nursing homes, homeless shelters, or correctional facilities)
- Mycobacteriology laboratory workers
- People with medical conditions, other than HIV, that increase the risk for developing TB disease (see Table 3.1)
- Children younger than 4 years
- Infants, children, or adolescents exposed to adults in high-risk categories



.....
An induration of 15 or more millimeters is considered a positive reaction for people with no known risk factors for TB.
.....

An induration of **15 or more millimeters** is considered a positive reaction for people with no known risk factors for TB. However, targeted testing should be done only in high-risk groups since a positive test result in low-risk groups can be inaccurate.

Most people who have a positive TST reaction will usually have a positive reaction every time they are tested, regardless of whether they receive treatment. This is because the TST detects the immune response to tuberculin, not the presence of tubercle bacilli in the body.

Thus the TST should not be performed on a person who has a documented history of either a positive TST result or treatment for TB disease.

Interpreting the TST Reaction for Occupational Exposure



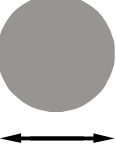
For **people who may be exposed to TB on the job** (such as health care workers and staff of nursing homes or correctional facilities), the interpretation of the TST reaction as positive or negative depends on

- The employee's individual risk factors for TB
- The risk of exposure to TB in the person's job

Therefore, in facilities where the risk of exposure to TB is very low, 15 or more millimeters of induration may be considered a positive reaction for employees with no other risk factors for TB. In facilities where TB patients receive care, 10 or more millimeters of induration may be considered a positive reaction for employees with no other risk factors for TB.



Table 3.2
Interpreting the TST Reaction

 5 or more millimeters	 10 or more millimeters	 15 or more millimeters
<p>An induration of 5 or more millimeters is considered positive for</p> <ul style="list-style-type: none"> ▪ People living with HIV ▪ Recent contacts of persons with infectious TB ▪ People who have previously had TB disease ▪ Patients with organ transplants and other immunosuppressed patients (including patients taking a prolonged course of oral or intravenous corticosteroids or TNF-α antagonists) 	<p>An induration of 10 or more millimeters is considered positive for</p> <ul style="list-style-type: none"> ▪ People who have come to the U.S. within the last 5 years from areas of the world where TB is common (for example, Asia, Africa, Eastern Europe, Russia, or Latin America) ▪ People who inject illegal drugs ▪ Mycobacteriology lab workers ▪ People who live or work in high-risk congregate settings ▪ People with certain medical conditions that place them at high risk for TB (silicosis, diabetes mellitus, severe kidney disease, certain types of cancer, and certain intestinal conditions) ▪ Children younger than 4 years ▪ Infants, children, and adolescents exposed to adults in high-risk categories 	<p>An induration of 15 or more millimeters is considered positive for</p> <ul style="list-style-type: none"> ▪ People with no known risk factors for TB



Study Questions 3.6 – 3.7

- 3.6 What two factors determine the interpretation of a skin test reaction as positive or negative? What additional factor is considered for people who may be exposed to TB on the job?
- 3.7 For which groups of people is 5 or more millimeters of induration considered a positive reaction? Name four.

Answers to study questions are on pages 71 – 77.



Study Questions 3.8 – 3.9

- 3.8 For which groups of people is 10 or more millimeters of induration considered a positive reaction? Name seven.
- 3.9 For which group of people is 15 or more millimeters of induration considered a positive reaction?

Answers to study questions are on pages 71 – 77.

Case Study 3.1

Which of the following patients have a positive TST reaction? Circle the best answer(s).

- a) Mr. West, 36 years old, HIV infected, 8 mm of induration
- b) Ms. Hernandez, 26 years old, native of Mexico, 7 mm of induration
- c) Ms. Jones, 56 years old, has diabetes, 12 mm of induration
- d) Mr. Sung, 79 years old, resident of a nursing home, 11 mm of induration
- e) Mr. Williams, 21 years old, no known risk factors, 13 mm of induration
- f) Ms. Marcos, 42 years old, chest x-ray findings suggestive of previous TB, 06 mm of induration
- g) Ms. Rayle, 50 years old, husband has pulmonary TB, 9 mm of induration

Answers to case study questions are on pages 78 – 81.



False-Positive Reactions

The TST is a valuable tool, but it is not perfect. Several factors can cause people to have a positive reaction even if they do not have TB infection. This is called a **false-positive reaction**.

The causes of false positive reactions may include, but are not limited to, the following:

.....
False-positive reactions can be caused by infection with nontuberculous mycobacteria or vaccination with BCG.
.....

- Infection with nontuberculous mycobacteria (NTM) (mycobacteria other than *M. tuberculosis*)
- BCG vaccination
- Administration of incorrect antigen
- Incorrect measuring or interpretation of the TST reaction

Infection with NTM can sometimes cause a false positive reaction to the TST. Another cause of a false positive reaction is BCG (bacille Calmette-Guérin), a vaccine for TB disease that is used in many countries. BCG, however, is rarely used in the United States because studies have shown that it is not completely effective. People who have been vaccinated with BCG **may** have a positive reaction to the TST even if they do not have TB infection.

.....
People who have a positive reaction should be further evaluated for LTBI or TB disease, even if they were vaccinated with BCG.
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However, there is **no reliable way** to distinguish a positive TST reaction caused by vaccination with BCG from a reaction caused by true TB infection. When using the TST, people who have been vaccinated with BCG should always be further evaluated for LTBI or TB disease as if they were not vaccinated with BCG.

A false-positive reaction may also occur if an incorrect antigen is used or when the results are not measured or interpreted properly.



Study Questions 3.10 – 3.11

3.10 Name four factors that can cause false-positive reactions to the TST.

3.11 Is there a reliable way to distinguish a positive tuberculin reaction caused by vaccination with BCG from a reaction caused by true TB infection?

Answers to study questions are on pages 71 – 77.



False-Negative Reactions

Some people have a negative reaction to the TST even though they have TB infection. This is called a **false-negative reaction**. The reasons for these false-negative reactions may include, but are not limited to the following:

- Anergy
- Recent TB infection (within the past 8-10 weeks)
- Very young age (younger than 6 months)
- Recent live-virus measles or smallpox vaccination
- Incorrect method of giving the TST
- Incorrect measuring or interpretation of TST reaction

.....
Anergy is the inability to react to skin tests because of a weakened immune system.
.....

A common cause of false-negative reactions is anergy. **Anergy** is the inability to react to skin tests because of a weakened immune system. Many conditions, such as HIV infection, cancer, measles or other viral infections, or severe TB disease itself, can weaken the immune system and cause anergy. HIV infection is an important cause of anergy.

.....
People who do not react to any of the substances used for anergy testing (including tuberculin) are considered anergic.
.....

Anergy testing in conjunction with skin testing is **no longer** routinely recommended as a part of TB testing; however, in some situations anergy testing may be used to guide decisions regarding therapy for patients. Anergy testing can be done by giving skin tests using substances other than tuberculin. The recommended substances for anergy testing include mumps, *Candida* (a type of fungus), or tetanus extracts. Most healthy people will have a skin test reaction to one or more of these substances.

People who do not react to any of the substances, including tuberculin, are considered anergic. People who have a reaction to any of the substances are NOT anergic.



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It takes 2 to 8 weeks after TB infection for the body's immune system to be able to react to tuberculin.
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.....
Because their immune systems are not yet fully developed, children younger than 6 months old may have a false-negative reaction to the TST.
.....

.....
Any patient with symptoms of TB should be evaluated for TB disease, regardless of his or her skin test reaction.
.....

Another cause of false-negative reactions is **recent TB infection** (infection within the past 8 to 10 weeks). It can take 2 to 8 weeks after TB infection for the body's immune system to be able to react to tuberculin and for the infection to be detected by the TST. For this reason, it is recommended that close contacts of someone with infectious TB disease who have a negative reaction to the TST be retested 8 to 10 weeks after the last time they were in contact with the person who has TB disease.

A third cause of false-negative reactions is **very young age**. Because their immune systems are not yet fully developed, **children younger than 6 months** may have a false-negative reaction to the TST.

Vaccination with live viruses may also lead to a false-negative reaction. The Advisory Committee on Immunization Practices recommends that skin testing be done on either the same day as vaccination with live-virus measles vaccine or 4 to 6 weeks after vaccination to prevent possible false-negative reactions. Also, skin testing should not be done until at least 1 month after a smallpox vaccination.

A false-negative reaction may also occur when the TST is given incorrectly or the results are not measured or interpreted properly.

Both false-positive and false-negative reactions to the TST are summarized in Table 3.3.

Any patient with symptoms of TB should be evaluated for TB disease, regardless of his or her TST reaction. In fact, people with symptoms of TB should be evaluated for TB disease right away, at the same time that the TST is given. TB symptoms and the diagnosis of disease are discussed later in this module.



Table 3.3
False-Positive and False-Negative Reactions to the TST

Type of Reaction	Possible Cause	People at Risk
False-positive	Nontuberculous mycobacteria (NTM)	People infected with NTM
	BCG vaccination	People vaccinated with BCG
	Administering of incorrect antigen	Any person being tested
	Incorrect interpretation of TST result	Any person being tested
False-negative	Anergy	HIV-infected people, other people with weakened immune systems, severe TB disease, and some viral illness (e.g., measles and chicken pox)
	Recent TB infection	People infected with <i>M. tuberculosis</i> within the past 8 to 10 weeks
	Very young age	Children younger than 6 months
	Recent live-virus measles or small pox vaccination	Any person who will be or recently received a live-virus vaccination
	Incorrect method of giving TST	Any person being tested
	Incorrect interpretation of TST	Any person being tested



Study Questions 3.12 – 3.14

3.12 Name six factors that can cause false-negative reactions to the TST.

3.13 What is anergy?

3.14 After TB germs have been transmitted to someone, how long does it take before TB infection can be detected by the TST?

Answers to study questions are on pages 71 – 77.



Study Question 3.15

3.15 What should be done if a patient has a negative TST result, but has symptoms of TB disease?

Answers to study questions are on pages 71 – 77.

Case Study 3.3

Mr. Bell comes to the TB clinic for a TST. He believes that he has been exposed to TB, and he knows he is at high risk for TB because he is HIV infected. He is given a TST, and his reaction is read 48 hours later as 0 millimeters of induration.

- What are three ways to interpret this result?

Answers to case study questions are on pages 78 – 81.



TB Testing Programs, the Booster Phenomenon, and Two-Step Testing

.....
 Certain facilities have TB testing programs, in which employees and residents are periodically given TSTs.

