

Self-Study Modules on Tuberculosis



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

Module 3: Objectives

At completion of this module, learners 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 (TST)**
- 3. Describe how to interpret an interferon-gamma release assay (IGRA)**
- 4. Discuss considerations for using either the TST or IGRA for diagnosing latent tuberculosis infection (LTBI)**
- 5. Describe the components of a medical evaluation for diagnosing TB disease**

Module 3: Overview

- **Targeted Testing**
- **Diagnosis of latent tuberculosis infection (LTBI)**
 - **TST**
 - **IGRAs**
 - **TB Testing Programs, the Booster Phenomenon, and Two-Step Testing**
- **Diagnosis of TB Disease**
- **Reporting TB Cases**

Targeted Testing

Targeted Testing (1)

- **Targeted testing is a TB control strategy used to identify and treat persons:**
 - **At high risk for latent TB infection (LTBI)**
 - **At high risk for developing TB disease once infected with *M. tuberculosis***

Targeted Testing (2)

- **Identifying persons with LTBI is an important goal of TB elimination because LTBI treatment can:**
 - **Prevent the development of TB disease**
 - **Stop the further spread of TB to others**

Targeted Testing (3)

A Decision to Test is a Decision to Treat

- **TB testing activities should be done only when there is a plan for follow-up care**
- **Health care workers (HCWs) should identify and test persons who are at high risk**
 - **People who are not at high risk generally should not be tested**

Targeted Testing (4)

High-Risk Groups

- **High-risk groups can be divided into two categories:**
 - **People who are at high risk for exposure to or infection with *M. tuberculosis***
 - **People who are at high risk for developing TB disease once infected with *M. tuberculosis***

Targeted Testing (5)

High-Risk Groups for TB Infection

- **Contacts of people known or suspected to have TB disease**
- **People who have come to U.S. within 5 years from areas of the world where TB is common**
- **People who visit areas with a high prevalence of TB disease**
- **People who live or work in high-risk congregate settings**

Targeted Testing (6)

High-Risk Groups for TB Infection

- **HCWs who serve patients at increased risk for TB disease**
- **Populations defined locally as having an increased incidence of LTBI or TB disease (e.g., medically underserved, low income, or people who abuse drugs or alcohol)**
- **Infants, children, and adolescents exposed to adults in high-risk groups**

Targeted Testing (7)

High-Risk Groups for TB Disease after Infection with *M. tuberculosis*

- **People living with HIV**
- **Children younger than 5 years of age**
- **People recently infected with *M. tuberculosis* (within the past 2 years)**
- **People with a history of untreated or inadequately treated TB disease**
- **People receiving immunosuppressive therapy**

Targeted Testing (8)

High-Risk Groups for TB Disease after

Infection with *M. tuberculosis*

- **Persons with silicosis, diabetes mellitus, chronic renal failure, leukemia, or cancer of the head, neck, or lung**
- **Persons who have had a gastrectomy or jejunioileal bypass**
- **Low body weight**
- **Cigarette smokers and persons who abuse drugs and alcohol**
- **Persons defined locally as having an increased incidence of disease due to *M. tuberculosis***

Diagnosis of Latent TB Infection (LTBI)

Diagnosis of LTBI

- **Available testing methods for *M. tuberculosis* infection:**
 - **Mantoux tuberculin skin test (TST)**
 - **Blood tests known as interferon-gamma release assays (IGRAs):**
 - **QuantiFERON®-TB Gold In-Tube (QFT-GIT)**
 - **T-SPOT®.TB test (T-Spot)**

Diagnosis of Latent TB Infection (LTBI)

Mantoux Tuberculin Skin Test Administering the Test

Mantoux Tuberculin Skin Test (1)

- **TST is administered by injection**
- **Tuberculin is made from proteins derived from inactive tubercle bacilli**
- **Most people who have TB infection will have a reaction at injection site**



Syringe being filled with 0.1 ml of liquid tuberculin

Mantoux Tuberculin Skin Test (2)

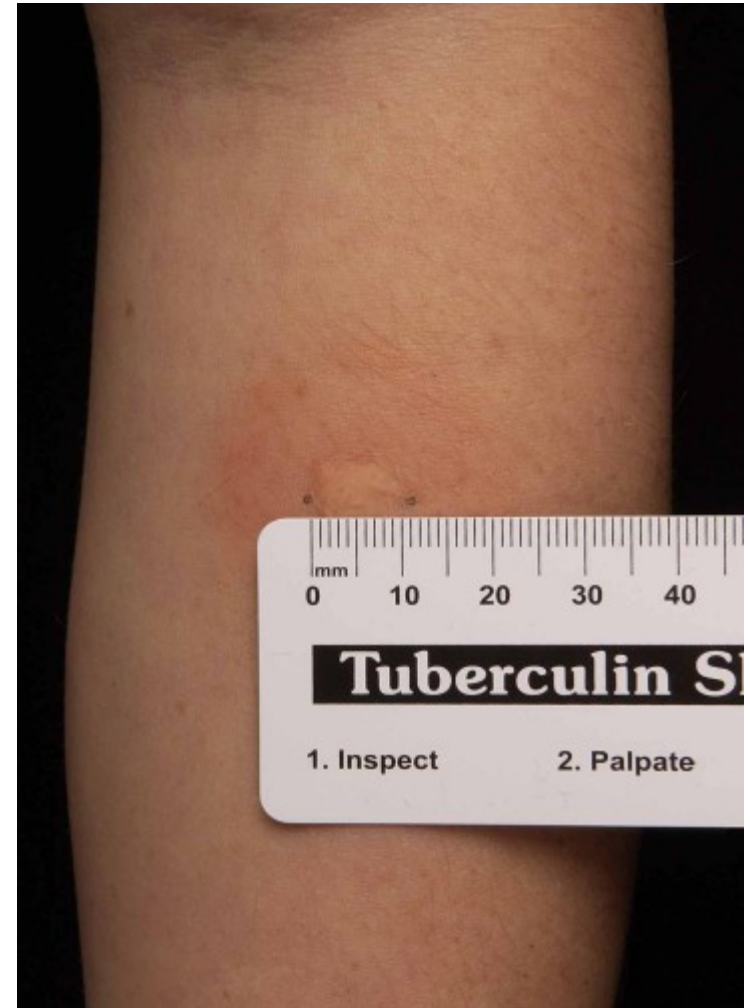
0.1 ml of 5 tuberculin units of liquid tuberculin are injected between the layers of skin on forearm



HCW administering Mantoux TST

Mantoux Tuberculin Skin Test (3)

- Forearm should be examined within 48 to 72 hours by HCW
- Reaction is an area of induration (swelling) around injection site
 - Induration is measured in millimeters
 - Erythema (redness) is not measured



Only the induration is measured

Mantoux Tuberculin Skin Test

Study Question 3.1

What is the TST used for?

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

Mantoux Tuberculin Skin Test

Study Question 3.2

How is the TST given?

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

Mantoux Tuberculin Skin Test

Study Question 3.3

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

The patient's arm is examined by a health care worker 48 to 72 hours after the tuberculin is injected.

Mantoux Tuberculin Skin Test

Study Question 3.4

How is the induration measured?

The diameter of the indurated area is measured across the forearm; erythema (redness) around the indurated area is not measured.

Diagnosis of Latent TB Infection (LTBI)

Mantoux Tuberculin Skin Test Interpreting the Reaction

Mantoux Tuberculin Skin Test (4)

Interpreting the Reaction

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



Mantoux Tuberculin Skin Test (5)

Interpreting the Reaction

- Induration of ≥ 5 mm is considered positive for:
 - People living with HIV
 - Recent contacts of people with infectious TB
 - People with chest x-ray findings suggestive of previous TB disease
 - People with organ transplants
 - Other immunosuppressed patients

Mantoux Tuberculin Skin Test (6)

Interpreting the Reaction

- Induration of ≥ 10 mm is considered a positive reaction for:
 - People who have recently come to U.S. from areas where TB is common
 - People who abuse drugs
 - Mycobacteriology laboratory workers
 - People who live or work in high-risk congregate settings

Mantoux Tuberculin Skin Test (7)

Interpreting the Reaction

- Induration of ≥ 10 mm is considered a positive reaction for:
 - People with certain medical conditions that increase risk for TB (e.g., silicosis, diabetes mellitus, severe kidney disease, certain types of cancer, and certain intestinal conditions)
 - Children younger than 5 years of age
 - Infants, children, or adolescents exposed to adults in high-risk categories

Mantoux Tuberculin Skin Test (8)

Interpreting the Reaction

- **Induration of ≥ 15 mm is considered a positive reaction for people who have no known risk factors for TB**

Occupational Exposure

- **For people who may be exposed to TB on the job (e.g., HCWs, staff of nursing homes or correctional facilities), interpretation of TST depends on:**
 - **The employee's individual risk factors for TB**
 - **The risk of exposure to TB in the person's job**

Mantoux Tuberculin Skin Test

Study Question 3.5

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?