Many residential facilities, health care facilities, and other settings have **TB testing programs**. This means that employees and residents are periodically given TSTs. The purposes of the testing programs are to

- Identify people who have LTBI and possibly TB disease, so that they can be given treatment as needed
- Determine whether TB is being transmitted in the facility

.....
 In a TB testing program, employees or residents are skin tested when they start their job or enter the facility; repeat testing is done at regular intervals thereafter.

In a TB testing program, employees or residents are TB tested when they start their job or enter the facility. If they are using a TST, this is called the **baseline skin test**. If they have a negative TST reaction, they may be retested at regular intervals thereafter. In some facilities, repeat testing should be done at least once a year. For more information on TB testing programs at health care and residential facilities, please refer to *Module 5, Infectiousness and Infection Control*.

.....
 In order to detect TB transmission and identify people who have skin test conversions, accurate information must be obtained for every employee's skin tests.

Employees or residents whose skin test reaction converts from negative to positive between testing intervals have probably become infected with *M. tuberculosis*. These **skin test conversions** may indicate that TB is being transmitted in the facility. People with skin test conversions are at high risk of developing TB disease because they were infected with *M. tuberculosis* relatively recently (within the past 2 years). (See *Module 1, Transmission and Pathogenesis of Tuberculosis*.) In order to detect TB transmission and identify people who have skin test conversions, accurate information must be obtained for every employee's baseline skin test, as well as for additional skin tests.



.....
The booster phenomenon can
affect the accuracy of the
baseline skin test.
.....

.....
The booster phenomenon
happens when a skin test
boosts the ability of the
immune system to react to
tuberculin.
.....

One factor that can affect the accuracy of the baseline skin test is the **booster phenomenon**. The booster phenomenon happens because in some people who have TB infection, the ability to react to tuberculin lessens over time. When these people are skin tested many years after they became infected with *M. tuberculosis*, they may have a negative reaction. However, if they are tested again within a year of the first test, they may have a positive reaction. This is because the first skin test “jogged the memory” of the immune system, **boosting** its ability to react to tuberculin. It may appear that these people were infected between the first and second skin tests (recent TB infection). Actually, the second, positive reaction is a **boosted reaction** (due to TB infection that occurred a long time ago). The booster phenomenon occurs mainly among older adults. The booster phenomenon is illustrated in Figure 3.4.



Targeted Testing and the Diagnosis of Latent Tuberculosis Infection and Tuberculosis Disease

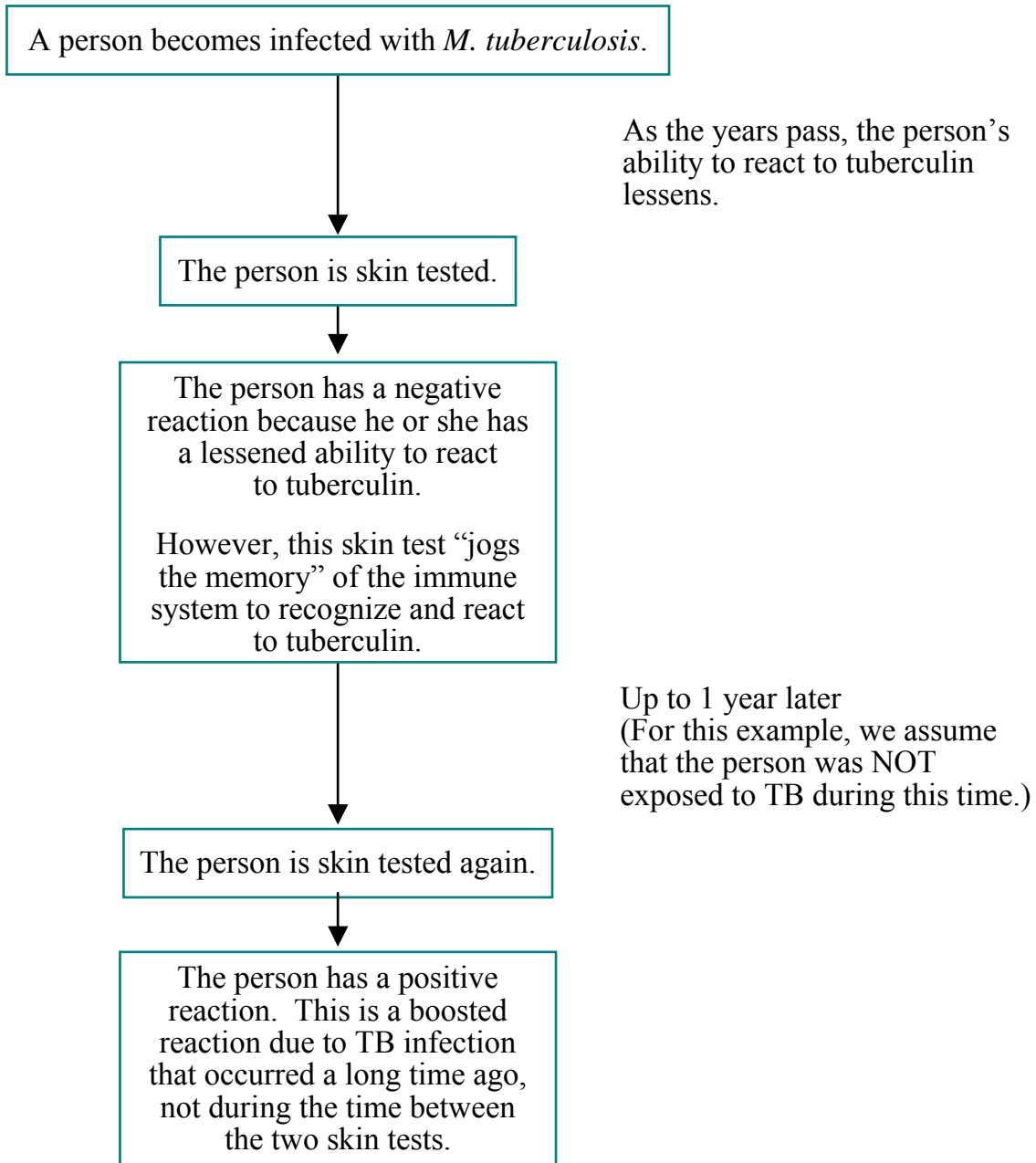


Figure 3.4 The booster phenomenon.



The booster phenomenon can present a problem in TB testing programs. This is because a negative reaction to the baseline skin test, followed by a positive reaction to a subsequent skin test that is given up to a year later, may be caused by either

- **Recent TB infection** in a person who was NOT infected at the time of the baseline skin test, or
- A **boosted reaction** in a person who WAS already infected before the baseline skin test

.....
Two-step testing is a strategy for telling the difference between boosted reactions and reactions caused by recent infection.
.....

To avoid misinterpretation, a strategy has been developed for telling the difference between boosted reactions and reactions caused by recent infection. This strategy, called **two-step testing**, should be used for the initial skin testing of persons who will be retested periodically, such as health care workers.

If a person has a negative reaction to an initial skin test, he or she is given a second test 1 to 3 weeks later.

- If the reaction to the second test is **positive**, it probably is a **boosted reaction** (due to TB infection that occurred a long time ago).
- If the reaction to the second test is **negative**, the person is considered **uninfected**. In this person, a positive reaction to a skin test given later on will probably be due to recent infection and be considered a skin test conversion.

.....
Two-step testing is used in many TB testing programs for skin testing employees when they start their job.
.....

Thus, because it provides accurate information about each employee's baseline skin test reaction, two-step testing is used in many TB testing programs for employees when they start their job. In particular, two-step testing is often used in hospitals and nursing homes. The procedure for two-step testing is shown in Figure 3.5.

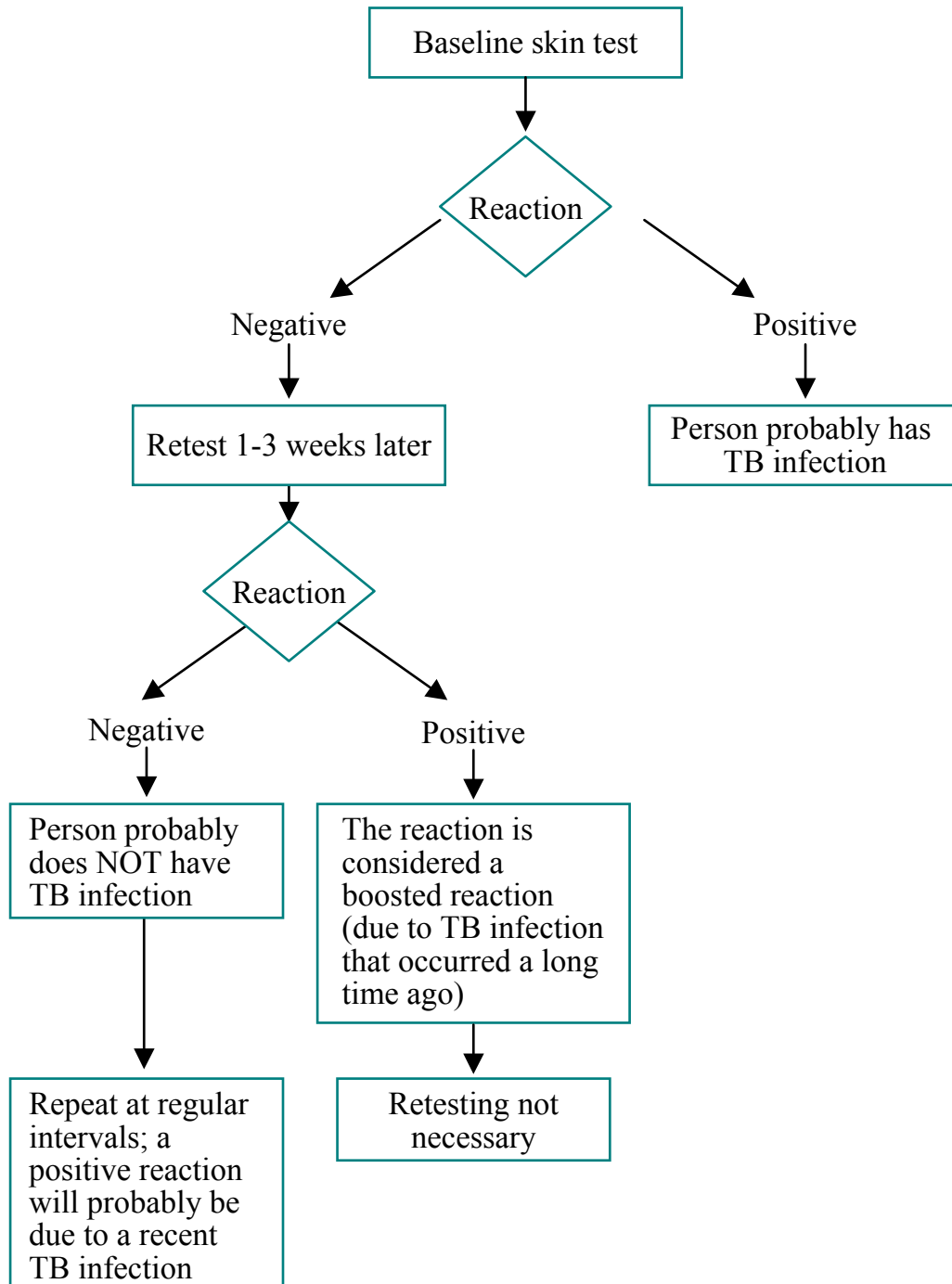


Figure 3.5 Two-step testing.