- **Size of induration and risk factors for TB**
- **An additional factor is the risk of exposure to TB in the person's job**

Mantoux Tuberculin Skin Test

Study Question 3.6

Name 5 groups of people for which ≥ 5 mm of induration is considered a positive reaction?

- **People living with HIV**
- **Recent contacts of people with infectious TB**
- **People with chest x-ray findings suggestive of previous TB disease**
- **Patients with organ transplants**
- **Other immunosuppressed patients**

Mantoux Tuberculin Skin Test

Study Question 3.7

Name seven groups of people for which ≥ 10 mm of induration is considered a positive reaction.

- **People who have recently come to the U.S. (within the last 5 years) from areas where TB is common**
- **People who abuse drugs**
- **Mycobacteriology lab workers**
- **People who live or work in high-risk congregate settings**
- **People with certain medical conditions**
- **Children younger than 5 years of age**
- **Infants, children, and adolescents exposed to adults in high-risk categories**

Mantoux Tuberculin Skin Test

Study Question 3.8

For which group of people is ≥ 15 mm of induration considered a positive reaction?

People with no risk factors for TB.

Diagnosis of Latent TB Infection (LTBI)

Mantoux Tuberculin Skin Test Factors that Affect the Reaction

Mantoux Tuberculin Skin Test (9)

False-Positive Reaction

- **Factors that may cause people to have a positive reaction even if they do not have TB infection:**
 - **Infection with nontuberculous mycobacteria (NTM)**
 - **BCG vaccination**
 - **Administration of incorrect antigen**
 - **Incorrect measuring or interpretation of TST reaction**

Mantoux Tuberculin Skin Test (10)

BCG Vaccine

- **People who have been vaccinated with BCG may have a false-positive TST reaction**
 - **However, there is no reliable way to distinguish a positive TST reaction caused by BCG vaccination from a reaction caused by true TB infection**
- **Individuals should always be further evaluated if they have a positive TST reaction**

Mantoux Tuberculin Skin Test (11)

False-Negative Reaction

- **Factors that may cause false-negative reactions:**
 - **Anergy**
 - **Recent TB infection (within past 8 to 10 weeks)**
 - **It can take 2 to 8 weeks after TB infection for body's immune system to react to tuberculin**
 - **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**

Mantoux Tuberculin Skin Test (12)

Anergy

- **Inability to react to skin tests due to weakened immune system**
- **Anergy testing is no longer routinely recommended**

Mantoux Tuberculin Skin Test (13)

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



Mantoux Tuberculin Skin Test

Study Question 3.9

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

- **Infection with nontuberculous mycobacteria (NTM)**
- **BCG vaccination**
- **Administration of incorrect antigen**
- **Incorrect measuring or interpretation of TST reaction**

Mantoux Tuberculin Skin Test

Study Question 3.10

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

No. Individuals who have had the BCG vaccine should be further evaluated for LTBI or TB disease the same as if they were not vaccinated with BCG.

Mantoux Tuberculin Skin Test

Study Question 3.11

Name 6 factors that may cause false-negative reactions to the TST.

- **Anergy**
- **Recent TB infection (within past 8 to 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**

Mantoux Tuberculin Skin Test

Study Question 3.12

What is anergy?

The inability to react to skin tests because of a weakened immune system.