Study Questions 3.16 – 3.19

3.16 What is the booster phenomenon?

3.17 What is the purpose of two-step testing?

3.18 In what type of situation is two-step testing used?

3.19 How is two-step testing done?

Answers to study questions are on pages 71 – 77.



Case Study 3.4

Ms. Wilson is a 60-year-old nurse. When she started a job at the local hospital, she was given a TST, her first test in 25 years. Her reaction was read 48 hours later as 0 millimeters of induration. Six months later, she was retested as part of the TB testing program in the unit where she works. Her skin test reaction was read 48 hours later as 11 millimeters of induration.

- **What are two ways to interpret this result?**

Answers to case study questions are on pages 78 – 81.



.....
The QFT-G is used to help
diagnose *M. tuberculosis*
infection.
.....

The QuantiFERON®-TB Gold Test

The QuantiFERON®-TB Gold (QFT-G) is a blood test referred to as an **interferon-gamma release assay (IGRA)**. IGRAs are blood tests that measure a person’s immune reactivity to *M. tuberculosis*. The QFT-G was approved by the Food and Drug Administration in 2005. The QFT-G is used to help diagnose *M. tuberculosis* infection in persons suspected of having either LTBI or TB disease. The **QuantiFERON®-TB Gold In-Tube** was approved by the Food and Drug Administration in October 2007; however, CDC guidelines for use in determining TB infection are currently being established.

Conducting a QFT-G

To conduct a QFT-G, first read the instructions from the manufacturer and then follow the steps below:

- Confirm arrangements for delivery and testing of blood in a qualified laboratory within 12 hours of blood sample collection.
- Draw a sample of whole blood from the patient into a tube with heparin, according to manufacturer’s instructions.
- Schedule an appointment for the patient to receive test results and, if then needed, medical evaluation and possible treatment for LTBI or TB disease.

.....
Before conducting a QFT-G,
arrangements should be
made with a qualified
laboratory.
.....

Before conducting a QFT-G, arrangements should be made with a laboratory qualified for processing blood for a QFT-G analysis. Next a sample of whole blood should be drawn from a patient into a tube with **heparin**, an anti-clotting agent. The patient’s blood samples must then be delivered to the laboratory within 12 hours for analysis. An appointment should be scheduled with the patient to discuss test results, and if needed, medical evaluation and possible treatment for TB disease or LTBI.



.....
Patient's blood samples are
mixed with antigens found in
M. tuberculosis strains.
.....

How a QFT-G Works

At the laboratory, the patient's blood samples are mixed with antigens (protein substances that can produce an immune response) and incubated for 16 to 24 hours. The antigens used, **ESAT-6 and CFP-10**, are found in *M. tuberculosis* strains. If a person is infected with *M. tuberculosis*, the blood cells in the sample will recognize the antigens and release **interferon-gamma (IFN- γ)** in response. IFN- γ is a protein that is normally produced by the body in response to infections.

Blood samples are also mixed with **control** substances, **mitogen and saline**. These controls are used for comparison purposes to help verify test results and to determine a person's background level of IFN- γ .

.....
QFT-G results are based on
the amount of IFN- γ released
in response to the antigens
and control substances.
.....

The QFT-G results are based on the amount of IFN- γ that is released in response to the *M. tuberculosis* antigens and control substances. As with the TST, medical evaluation and additional tests (such as chest x-rays, sputum smears, and culture) are needed to confirm the diagnosis of LTBI or TB disease.

Interpreting QFT-G Results

Interpretation of the QFT-G result is based on the IFN- γ concentrations in the test samples. To help calculate the results, laboratories can use software provided by the manufacturer. The laboratory conducting the analysis of the QFT-G will then submit a report of the results back to the health care provider who requested the test.

The laboratory report should include interpretation of QFT-G results and indicate the concentration of IFN- γ in each blood sample. The test results will be reported as positive, negative, or indeterminate (Table 3.4).



Table 3.4
Report of QFT-G Results

QFT-G Result	Report/Interpretation
Positive	<i>M. tuberculosis</i> infection likely
Negative	<i>M. tuberculosis</i> infection unlikely, but cannot be excluded especially if <ol style="list-style-type: none"> 1. Patient has TB signs and symptoms 2. Patient has a high risk for developing TB disease once infected with <i>M. tuberculosis</i>
Indeterminate	Test did not provide useful information about the likelihood of <i>M. tuberculosis</i> infection. Options are to repeat QFT-G test or give a TST or do no additional testing

.....
Persons who test positive for TB infection should be evaluated for TB disease and, if disease is ruled out, they should be considered for LTBI treatment.
.....

If the result is positive, then it is likely that the patient has *M. tuberculosis* infection. There is no reason to follow a positive QFT-G result with a TST. However, TB disease should be ruled out by medical evaluation before LTBI is diagnosed.

If the result is negative, then the patient is unlikely to have *M. tuberculosis* infection and may not require further evaluation unless they have signs and symptoms of TB disease. Moreover, as with the TST, persons who have a negative test result can still have LTBI.

If the result is indeterminate, that means that test could not be interpreted. The options then are to retest the patient with the QFT-G again or a TST or do no additional testing.

Each QFT-G result and its interpretation should be considered in conjunction with other epidemiologic, historical, physical, and diagnostic findings. Regardless of test results, if a patient has signs and symptoms of TB disease or if they are at high risk for developing TB disease, they should be further evaluated.

As with the TST, negative QFT-G results for contacts to persons with infectious TB should be confirmed with a repeat test 8 to 10 weeks after the end of exposure.



.....
QFT-G can be used in all
circumstances in which the
TST is currently used.
However, caution should be
used when testing in certain
populations because of
limited data.
.....

QFT-G Recommendations

QFT-G can be used in all circumstances in which the TST is currently used, including contact investigations, evaluation of recent immigrants who have had BCG vaccination, and TB testing of health care workers and others undergoing serial evaluation for *M. tuberculosis*. However, caution should be used when testing certain populations because of limited data in the use of QFT-G. Furthermore, as with the TST, testing programs using QFT-G should only be implemented if plans are also in place for the necessary follow-up medical evaluation (such as chest x-rays) and treatment for persons diagnosed with LTBI or TB disease.

Advantages of Using QFT-G

The QFT-G and the TST measure different components of a patient's immune response to determine if he or she is infected with *M. tuberculosis*. Furthermore, both tests are conducted differently. The QFT-G is conducted with blood samples analyzed in a laboratory, where as the TST is conducted by injecting tuberculin intradermally in the patient's forearm. There are advantages and limitations to using the QFT-G. Below are listed some advantages to using the QFT-G compared to using the TST (Table 3.5).

- Requires a single patient visit to conduct the test
- Results can be available in 24 hours
- Does not cause the booster phenomenon which can happen with repeat TSTs
- Less likely to have incorrect reading of results compared to the TST
- BCG vaccination does not affect QFT-G results



.....
The QFT-G results can be
available in 24 hours.
.....

An advantage of using the QFT-G compared to the TST is that it only requires one patient visit to conduct the test and results can be available in 24 hours. The TST requires two visits by the patient to obtain test results. The patient must come for their first visit to receive the TST and then for a second visit, 48 to 72 hours later, to have their TST reaction read.

.....
The QFT-G does not cause
the booster phenomenon.
.....

Since the QFT-G is a blood test conducted in a laboratory the patient is not exposed to the antigens. Thus, unlike the TST, there is no booster phenomenon when using the QFT-G. Since the QFT-G does not cause the booster phenomenon, there is no need to use two-step testing.

Another advantage of using the QFT-G is that there is less chance of reader bias since it is a laboratory-based test. The TST requires that the health care worker place, measure, and interpret the test. Thus there is more of a chance of an incorrect reading of the results of the test.

.....
BCG vaccination does not
affect QFT-G results.
.....

The antigens used in QFT-G, ESAT-6 and CFP-10, are not found in the BCG vaccine strains. Therefore, previous vaccination with BCG will not cause false-positive results when using the QFT-G as compared to using a TST.

**Table 3.5
Advantages of Using the QFT-G Compared to Using the TST**

QFT-G Result	TST
Requires 1 patient visit to conduct the test	Requires 2 patient visits to conduct the test (3 to 4 visits if 2 step testing)
Results can be available in 24 hours	Results are available 48 to 72 hours later
Does not cause booster phenomenon	Can cause booster phenomenon
Not as likely to have incorrect reading	More likely to have incorrect reading
BCG vaccination does not cause false-positive result	BCG vaccination can cause false-positive result



.....
 Since the QFT-G is a new test for diagnosing infection with *M. tuberculosis*, there is still limited laboratory and medical experience with using the test.

Disadvantages and Limitations

Since the QFT-G is a new test for diagnosing infection with *M. tuberculosis*, there is still limited laboratory and medical experience with using the test. Following are some known disadvantages and limitations to using the QFT-G.

- Blood samples must be processed within 12 hours after collection
- Errors in running and interpreting the test can decrease the accuracy of the QFT-G
- There are limited data on the use of QFT-G in children younger than 17 years of age, among persons recently exposed to *M. tuberculosis*, persons living with HIV/AIDS, persons on immunosuppressive therapy (including corticosteroids or TNF- α antagonists), persons with certain blood disorders, cancers, diabetes, silicosis, or severe kidney disease.
- There are limited data on the use of QFT-G to determine who is at risk for developing TB disease

A potential disadvantage to using the QFT-G is that samples must be processed within 12 hours after collecting the blood. If they are not processed in 12 hours, the accuracy of the test is decreased. The accuracy of the test can also be affected by laboratory errors in running and interpreting the test. Therefore, efforts should be made to ensure that blood specimens are properly collected and promptly transported to a laboratory qualified in analyzing a QFT-G.