Mantoux Tuberculin Skin Test

Study Question 3.13

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

2 to 8 weeks

Mantoux Tuberculin Skin Test

Study Question 3.14

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

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

Diagnosis of Latent TB Infection (LTBI)

Interferon-Gamma Release Assays (IGRAs)

Types of IGRAs

- **QuantiFERON®-TB Gold In-Tube (QFT-GIT)**
 - Approved in 2007
- **T-Spot®.TB test (T-SPOT)**
 - Type of ELISpot assay
 - Approved in 2008
- **CDC guidelines for IGRAs published in 2010**



QFT-GIT testing materials



T-Spot®.TB testing materials

IGRAs (1)

- **Blood tests that help diagnose *M. tuberculosis* infection**
- **Measures a person's immune reactivity to *M. tuberculosis***

IGRAs (2)

Conducting the Test

- **Confirm arrangements for testing in a qualified laboratory**
- **Arrange for delivery of the blood sample to the laboratory in the time the laboratory specifies**
- **Draw a blood sample from the patient according to the manufacturer's instructions**
- **Schedule follow-up appointment for patient to receive test results**
- **Based on test results, provide follow-up evaluation and treatment as needed**

IGRAs (3)

How it Works

- **Blood samples are mixed with antigens (protein substances that can produce an immune response) and incubated**
- **If the person is infected with *M. tuberculosis*, blood cells will recognize antigens and release interferon gamma (IFN- γ) in response**

IGRAs (4)

Interpreting Results

- **QFT-GIT Results**
 - Based on amount of IFN- γ released in response to *M. tuberculosis* antigens and control substances
- **T-Spot Results**
 - Based on number of IFN- γ producing cells (spots) produced

IGRAs (5)

Interpreting Results

- **Qualitative test interpretation and quantitative assay measurements should be reported**
- **Laboratories use software provided by manufacturer to calculate results**
- **Results are sent to requesting health care provider**

Nil	Mit - Nil	Interpretation	Irregularity:	Comp
	12.503	Negative		
	12.507	Positive		

QFT-GIT Results

IGRAs (6)

Report of Results

IGRA Result	Interpretation
Positive	<i>M. tuberculosis</i> infection likely
Negative	<p><i>M. tuberculosis</i> infection unlikely, but cannot be excluded especially if</p> <ol style="list-style-type: none"> 1. Patient has signs and symptoms of TB 2. Patient has a high risk for developing TB disease once infected with <i>M. tuberculosis</i>
Indeterminate	<p>The test did not provide useful information about the likelihood of <i>M. tuberculosis</i> infection. Repeating an IGRA or performing a TST may be useful.</p>
Borderline (T-Spot only)	<p>The test did not provide useful information about the likelihood of <i>M. tuberculosis</i> infection. Repeating an IGRA or performing a TST may be useful.</p>

IGRA Recommendations (1)

- **IGRAs are the preferred method of testing in**
 - **Groups of people who might be less likely to return for TST reading and interpretation**
 - **Persons who have received the BCG vaccine**
- **TST is the preferred method of testing for children younger than 5 years of age**

IGRA Recommendations (2)

- Routine testing using both TST and IGRAs is **NOT** recommended
- Certain situations where results from both tests may be useful:
 - When the initial test is negative and:
 - Risk for infection, progression to disease, or a poor outcome is high
 - There is clinical suspicion for TB disease and confirmation of *M. tuberculosis* infection is **desired**

IGRA Recommendations (3)

- **Certain situations where results from both tests may be useful**
 - **When the initial test is positive and:**
 - **Additional evidence of infection is required to encourage the patient's acceptance and adherence to treatment**
 - **Person has a low risk of both infection and progression from infection to TB disease**

IGRA Advantages

- **Requires single patient visit to conduct test**
- **Results can be available in 24 hours**
- **Does not cause booster phenomenon which can happen with repeat TSTs**
- **BCG vaccination does not affect IGRA results**

IGRA Disadvantages and Limitations (1)

- **Blood samples must be processed within 8 to 30 hours after collection**
- **Errors in collecting or transporting blood specimens or in running and interpreting test can decrease accuracy**
- **Limited data on its use in certain populations (e.g., children younger than 5, persons recently infected, immunocompromised persons, and serial testing)**

IGRA Disadvantages and Limitations (2)

- **Limited data on its use to predict who will progress to TB disease**
- **Tests may be expensive**

IGRAs

Study Question 3.15

What are the steps for conducting an IGRA?

- **Confirm arrangements for testing in a qualified laboratory**
- **Arrange for delivery of the blood sample to the laboratory**
- **Draw a blood sample from the patient according to the manufacturer's instructions**
- **Schedule follow-up appointment for patient to receive test results**
- **Based on test results, provide follow-up evaluation and treatment as needed**

IGRAs

Study Question 3.16

How are IGRA results interpreted?