Since the QFT-G is a new test, there are currently limited data on the use of QFT-G in certain populations. These include children younger than 17 years of age, persons living with HIV/AIDS, or other medical conditions including certain blood disorders and cancers, diabetes, silicosis, severe kidney disease, and immunosuppressive therapy [for example, corticosteroids or TNF- α antagonists]. There are also limited data on using QFT-G on persons who have been recently exposed to TB (contacts) and other populations at high risk of LTBI.



Study Questions 3.20 – 3.23

3.20 What are the steps for conducting a QFT-G?

3.21 How are the QFT-G results interpreted?

3.22 How should a negative QFT-G result be interpreted?

3.23 What are five advantages for using QFT-G as compared to the TST?

Answers to study questions are on pages 71 – 77.



Diagnosis of TB Disease

The key to diagnosing TB is for clinicians to “think TB” when they see a patient with symptoms of the disease or abnormal chest x-ray findings. Because TB is not as common as it was many years ago in the United States, many clinicians do not consider the possibility of TB when making diagnoses for patients who have symptoms of TB. When this happens, the diagnosis of TB may be delayed or even overlooked, and the patient will remain ill and possibly infectious.

.....
Anyone with symptoms of TB
or a positive TST or QFT-G
result should be medically
evaluated for TB disease.
.....

Anyone with **symptoms of TB** or anyone found to have a **positive TST or QFT-G result** should be medically evaluated for TB disease.

There are five components for conducting a complete medical evaluation for diagnosing TB disease.

1. Medical history
2. Physical examination
3. Test for TB infection
4. Chest x-ray
5. Bacteriological examinations

1. The Medical History

A medical history is the part of a patient’s life history that is important for diagnosing and treating the patient’s medical condition. It includes social, family, medical, and occupational information about the patient.



To obtain a medical history, the clinician should ask whether the patient has:

- a. Any symptoms of TB disease
- b. Been exposed to a person with infectious TB or has risk factors for exposure to TB
- c. Risk factors for developing TB disease
- d. Had LTBI or TB disease before

Clinicians should suspect TB disease in patients with any of these factors.

a. Symptoms of TB disease

People with TB disease may or may not have symptoms. However, most patients with TB disease have one or more symptoms that led them to seek medical care. Occasionally, TB is discovered during a medical examination for an unrelated condition (for example, when a patient is given a chest x-ray before undergoing surgery). Usually, when patients do have symptoms, the symptoms have developed gradually, and they have been present for weeks or even months. Pulmonary TB disease usually causes one or more of the following symptoms:

- A cough (lasting for 3 or more weeks)
- Chest pain when breathing or coughing
- Coughing up sputum (phlegm from deep in the lungs) or blood
- Fever
- Chills
- Night sweats
- Weight loss
- Appetite loss
- Fatigue
- Malaise

.....
People with TB disease may
or may not have symptoms.
.....



.....
The symptoms of
extrapulmonary TB disease
depend on the part of the
body that is affected by the
disease.
.....

The symptoms of extrapulmonary TB disease depend on the part of the body that is affected by the disease. For example, TB of the spine may cause pain in the back; TB of the kidney may cause blood in the urine.

All of these symptoms may be caused by other diseases, but they should prompt the clinician to suspect TB disease.

b. Exposure to TB

.....
Some people may have been
exposed to TB in the distant
past, when they were
children. Others may have
been exposed more recently.
.....

Another important part of the medical history is asking the patient about his or her **exposure** to TB. Patients should be asked whether they have spent time with someone who has infectious TB. Some people may have been exposed to TB in the distant past, when they were children. Others may have been exposed more recently.

Anyone who has been exposed to TB may have TB infection. Some people become infected with *M. tuberculosis* without knowing that they were exposed to it. The risk of being exposed to TB is higher for some people. It is important to consider demographic factors (for example, country of origin, age, ethnic or racial group, or occupation) that may increase the patient’s risk for exposure to TB. For more details on groups at high risk for exposure to TB, see the Targeted Testing Section of this module, page 6.

c. Risk factors for developing TB disease

.....
Some conditions appear to
increase the risk that TB
infection will progress to
disease.
.....

A third part of the medical history is checking for **risk factors** for developing TB disease. Also, clinicians should see whether the patient has medical conditions, especially HIV infection, which can increase the risk of LTBI progressing to TB disease. All patients who do not know their current HIV status should be referred for HIV counseling and testing.

For more information on factors and medical conditions that can increase the risk of patients developing TB disease, see the Targeted Testing section of this module.



.....
The clinician should ask the patient whether he or she has ever been diagnosed with or treated for TB infection or disease.
.....

d. Previous TB infection or TB disease

During the medical history, the clinician should ask the patient whether he or she has ever been diagnosed with or treated for TB infection or disease.

- Patients known to have a positive TST or QFT-G result probably have TB infection. If they were infected within the past 2 years, they are at high-risk for developing TB disease.
- Patients who have had TB disease before should be asked when they had the disease and how the disease was treated.

Clinicians may also contact the local health department for information about whether a patient has received TB treatment in the past. If the treatment regimen that was prescribed was inadequate or if the patient did not follow the recommended treatment, the TB disease may come back, and it may be resistant to one or more of the drugs used.

2. Physical Examination

A physical examination is an essential part of the evaluation of any patient. It cannot confirm or rule out TB disease, but it can provide valuable information about the patient's overall condition and factors that may affect how TB disease is treated if it is diagnosed.



.....
Patients with symptoms of TB disease should always be evaluated for TB disease, regardless of their skin test results.
.....

3. Testing for TB Infection

Currently, there are two tests available for diagnosing TB infection in the U.S.

- Mantoux tuberculin skin test (TST)
- QuantiFERON[®]-TB Gold test (QFT-G)

Patients with symptoms of TB disease are sometimes given a TST or QFT-G to detect infection with TB. However, some patients with TB disease may have a negative TST or QFT-G result. **Patients with symptoms should always be evaluated for TB disease, regardless of their TST or QFT-G results.** Furthermore, for patients with symptoms of TB disease, clinicians should not wait for TST or QFT-G results before starting other diagnostic tests. Instead, the patient should be given a TST or QFT-G at the same time as the other steps in the diagnosis of TB disease.

For more information on testing for TB infection, see Diagnosis of LTBI section of this module, page 8.

Study Questions 3.24

3.24 What are the five components for conducting a medical evaluation for diagnosing TB disease?

Answers to study questions are on pages 71 – 77.



Study Questions 3.25 – 3.27

3.25 What parts of a patient’s medical history should lead a clinician to suspect TB?

3.26 What are the symptoms of pulmonary TB disease? What are the symptoms of extrapulmonary TB disease (pulmonary or extrapulmonary)?

3.27 For patients with symptoms of TB disease, should clinicians wait for TST or QFT-G results before starting other diagnostic tests?

Answers to study questions are on pages 71 – 77.



.....
Chest x-rays are useful for
diagnosing TB disease
because pulmonary TB is
the most common form of
the disease.
.....

4. The Chest X-Ray

The chest x-ray is useful for diagnosing TB disease because pulmonary TB is the most common form of the disease. Usually, when a person has TB disease in the lungs, the chest x-ray appears abnormal (Figure 3.6). It may show **infiltrates** (collections of fluid and cells in the tissues of the lung) or **cavities** (hollow spaces within the lung that may contain many tubercle bacilli).



*Figure 3.6 Abnormal chest x-ray.
Arrow points to cavity in patient's
right upper lobe. Left lung is normal.*

The purposes of the chest x-ray are to

- Help rule out the possibility of pulmonary TB disease in a person who has a positive TST or a QFT-G result
- Check for lung abnormalities in people who have symptoms of TB disease



.....
The results of a chest x-ray
cannot confirm that a person
has TB disease.
.....

The results of a chest x-ray, however, **cannot confirm that a person has TB disease**. A variety of illnesses may produce abnormalities whose appearance on a chest x-ray resembles TB. Although an abnormality on a chest x-ray may lead a clinician to suspect TB, **only a bacteriologic culture that is positive for *M. tuberculosis* proves that a patient has TB disease**. Moreover, a chest x-ray cannot detect TB infection.

.....
In HIV-infected patients,
pulmonary TB may have an
unusual appearance on the
chest x-ray.
.....

In persons living with HIV, pulmonary TB disease may have an unusual appearance on the chest x-ray. The chest x-ray may even appear entirely normal.

A chest x-ray may be used to rule out the possibility of pulmonary TB in a person who has had a positive TST result and no symptoms of disease.

Study Questions 3.28 – 3.29

3.28 Name two purposes of the chest x-ray.

3.29 Can the results of a chest x-ray confirm that a person has TB disease? Why or why not?

Answers to study questions are on pages 71 – 77.



.....
A bacteriologic examination is done in a laboratory that specifically deals with *M. tuberculosis* and other mycobacteria.
.....

.....
Patients suspected of having pulmonary TB disease may cough up sputum, which is collected in a sterile container for processing and examination.
.....

5. The Bacteriologic Examination

Clinical specimens (for example, sputum or urine) are examined and cultured (grown) in the laboratory for the **bacteriologic examination**. TB bacteriologic examination is done in a laboratory that specifically deals with *M. tuberculosis* and other mycobacteria (a **mycobacteriology laboratory**). The bacteriologic examination has five parts:

- a. **Specimen collection**
- b. **Examination of the acid-fast bacilli smears**
- c. **Direct identification of specimen (nucleic acid amplification)**
- d. **Specimen culturing and identification**
- e. **Drug susceptibility testing**

a. Specimen collection

Specimens that will be sent to the laboratory can be obtained in several ways. Usually, patients who are suspected of having pulmonary TB disease simply cough up **sputum** (phlegm from deep in the lungs) into a sterile container for processing and examination (Figure 3.7). This is the least expensive and easiest procedure. A health care worker should coach and directly supervise the patient when sputum is collected. Patients who are not supervised are not always successful in providing an adequate specimen, especially in their first attempt.



Figure 3.7 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 prevents the spread of tubercle bacilli.



.....
For an induced sputum sample, the patient inhales a saline mist, causing him or her to cough deeply.
.....

Patients should have at least three sputum specimens examined. It is best to try and get the specimens early in the morning. If a patient cannot cough up sputum on his or her own, other techniques can be used to obtain a specimen. An **induced sputum** sample can be obtained by having the patient inhale a saline (salt water) mist, which causes the patient to cough deeply. This procedure is easily done, and it should be used to help patients cough up sputum if they cannot do so on their own. Induced specimens are often clear and watery; they should be labeled “induced specimen” so that they will not be confused with saliva. Laboratories will not accept saliva as a specimen.

.....
A bronchoscopy is done to obtain pulmonary secretions or lung tissue.
.....

Another procedure, **bronchoscopy**, can be used to obtain pulmonary secretions or lung tissue. In this procedure, an instrument called the bronchoscope is passed through the mouth directly into the diseased portion of the lung, and some sputum or lung tissue is removed. Bronchoscopy should be used only when patients cannot cough up sputum on their own and an induced specimen cannot be obtained.

.....
For gastric washing, a tube is inserted through the patient’s nose and passed into the stomach to collect a sample of sputum that has been coughed into the throat and then swallowed.
.....

A fourth procedure, **gastric washing**, involves inserting a tube through the patient’s nose and passing it into the stomach. The idea is to get a sample of gastric secretions that contains sputum that has been coughed into the throat and then swallowed. Gastric washings are done in the morning because patients usually swallow sputum during the night. This procedure is usually used only when patients cannot cough up sputum on their own, an induced specimen cannot be obtained, and bronchoscopy cannot be done. However, gastric washings are often used for obtaining samples from children. Most children produce little or no sputum when they cough.



.....
Health care workers should use precautions to control the spread of tubercle bacilli during sputum collection procedures.
.....

It is very important for health care workers to use infection control precautions to control the spread of tubercle bacilli during these procedures and any other procedures that may cause persons who have pulmonary TB disease to cough. This is discussed further in *Module 5, Infectiousness and Infection Control*.

.....
In patients with extrapulmonary TB disease, specimens are obtained in various ways, depending on the part of the body that is affected.
.....

In patients who have **extrapulmonary TB disease, specimens other than sputum** may be obtained. The specimen obtained from these patients depends on the part of the body that is affected. For example, urine samples are obtained from patients suspected of having TB disease of the kidney, and fluid samples are obtained from the area around the spine in patients suspected of having TB meningitis (TB disease in the membranes surrounding the brain and spinal cord).

The methods of obtaining a sputum specimen are summarized in Table 3.6.



Table 3.6
Methods of Obtaining a Sputum Specimen

Method	Description	Advantage	Disadvantage
Coughing up sputum	Patient coughs up sputum	Inexpensive Easy to do	Patient may not be able to cough up sputum on his or her own, or may spit up saliva instead of sputum
Inducing sputum	Patient inhales a saline mist, causing him or her to cough deeply	Easy to do	Specimens may be watery and may be confused with saliva (should be labeled “induced specimen”) Requires special equipment
Bronchoscopy	Bronchoscope is passed through the mouth or nose directly into the diseased portion of the lung, and some sputum or lung tissue is removed	Useful for obtaining sputum when coughing or inducing sputum does not work	Most expensive and invasive procedure Requires special equipment Must be done by a specialized physician in a hospital or clinic
Gastric washing	Tube is inserted through the patient’s 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	Useful for obtaining samples in children, who usually produce little or no sputum when they cough	Must be done as soon as patient wakes up in the morning; patient may be required to stay in hospital Can be uncomfortable for the patient



Case Study 3.5

Mr. Lee has a cough and other symptoms of TB disease, and he is evaluated with a chest x-ray. However, he is unable to cough up any sputum on his own for the bacteriologic examination.

- **What should be done?**

Answers to case study questions are on pages 78 – 81.



.....
Specimens are smeared onto a glass slide and stained so that they can be examined for acid-fast bacilli (AFB) under a microscope.
.....

b. Examination of AFB smears

Before the specimen is examined under a microscope, it is smeared onto a glass slide and stained with a dye. This is called a **smear**. Then laboratory personnel use the microscope to look for **acid-fast bacilli** (AFB) on the smear (Figure 3.8). AFB are mycobacteria that stay stained even after they have been washed in an acid solution. Tubercle bacilli are one kind of AFB.

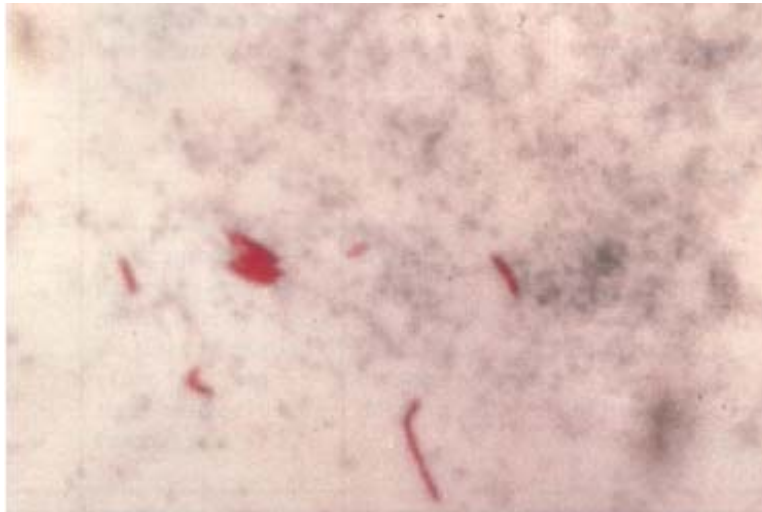


Figure 3.8 AFB smear. In this photograph, the AFB (shown in red) are tubercle bacilli.

.....
When AFB are seen in a smear, they are counted and classified according to the number of AFB seen.
.....

When AFB are seen in a smear, they are counted. There is a system for reporting the number of AFB that are seen at a certain magnification. According to the number of AFB seen, the smears are classified as 4+, 3+, 2+, or 1+. In smears classified as 4+, 10 times as many AFB were seen as in smears classified as 3+; in 3+ smears, 10 times as many as in 2+ smears; and in 2+ smears, 10 times as many as in 1+ smears.



.....
A negative smear does not rule out the possibility of TB because there can be AFB in the smear that were not seen.
.....

Smears that are classified as 4+ and 3+ are considered strongly positive; 2+ and 1+ smears are considered moderately positive. If very few AFB are seen, the smear is classified by the actual number of AFB seen (no plus sign). For example, if only 2 AFB were seen in the entire smear, the smear is classified as “2 AFB seen.” Smears classified in this way are considered weakly positive. Finally, if no AFB are seen, the smear is called negative. But a negative smear **does not rule out** the possibility of TB because there can be AFB in the smear that were not seen.

It takes only a few hours to prepare and examine a smear. Therefore, the results of the smear examination should be available to the clinician within 1 day.

.....
The results of the smear examination can be used to help determine the infectiousness of the patient.
.....

The results of the smear examination can be used to help determine the infectiousness (contagiousness) of the patient. Patients who have many tubercle bacilli in their sputum have a positive smear. Patients who have positive smears are considered infectious because they can cough many tubercle bacilli into the air. (This is discussed in more detail in *Module 5, Infectiousness and Infection Control.*) However, because AFB are not always tubercle bacilli, patients who have positive smears do not necessarily have TB. Furthermore, as mentioned previously, patients who have negative smears may have TB.

Laboratories should report initial positive smears and positive *M. tuberculosis* cultures within 24 hours of specimen receipt by telephone or fax to the primary health care provider. Out-of-state laboratories who receive referral specimens must contact the health care provider 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 promptly report all suspected or confirmed cases of TB to the state or local health department. This will ensure that a contact investigation can be initiated quickly to interrupt the potential ongoing transmission.

The classification of smears is summarized in Table 3.7.



Table 3.7
Smear Classifications and Results

Classification of Smear	Smear Result	Infectiousness of Patient
4+	Strongly positive	Probably very infectious
3+	Strongly positive	Probably very infectious
2+	Moderately positive	Probably infectious
1+	Moderately positive	Probably infectious
Actual number of AFB seen (no plus sign)	Weakly positive	Probably infectious
No AFB seen	Negative	May not be infectious*

* The criteria for determining whether a patient may be considered noninfectious are discussed in *Module 5, Infectiousness and Infection Control*.

Study Questions 3.30 – 3.32

3.30 What are the four ways to collect sputum specimens? Indicate which procedure is the least expensive and easiest to perform.

3.31 What do laboratory personnel look for in a smear?

3.32 What does a positive smear indicate about a patient's infectiousness?

Answers to study questions are on pages 71 – 77.



Case Study 3.6

Ms. Thompson 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+.

- **What do these results tell you about Ms. Thompson's diagnosis and her infectiousness?**

Answers to case study questions are on pages 78 – 81.



Case Study 3.7

Mr. Sagoo has symptoms of TB disease and a cavity on his chest x-ray, but all of his sputum smears are negative for acid-fast bacilli.

■ **Does this rule out the diagnosis of pulmonary TB disease?**

■ **Why or why not?**

Answers to case study questions are on pages 78 – 81.



.....
Nucleic acid amplification test can be used to identify *M. tuberculosis* from sputum specimens.
.....

.....
NAA test results can help guide clinicians decisions for patient therapy and isolation; however, they do not replace the need for AFB smear, culture, or clinical judgment.
.....

.....
All specimens should be cultured, regardless of whether the smear is positive or negative.
.....

.....
Culturing the specimen is necessary to determine if it contains *M. tuberculosis* and to confirm a diagnosis of TB disease.
.....

c. Direct identification of the specimen (nucleic acid amplification)

Nucleic acid amplification (NAA) tests can be used for directly identifying *M. tuberculosis* from sputum specimens. NAA tests, including **polymerase chain reaction (PCR)** and other methods, are used to amplify (copy) DNA and RNA segments, in to order to rapidly identify the microorganisms in the specimen.

A patient can be **presumed to have TB** if they have a positive NAA test with an AFB positive smear. If a patient has a negative NAA test with an AFB positive smear, the patient may have a nontuberculous mycobacteria infection. NAA test results can help guide clinicians decisions for patient therapy and isolation; however, they do not replace the need for AFB smear, culture, or clinical judgment.

Decisions about when and how to use NAA tests for TB diagnosis should be based on each individual situation. Laboratories should not reserve specimens for NAA tests if it will compromise the ability to perform the other diagnostic tests.

d. Culturing and identifying the specimen

Culturing the specimen means growing the mycobacteria on solid **media** and in liquid media, substances that contain nutrients, in the laboratory (Figure 3.9). When the mycobacteria have formed **colonies** (groups), or when there is sufficient growth in the liquid media, they can be identified. All specimens should be cultured, regardless of whether the smear is positive or negative.