- **QFT-GIT results are based on the amount of IFN- γ that is released in response to the antigens and control substances**
- **T-Spot results are based on the number of IFN- γ producing cells (spots) produced**
- **Both the standard qualitative test interpretation and the quantitative assay measurements should be reported**

IGRAs

Study Question 3.17

How should negative IGRA results be interpreted?

- **If the result is negative, the patient is unlikely to have *M. tuberculosis* infection**
- **Patient may not require further evaluation unless they have signs and symptoms of TB disease**

IGRAs

Study Question 3.18

What are 5 advantages for using an IGRA as compared to the TST?

- **Requires a single patient visit**
- **Results can be available in 24 hours**
- **Does not cause the booster phenomenon**
- **Laboratory test not affected by health care worker perception or bias**
- **BCG vaccine does not affect IGRA results**

Diagnosis of Latent TB Infection (LTBI)

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

TB Testing Programs (1)

- **Many residential facilities, health care settings, and other settings have TB testing programs**
 - **Employees and residents are periodically given TSTs or IGRAs**
- **Testing programs:**
 - **Identify people who have LTBI or TB disease so they can be given treatment as needed**
 - **Determine whether TB is being transmitted in facility**

TB Testing Programs (2)

Baseline Test

- **Employees or residents are given TSTs or IGRAs when they first enter facility**
 - **If person is negative, they may be retested at regular intervals thereafter**

TB Testing Programs (3)

Conversion

- **Persons whose TST or IGRA result converts from negative to positive may have been infected with *M. tuberculosis***
 - **TST or IGRA conversions may indicate that TB is being transmitted in facility**

Booster Phenomenon

- **Phenomenon in which people who are skin tested many years after they became infected with TB have:**
 - **Negative reaction to initial TST**
 - **Positive reaction to subsequent TST given up to one year later**
- **Occurs mainly in older adults**
- **May affect accuracy of baseline skin test**
- **TST can boost subsequent IGRA results**

Figure 3.6

The booster phenomenon with the TST

Person becomes infected with *M. tuberculosis*

Person is skin tested years later

Person has negative reaction due to lessened ability to react to tuberculin

However, this skin test “jogs the memory” of the immune system to recognize and react to tuberculin

Person is skin tested again, up to 1 year later. For this example, we assume that the person was NOT exposed to TB during this time

As years pass, person’s ability to react to tuberculin lessens

Occurs mainly in previously infected, older adults whose ability to react to tuberculin has decreased over time

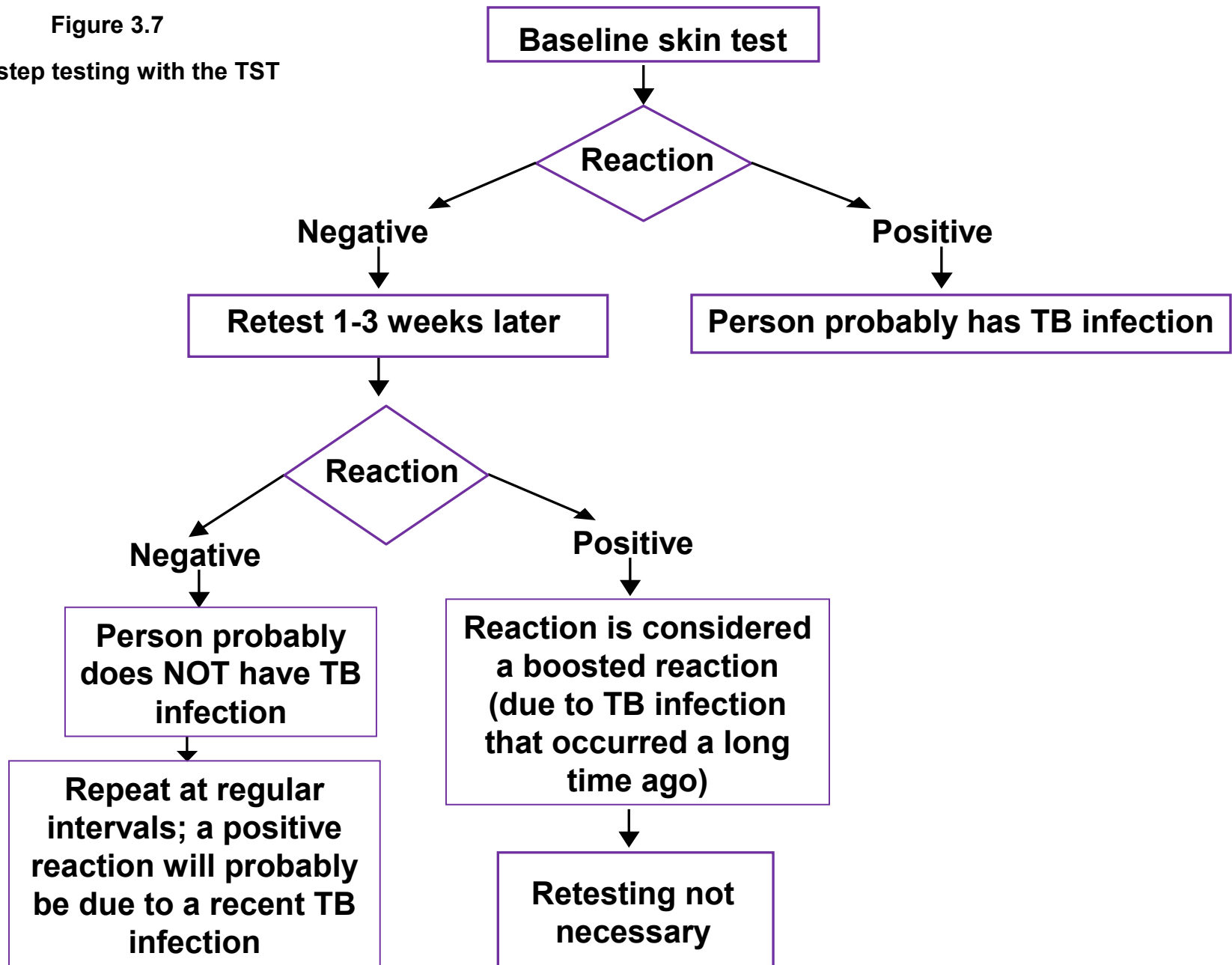
Person has a positive reaction. This is a boosted reaction due to TB infection that occurred a long time ago, not during the time between the two skin tests