Culturing the specimen is necessary to determine if it contains *M. tuberculosis* and to confirm a diagnosis of TB disease. However, in some cases, patients are diagnosed with TB disease on the basis of their clinical presentation (i.e., signs and symptoms), even if their specimen does not contain *M. tuberculosis*.



Figure 3.9 Colonies of M. tuberculosis growing on media.



.....
The first procedure in
culturing the specimen is to
detect the growth of the
mycobacteria.
.....

The first procedure in culturing the specimen is to detect the growth of the mycobacteria. Mycobacteria grow very slowly. When solid media are used to culture the specimen, it can take as long as 3 to 6 weeks for the growth of the mycobacteria to be detected. However, rapid culturing methods that involve liquid media can decrease this time to 4 to 14 days.

.....
The second procedure is to
identify the organism that has
grown.
.....

The second procedure is to identify the organism that has grown in the culture. All types of mycobacteria will grow in solid or liquid media. For this reason, laboratory tests must be done to determine whether the organism is *M. tuberculosis* or one of the nontuberculous mycobacteria. Nucleic acid probes can rapidly identify the type of mycobacteria present in the specimens in 2 to 4 hours. Thus, using liquid media and nucleic acid probes, it is usually possible to culture and to identify *M. tuberculosis* within two weeks.

If traditional solid medium and biochemical tests are used for both the isolation and identification of the organism, the entire process can take 6 to 12 weeks.

.....
A positive culture for
M. tuberculosis confirms the
diagnosis of TB disease.
.....

When *M. tuberculosis* is identified in a patient's culture, the patient is said to have a **positive culture** for *M. tuberculosis*. A positive culture for *M. tuberculosis*, also called a *M. tuberculosis* **isolate**, confirms the diagnosis of TB disease.

When *M. tuberculosis* is NOT identified in a patient's culture, the patient is said to have a **negative culture** for *M. tuberculosis*. A negative culture does not necessarily rule out the diagnosis of TB disease; as mentioned earlier, some patients with negative cultures are diagnosed with TB disease on the basis of their clinical presentation (i.e., signs and symptoms).



Follow-up bacteriological examinations are important for assessing the patients infectiousness and response to treatment. Specimens should be obtained monthly until 2 consecutive specimen cultures are negative. Culture conversion is the most important objective measure of response to treatment.

The differences between sputum smears and cultures are summarized in Table 3.8.

Table 3.8
Differences Between Sputum Smears and Cultures

Feature	Smears (see Figure 3.8)	Cultures (see Figure 3.9)
Equipment needed	Microscope, glass slides, special dyes	Incubators, safety cabinet, culture plates or tubes, culture media, biochemicals for tests
Time needed to make report	1 day	4 days to 12 weeks (depending on method used)
Basis of procedure	Looking for AFB on slide under microscope	Growth and identification of tubercle bacilli or other mycobacteria on culture media in incubator
Significance of a negative report	<p>Patient is less likely to be infectious</p> <p>Does not rule out TB disease (culture may be positive)</p>	<p>No live tubercle bacilli found in the specimen</p> <p>Does not rule out TB disease (live tubercle bacilli may be in other specimens and/or in the patient)</p>
Significance of a positive report	<p>Patient is more likely to be infectious (if AFB are tubercle bacilli)</p> <p>AFB could be nontuberculous mycobacteria</p>	Confirms diagnosis of TB disease



Criteria for Reporting TB Cases

All 50 states, the District of Columbia, New York City, Puerto Rico, and seven other jurisdictions in the Pacific and the Caribbean report TB cases to the federal Centers for Disease Control and Prevention (CDC) using a standard case report form called the *Report of Verified of Case of Tuberculosis (RVCT)*. Each reported TB case is checked to make sure that it meets certain criteria. All cases that meet the criteria, called *verified TB cases*, are counted each year.

Cases that meet *one* of these four sets of criteria are counted as verified TB cases:

1. The patient has a positive culture for *M. tuberculosis*

or

2. The patient has a positive NAA test for *M. tuberculosis**

or

3. The patient has a positive smear for AFB, but a culture has not been done or cannot be done

or

4. If in the absence of laboratory confirmation, the patient has a positive TST reaction, has other signs and symptoms of TB disease, is being treated with two or more TB drugs, and has been given a complete diagnostic evaluation

In addition, cases that do not meet any of these sets of criteria (for example, a patient who is anergic and has a negative culture for *M. tuberculosis* but who has signs and symptoms of TB disease) may be counted as a verified TB case if a health care provider has reported the case and decided to treat the patient for TB disease.

*Nucleic acid amplification (NAA) tests must be accompanied by culture for mycobacteria species. However, for surveillance purposes, CDC will accept results obtained from NAA tests approved by the Food and Drug Administration (FDA) and used according to the approved product labeling on the package insert.



.....
Drug susceptibility tests should be done when a patient is first found to have a positive culture for *M. tuberculosis*.
.....

.....
Drug susceptibility tests determine which drugs will kill the tubercle bacilli that are causing disease in a particular patient.
.....

.....
The results of drug susceptibility tests can help clinicians choose the appropriate drugs for each patient.
.....

.....
Drug susceptibility tests should be repeated if a patient has a positive culture for *M. tuberculosis* after 3 months of treatment or if a patient does not seem to be getting better.
.....

e. Drug susceptibility testing

For all patients, drug susceptibility tests should be done when the patient is first found to have a positive culture for *M. tuberculosis* (that is, the first isolate of *M. tuberculosis*). Drug susceptibility tests are done to determine which drugs will kill the tubercle bacilli that are causing disease in a particular patient. Tubercle bacilli that are killed by a particular drug are said to be **susceptible** to that drug, whereas those that can grow even in the presence of a particular drug are said to be **resistant** to that drug. The **drug susceptibility pattern** of a strain of tubercle bacilli is the list of drugs to which the strain is susceptible and to which it is resistant.

Drug-resistant TB can be **mono-resistant** if the tubercle bacilli are resistant to any one TB treatment drug, or **poly-resistant** if resistant to at least two TB drugs (but not both isoniazid and rifampin). A patient is diagnosed with **multidrug-resistant TB (MDR TB)** if the tubercle bacilli are resistant to at least isoniazid and rifampin, the two best first-line TB treatment drugs. A patient is diagnosed with **extensively drug-resistant TB (XDR TB)** if the tubercle bacilli are resistant to isoniazid and rifampin, plus resistant to any fluoroquinolone and at least one of three injectable second-line drugs (such as amikacin, kanamycin, or capreomycin).

The results of drug susceptibility tests can help clinicians choose the appropriate drugs for treating each patient. This is very important. Patients with TB disease who are treated with drugs to which their strain of TB is resistant may not be cured. In fact, their strain of TB may become resistant to additional drugs.

Drug susceptibility tests should be repeated if a patient has a positive culture for *M. tuberculosis* after 3 months of treatment or if a patient does not seem to be getting better. That way, the clinician can find out whether the patient's strain of TB has become resistant to certain drugs; if necessary, the clinician may change the drugs used for treating the patient. Clinicians treating patients with drug-resistant TB should always consult with a medical expert



who is familiar with the treatment of drug-resistant TB.

In the laboratory, drug susceptibility testing can be done using a liquid medium or a solid medium method. In a drug susceptibility test, organisms that grow in media containing a specific drug are considered resistant to that drug (Figure 3.10). Liquid medium methods are faster than solid media methods for determining susceptibility to first-line TB medications. Usually the susceptibility results can be obtained within 7 to 14 days with the liquid medium method. Traditional solid medium methods, can take as long as 21 days.



Figure 3.10 Drug susceptibility testing on solid media. Organisms are resistant to the drug in the upper right compartment and susceptible to the drugs in the lower compartments. Upper left contains no drugs.



Study Questions 3.33 – 3.36

3.33 Why is it necessary to culture a specimen?

3.34 What does a positive culture for *M. tuberculosis* mean? How is this important for the TB diagnosis?

3.35 Why are drug susceptibility tests done?

3.36 How often should drug susceptibility tests be done?

Answers to study questions are on pages 71 – 77.



SUMMARY

Targeted testing for TB infection is done to identify persons who are at high risk of developing TB disease and who would benefit from LTBI treatment. All testing activities should be accompanied by a plan for follow-up care, medical evaluation, and treatment for persons with latent TB infection or TB disease. Persons with a positive test for TB infection should be evaluated for TB disease and, if disease is ruled out, considered for treatment for LTBI.

Flexibility is needed in defining high-risk groups for testing. High-risk groups can be divided into two categories:

- People at high risk for TB exposure or infection with *M. tuberculosis*
- People at high risk for developing TB disease once infected with *M. tuberculosis*

Currently, the available methods for detecting *M. tuberculosis* infection are the TST and interferon-gamma release assays (IGRA) such as the QFT-G. The TST and the QFT-G measure different components of the immune response and are administered differently.

The TST is more commonly used and is done by using a needle and syringe to inject tuberculin between the layers of the skin, usually on the forearm. After 48 to 72 hours, the patient's arm is examined for a reaction (an induration). The diameter of the indurated area (the swelling, not the redness) is measured across the forearm. Most people with TB infection have a positive reaction to the tuberculin. The interpretation of the TST depends on the size of the induration, the person's risk factors for TB, and for people who may be exposed to TB on the job, the risk of exposure to TB in the person's job.

Several factors can affect how the TST reaction is interpreted. Some factors, such as infection with non-tuberculosis mycobacteria (NTM) and vaccination with BCG, can cause false-positive reactions. Other factors, such as anergy, recent infection, very young age, and recent live virus vaccination can cause false-negative reactions. Incorrect methods for giving and interpreting the TST can lead to either a false-positive or false-negative reaction.

In many TB testing programs, two-step testing is used for skin testing employees when they start their job. Two-step testing is a strategy for telling the difference between boosted reactions and reactions caused by recent infection.

The QFT-G is a FDA approved interferon-gamma release assay. It is a blood test for TB infection. The patient's blood samples must be delivered to a qualified laboratory within 12 hours of collecting the samples.

At the laboratory, the blood samples are mixed and incubated with antigens found in *M. tuberculosis* strains and with control substances. The results of the QFT-G depend upon the amount of IFN- γ released in response to the antigens and the control substances. The laboratory will submit a report with the results back to the health care worker who requested the test.



The QFT-G can be used in all circumstances in which the TST is currently used. However, there are both advantages and limitations for using the QFT-G. Advantages include that the QFT-G requires only one patient visit to conduct the test and that the results are available in 24 hours. Also, unlike the TST, the QFT-G does not cause the booster phenomenon and results are not affected by BCG vaccination. Furthermore, since the QFT-G is a laboratory-based test, there is less chance of having an incorrect reading of the results as compared to the TST.

There are some disadvantages and limitations for using the QFT-G. For a QFT-G, blood samples must be processed within 12 hours at a qualified laboratory. Thus efforts must be made to ensure that the specimens are promptly delivered to a qualified laboratory. Also, since the QFT-G is a new test, there are currently limited data on the use of it in certain populations. These include children younger than 17 years of age and persons living with HIV/AIDS and other medical conditions. There is also limited data on using QFT-G on persons who have been recently exposed to TB (contacts) and other populations at high risk for LTBI.

Regardless of the testing method used (TST or QFT-G), patients with positive results should be evaluated for TB disease. If TB disease is ruled-out, the patient should be considered for LTBI treatment. Furthermore, recent contacts of someone with infectious TB disease who have a negative TST or QFT-G result should be retested 8 to 10 weeks after the last time they were in contact with the person who has TB.

There are five components for conducting a complete medical evaluation for diagnosing TB disease. The first is the medical history. This means checking if the person has symptoms of TB disease; has been exposed to a person with TB or has other exposure risk factors; has had TB infection or TB disease before; or has risk factors for developing TB disease. The symptoms of pulmonary TB disease include coughing lasting for 3 or more weeks, pain in the chest when breathing or coughing, and coughing up sputum or blood. The general symptoms of TB disease (pulmonary or extrapulmonary) include weight loss, fatigue, malaise, fever, and night sweats. Other symptoms of extrapulmonary TB disease depend on the part of the body that is affected by the disease.

The second component of the medical evaluation is the physical examination. Patients should be given a physical examination to determine their overall condition and factors that may affect how TB disease is treated if it is diagnosed.

For the third component of the medical evaluation, patients with symptoms of TB disease may be given a TST or a QFT-G to help confirm infection with *M. tuberculosis*. However, patients should always be evaluated for TB disease, regardless of their TST or QFT-G results.

The fourth component of the medical evaluation is the chest x-ray. A chest x-ray helps rule out the possibility of pulmonary TB disease in a person who has a positive result to the TST or QFT-G. Chest x-rays also help determine if there are lung abnormalities in people who have symptoms of TB disease. However, chest x-ray results cannot confirm that a person has TB disease. Furthermore, chest x-rays for people living with HIV and pulmonary TB may appear unusual or even normal.



The fifth component of the medical evaluation is the bacteriologic examination. First, a sputum specimen is obtained from patients suspected of having pulmonary TB disease; other specimens are obtained from patients suspected of having extrapulmonary TB disease. The specimen is smeared onto a slide, stained, and examined under a microscope for the presence of acid-fast bacilli. When acid-fast bacilli are seen in a smear, they are counted, and the smear is classified according to this number. Patients with positive smears are considered infectious.

Next, based on clinical and laboratory decision, nucleic acid amplification (NAA) tests can be used on the sputum specimen to help identify the microorganisms. NAA test results can help guide clinicians decisions for patient therapy and isolation; however, they do not replace the need for AFB smear, culture, or clinical judgment.

The specimen is then cultured, or grown, so that laboratory personnel can determine whether it contains *M. tuberculosis*. Special tests are used to identify the mycobacteria once they have grown enough to be detected. A positive culture for *M. tuberculosis* confirms the diagnosis of TB disease.

Finally, the cultured specimen is also tested for drug susceptibility. The results of drug susceptibility tests can help clinicians choose the appropriate drugs for each patient and determine if the patient has drug-resistant TB.



Additional Reading

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ANSWERS TO STUDY QUESTIONS

3.1. What is the TST used for? (page 8)

The TST is used to determine whether a person has TB infection.

3.2. How is the Mantoux TST given? (page 8)

The Mantoux TST is given by using a needle and syringe to inject 0.1 ml of 5 tuberculin units of liquid tuberculin between the layers of the skin (intradermally), usually on the forearm.

3.3. With the TST, when is the patient's arm examined? (page 9)

The patient's arm is examined 48 to 72 hours after the tuberculin is injected.

3.4. How is the induration measured? (pages 9-10)

The diameter of the indurated area is measured across the forearm; erythema (redness) around the indurated area is not measured, because the presence of erythema does not indicate that a person has TB infection.

3.5. Why is the Mantoux TST preferable to multiple-puncture tests? (page 9)

The Mantoux TST is preferable to multiple-puncture tests because it is more accurate. In multiple-puncture tests, the amount of tuberculin that actually enters the skin cannot be measured. In the Mantoux TST, however, the amount of tuberculin can always be measured. Therefore, the Mantoux TST is more accurate, and it is the preferred method.

3.6. What two factors determine the interpretation of a TST reaction as positive or negative? What additional factor is considered for people who may be exposed to TB on the job? (pages 13-14)

The two factors that are used to determine the interpretation of a TST include the size of the induration and the person's risk factors for TB.

For people who may be exposed to TB on the job, an additional factor is considered is the risk of exposure to TB in the person's job.



3.7. For which groups of people is 5 or more millimeters of induration considered a positive reaction? Name four. (page 15)

An induration of 5 or more millimeters is considered a positive reaction for

- People living with HIV
- Recent contacts of people with infectious TB
- People who have had TB disease before
- Patients with organ transplants and those taking a prolonged course of oral or intravenous corticosteroids or TNF- α

3.8. For which groups of people are 10 or more millimeters of induration considered a positive reaction? Name seven. (page 15)

An induration of 10 or more millimeters is considered a positive reaction for

- Recent arrivals to the United States (<5 years) from areas of the world where TB is common
- People who inject illegal drugs
- Mycobacteriology lab workers
- People who live or work in high-risk congregate settings
- People with certain medical conditions that place them at high risk for TB
- Children younger than 4 years
- Infants, children and adolescents exposed to adults in high-risk categories

3.9. For which group of people is 15 or more millimeters of induration considered a positive reaction? (page 15)

An induration of 15 or more millimeters is considered a positive reaction for people with no risk factors for TB.

3.10. Name four factors that can cause false-positive reactions to the TST. (page 18)

- Infection with nontuberculous mycobacteria (NTM) (mycobacteria other than *M. tuberculosis*)
- BCG vaccination
- Administration on incorrect antigen
- Incorrect measuring or interpretation of the TST reaction



3.11. Is there a reliable way to distinguish a positive TST reaction caused by vaccination with BCG from a reaction caused by true TB infection? (page 18)

No, there is no reliable way to distinguish a positive TST reaction caused by vaccination with BCG from a reaction caused by true TB infection. When using the TST, people who have been vaccinated with BCG should always be further evaluated for LTBI or TB disease the same as if they were not vaccinated with BCG.

3.12. Name six factors that can cause false-negative reactions to the TST. (page 21)

False-negative reactions may be due to

- Anergy
- Recent TB infection (within the past 8- 10 weeks)
- Very young age (younger than 6 months)
- Recent live-virus measles or smallpox vaccination
- Incorrect method of giving the TST
- Incorrect measuring or interpretation of TST reaction

3.13. What is anergy? (page 21)

Anergy is the inability to react to skin tests because of a weakened immune system. Many conditions, such as HIV infection, cancer, or severe TB disease itself, can weaken the immune system and cause anergy. HIV infection is an important cause of anergy.

3.14. After TB has been transmitted, how long does it take before TB infection can be detected by the TST? (page 22)

After TB has been transmitted, it takes 2 to 8 weeks before TB infection can be detected by the TST.

3.15. What should be done if a patient has a negative TST reaction but has symptoms of TB disease? (page 22)

Any patient with symptoms of TB disease should be evaluated for TB disease, regardless of his or her skin test reaction. In fact, people with symptoms of TB disease should be evaluated for TB disease right away, at the same time that the TST is given.

3.16. What is the booster phenomenon? (page 27)

The booster phenomenon is a phenomenon in which people (especially older adults) who are skin tested many years after becoming infected with *M. tuberculosis* may have a negative reaction to an initial skin test, followed by a positive reaction to a skin test given up to a year later. This happens because in some people who have TB infection, the ability to react to tuberculin lessens over time. The first skin test “jogs the memory” of the immune system, boosting its ability to react to tuberculin.



3.17. What is the purpose of two-step testing? (page 29)

The purpose of two-step testing is to tell the difference between boosted reactions and reactions caused by recent infection. Because it provides accurate information about each employee's baseline skin test reaction, two-step testing is used in many TB screening programs for skin testing employees when they start their job.

3.18. In what type of situation is two-step testing used? (page 29)

Two-step testing is used in many TB screening programs for skin testing employees when they start their job.

3.19. How is two-step testing done? (page 29)

If a person has a negative reaction to an initial skin test, he or she is given a second test 1 to 3 weeks later.

- If the reaction to the second test is positive, it is considered a boosted reaction (due to TB infection that occurred a long time ago).
- If the reaction to the second test is negative, the person is considered uninfected. In this person, a positive reaction to a TST given later on will probably be due to recent TB infection.

3.20. What are the steps for conducting a QFT-G? (page 33)

To conduct a QFT-G, first read the instructions from the manufacturer and then follow the steps below:

- Confirm arrangements for delivery and testing of blood in a qualified laboratory within 12 hours of blood sample collection.
- Draw a sample of whole blood from the patient into a tube with heparin, according to manufacturer's instructions.
- Schedule an appointment for the patient to receive test results and, if then needed, medical evaluation and possible treatment for LTBI or TB disease.

3.21. How are the QFT-G results interpreted? (page 34)

Interpretation of the QFT-G result is based on the IFN- γ concentrations in the test samples. To help calculate the results, laboratories can use software provided by the manufacturer. A report of the results is submitted to the requesting clinician.

3.22. How would a negative QFT-G result be interpreted (page 35)

If the result is negative, then the patient is unlikely to have *M. tuberculosis* infection and may not require further evaluation unless they have signs and symptoms of TB disease. Moreover, as with the TST, persons who have a negative test result can still have LTBI.