Two-Step Testing

- **Only conducted when TST is used**
- **Distinguishes between boosted reactions and reactions caused by recent infections**
- **Should be used for initial skin testing of persons who will be retested periodically**
- **If person's initial skin test is negative, they should be given a second test 1 to 3 weeks later**
 - **Second test positive: probably boosted reaction**
 - **Second test negative: considered uninfected**

Figure 3.7

Two-step testing with the TST



Booster Phenomenon

Study Question 3.19

What is the booster phenomenon?

- Phenomenon in which people who are skin tested many years after becoming infected with *M. tuberculosis* have a negative reaction to an initial skin test, followed by a positive reaction to a skin test given up to a year later
- Occurs because the ability to react to tuberculin lessens over time in some people

Two-Step Testing

Study Question 3.20

What is the purpose of two-step testing?

To distinguish between boosted reactions and reactions caused by recent infection.

Two-Step Testing

Study Question 3.21

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

It is used in many programs for skin testing employees when they start their job.

Two-Step Testing

Study Question 3.22

How is two-step testing done?

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 reaction to second test is positive, it is considered a boosted reaction**
- If reaction to second test is negative, person is considered to be uninfected**

Diagnosis of TB Disease

Medical Evaluation

- **Anyone with TB symptoms or positive TST or IGRA result should be medically evaluated for TB disease**
- **Components of medical evaluation:**
 1. **Medical history**
 2. **Physical examination**
 3. **Test for TB infection**
 4. **Chest x-ray**
 5. **Bacteriological examination**

Diagnosis of TB Disease

Medical Evaluation

1. **Medical History**
2. **Physical Examination**
3. **Test for TB Infection**

1. Medical History (1)

- **Clinicians should ask patients if they have:**
 - **Symptoms of TB disease**
 - **Been exposed to a person with infectious TB or have risk factors for exposure to TB**
 - **Any risk factors for developing TB disease**
 - **Had LTBI or TB disease before**

1. Medical History (2)

General Symptoms of TB Disease

- **Fever**
- **Chills**
- **Night sweats**
- **Weight loss**
- **Appetite loss**
- **Fatigue**
- **Malaise**

1. Medical History (3)

Symptoms of Pulmonary TB Disease

- **Cough lasting 3 or more weeks**
- **Chest pain**
- **Coughing up sputum or blood**