3.23. What are the five advantages for using QFT-G compared to the TST (page 36)

- Requires a single patient visit to conduct the test
- Results can be available in 24 hours
- Does not cause the booster phenomenon which can happen with repeat TSTs
- Less likely to have incorrect reading of results compared to the TST
- BCG vaccination does not affect QFT-G results

3.24. What are the five components for conducting a complete medical evaluation for diagnosing TB disease? (page 40)

- Medical history
- Physical examination
- Test for TB infection
- Chest x-ray
- Bacteriologic examinations

3.25. What parts of a patient's medical history should lead a clinician to suspect TB? (page 41)

Clinicians should suspect TB disease in patients who have

- Symptoms of TB disease
- Been exposed to a person who has infectious TB or has other risk factors for exposure to TB
- Risk factors for developing TB disease
- Had TB infection or TB disease before

3.26. What are the symptoms of pulmonary TB disease? What are the symptoms of extrapulmonary TB disease? (pages 41-42)

Pulmonary TB disease usually causes one or more of the following symptoms:

- Coughing
- Pain in the chest when breathing or coughing
- Coughing up sputum or blood
- Weight loss
- Fatigue
- Malaise
- Fever
- Night sweats

The symptoms of extrapulmonary TB disease depend on the part of the body that is affected by the disease. For example, TB of the spine may cause pain in the back; TB of the kidney may cause blood in the urine.



3.27. For patients with symptoms of TB disease, should clinicians wait for TST or QFT-G results before starting other diagnostic tests? (page 43)

No. For patients with symptoms of TB disease, clinicians should not wait for TST or QFT-G results before starting other diagnostic tests.

3.28. Name two purposes of the chest x-ray. (page 46)

The purposes of the chest x-ray are to

- Help rule out the possibility of pulmonary TB disease in a person who has a positive TST or QFT-G result and no symptoms of TB
- Check for lung abnormalities in people who have symptoms of TB disease

3.29. Can the results of a chest x-ray confirm that a person has TB disease? Why or why not? (page 47)

No, the results of a chest x-ray cannot confirm that a person has TB disease. This is because a variety of illnesses may produce abnormalities whose appearance on a chest x-ray resembles TB. Although an abnormality on a chest x-ray may lead a clinician to suspect TB, only a bacteriologic culture that is positive for *M. tuberculosis* proves that a patient has TB disease.

3.30. What are the four ways to collect sputum specimens? Indicate which procedure is the least expensive and easiest to perform. (pages 48-51)

- Usually, patients who are suspected of having pulmonary TB disease simply cough up sputum and the sputum is collected in a sterile container for processing and examination. This is the least expensive and easiest procedure. If a patient cannot cough up sputum on his or her own, other techniques can be used to obtain a specimen.
- An induced sputum sample can be obtained by having the patient inhale a saline (salt water) mist, which causes the patient to cough deeply.
- Bronchoscopy can be used to obtain pulmonary secretions or lung tissue. In this procedure, an instrument called the bronchoscope is passed through the nose or mouth directly into the diseased portion of the lung, and some sputum or lung tissue is removed.
- Gastric washing involves inserting a tube through the patient's nose and passing it into the stomach. The idea is to get a sample of gastric secretions that contain sputum that has been coughed into the throat and then swallowed.

3.31. What do laboratory personnel look for in a smear? (page 53)

Laboratory personnel use the microscope to look for acid-fast bacilli (AFB) on the smear. AFB are mycobacteria that stay stained even after they have been washed in an acid solution. Tubercle bacilli are one kind of AFB.



3.32. What does a positive smear indicate about a patient's infectiousness? (page 54)

The results of the smear examination can be used to help determine the infectiousness (contagiousness) of the patient. Patients who have many tubercle bacilli in their sputum have a positive smear. Patients who have positive smears are considered infectious because they can cough many tubercle bacilli into the air.

3.33. Why is it necessary to culture a specimen? (page 58)

Culturing the specimen is necessary to determine whether the specimen contains *M. tuberculosis* and to confirm a diagnosis of TB disease. (However, in some cases, patients are diagnosed with TB disease on the basis of their signs and symptoms, even if their specimen does not contain *M. tuberculosis*.)

3.34. What does a positive culture for *M. tuberculosis* mean? How is this important for the TB diagnosis? (page 60)

A positive culture for *M. tuberculosis* means that *M. tuberculosis* has been identified in a patient's culture. A positive culture for *M. tuberculosis* confirms the diagnosis of TB disease.

3.35. Why are drug susceptibility tests done? (page 63)

Drug susceptibility tests are done to determine which drugs will kill the tubercle bacilli that are causing disease in a particular patient. The results of drug susceptibility tests can help clinicians choose the appropriate drugs for each patient.

3.36. How often should drug susceptibility tests be done? (page 63)

Drug susceptibility tests should be done when a patient is first found to have a positive culture for *M. tuberculosis*. In addition, drug susceptibility tests should be repeated if a patient has a positive culture for *M. tuberculosis* after 3 months of treatment or if a patient does not seem to be getting better.



ANSWERS TO CASE STUDIES

- 3.1. Which of the following patients have a positive TST reaction?
- Mr. West, 36 years old, HIV infected, 8 mm of induration
 - Ms. Hernandez, 26 years old, native of Mexico, 7 mm of induration
 - Ms. Jones, 56 years old, has diabetes, 12 mm of induration
 - Mr. Sung, 79 years old, resident of a nursing home, 11 mm of induration
 - Mr. Williams, 21 years old, no known risk factors, 13 mm of induration
 - Ms. Marcos, 42 years old, chest x-ray findings suggestive of previous TB, 6 mm of induration
 - Ms. Rayle, 50 years old, husband has pulmonary TB, 9 mm of induration
- (a, c, d, f, g)
- 3.2. A 30-year-old man who recently immigrated from India is given a TST and found to have 14 millimeters of induration. He reports that he was vaccinated with BCG as a child. He also says that his wife was treated for pulmonary TB disease last year.
- **How should this man's results be interpreted?**

This man does have a positive reaction to the TST (10 or more millimeters is considered a positive reaction for a person from a country where TB is common). Also, he is a contact of person with pulmonary TB (his wife). However, because he was vaccinated with BCG there is a possibility that this may be a false-positive reaction. However, since there is no reliable way to distinguish a positive TST reaction caused by BCG or from a true TB infection, this man should be further evaluated for LTBI or TB disease as if he was not vaccinated with BCG.
 - **What factors make it more likely that this man's positive reaction is due to TB infection?**

This man is from an area of the world where TB is common, so he was probably exposed to TB in his native country. Therefore, he is at increased risk for TB infection. Also, his wife has had pulmonary TB, which further increases the probability that he has been exposed to TB. Since there is no reliable way to distinguish a positive TST reaction caused by BCG from a true TB infection, this man should be further evaluated for LTBI or TB disease as if he was not vaccinated with BCG.



3.3. Mr. Bell comes to the TB clinic for a TST. He believes that he has been exposed to TB, and he knows he is at high risk for TB because he is HIV infected. He is given a TST, and his reaction is read 48 hours later as 0 millimeters of induration.

■ **What are three ways to interpret this result?**

There are three possible reasons why Mr. Bell had no reaction to the tuberculin skin test.

- First, he may not have TB infection.
- Second, he may be anergic. People who are HIV infected are more likely to be anergic than persons who are not HIV infected. If Mr. Bell is anergic, he would be unable to react to tuberculin even if he did have TB infection.
- Third, it may be less than 8 to 10 weeks since he was exposed to TB. After TB has been transmitted, it takes 2 to 8 weeks before TB infection can be detected by the tuberculin skin test. Mr. Bell should be retested 8 to 10 weeks after he was last exposed to TB.

3.4. Ms. Wilson is a 60-year-old nurse. When she started a job at the local hospital, she was given a TST, her first test in 25 years. Her reaction was read 48 hours later as 0 millimeters of induration. Six months later, she was retested as part of the TB screening program in the unit where she works. Her TST reaction was read 48 hours later as 11 millimeters of induration.

■ **What are two ways to interpret this result?**

There are two possible explanations for this result.

- One explanation is that Ms. Wilson may have been exposed to and infected with *M. tuberculosis* sometime in the 6 months between her first and second skin tests.
- The other explanation is the booster phenomenon. If Ms. Wilson was infected with *M. tuberculosis* many years ago, her ability to react to tuberculin may have decreased. This would explain why she did not react to the first TST. Then the first tuberculin test may have boosted the ability of her immune system to react to tuberculin. This would explain why she had a positive reaction to the second TST, which was given within a year of the first test. If this scenario is true, Ms. Wilson's positive reaction would not mean that she was recently infected with *M. tuberculosis*.

This problem in interpreting Ms. Wilson's reaction would have been avoided if she had been tested with a two-step procedure when she first joined the hospital. In any event, because she has a positive reaction, Ms. Wilson should be evaluated for TB disease.



3.5. Mr. Lee has a cough and other symptoms of TB disease, and he is evaluated with a chest x-ray. However, he is unable to cough up any sputum on his own for the bacteriologic examination.

■ **What should be done?**

If a patient cannot cough up a sputum specimen, other techniques can be used to obtain sputum. First, clinicians can try to obtain an induced sputum sample. If they cannot obtain an induced sputum sample, a bronchoscopy or gastric washing may be done.

3.6. Ms. Thompson 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+.

■ **What do these results tell you about Ms. Thompson's diagnosis and her infectiousness?**

These results show that Ms. Thompson's sputum specimens contain many acid-fast bacilli. Because the smears are positive, clinicians should suspect that Ms. Thompson has TB disease. They should also consider her infectious. However, it is possible that these acid-fast bacilli are mycobacteria other than tubercle bacilli. Therefore, the diagnosis of TB disease cannot be proven until the culture results are available.

3.7. Mr. Sagoo has symptoms of TB disease and a cavity on his chest x-ray, but all of his sputum smears are negative for acid-fast bacilli.

■ **Does this rule out the diagnosis of pulmonary TB disease?**

No.

■ **Why or why not?**

M. tuberculosis may grow in the cultures even though there were no acid-fast bacilli on the smear. Mr. Sagoo's symptoms and his abnormal chest x-ray suggest that he does have pulmonary TB disease.



3.8. In the public health clinic, you see a patient, Ms. Sanchez, who 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.

■ **What parts of Ms. Sanchez's medical history lead you to suspect TB disease?**

Ms. Sanchez has symptoms of TB disease (weight loss, fever, and a persistent cough). Also, in the past she has been treated for TB disease. We don't know whether she completed therapy, but until we can prove otherwise, we should assume that she has TB disease again. Her history of injecting illegal drugs (heroin) is another risk factor for TB.

■ **What diagnostic tests should be done?**

People who have TB symptoms should be evaluated for TB disease. Because she has symptoms of pulmonary TB disease (coughing), Ms. Sanchez should be given a chest x-ray. In addition, a sputum specimen should be collected for smear and culture, and drug susceptibility testing should be done if the culture is positive for *M. tuberculosis*.