1. Medical History (4)

Symptoms of Extrapulmonary TB Disease

- **Symptoms of extrapulmonary TB disease depend on part of body that is affected**
- **For example:**
 - **TB disease in spine may cause back pain**
 - **TB disease in kidneys may cause blood in urine**

2. Physical Examination

A physical examination cannot confirm or rule out TB disease, but can provide valuable information



3. Test for TB Infection (1)

- **Types of tests available for diagnosing TB infection in U.S.:**
 - **Mantoux TST**
 - **IGRAs**
 - **QFT-GIT**
 - **T-SPOT**



3. Test for TB Infection (2)

- **Patients with symptoms of TB disease should always be evaluated for TB disease, regardless of their TST or IGRA test result**
 - **Clinicians should not wait for TST or IGRA results before starting other diagnostic tests**
 - **TST or IGRA should be given at the same time as other steps in the diagnosis of TB disease**

Diagnosis of TB Disease

Study Question 3.23

What are the 5 components for conducting a medical evaluation for diagnosing TB disease?

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

Diagnosis of TB Disease

Study Question 3.24

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

- **Symptoms of TB disease**
- **Exposure to a person who has infectious TB or has other risk factors for exposure to TB**
- **Risk factors for developing TB disease**
- **TB infection or TB disease in the past**

Diagnosis of TB Disease

Study Question 3.25

What are the symptoms of pulmonary TB disease? What are the symptoms of extrapulmonary TB disease?

- **General symptoms of TB disease: Weight loss, fatigue, malaise, fever, and night sweats**
- **Pulmonary: Coughing, pain in chest, coughing up sputum or blood**
- **Extrapulmonary: Depends 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.**

Diagnosis of TB Disease

Study Question 3.26

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

No, clinicians should not wait for TST or IGRA results before starting other diagnostic tests.

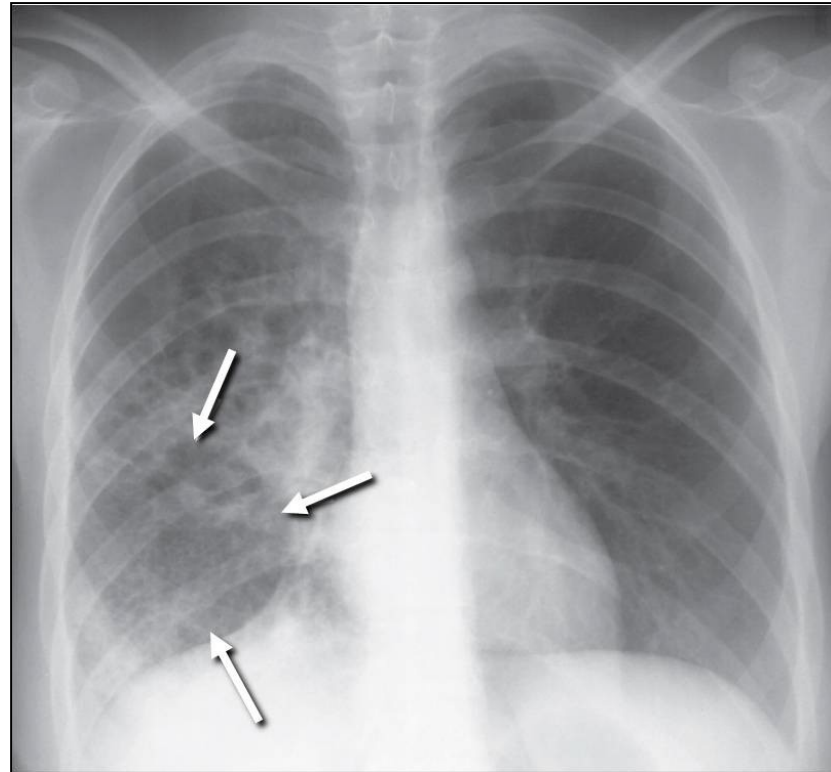
Diagnosis of TB Disease

Medical Evaluation

4. Chest X-Ray

4. Chest X-Ray (1)

- **When a person has TB disease in the lungs, the chest x-ray usually appears abnormal. It may show:**
 - **Infiltrates (collections of fluid and cells in lung tissue)**
 - **Cavities (hollow spaces within the lung)**



Abnormal chest x-ray with cavity

4. Chest X-Ray (2)

- **Chest x-rays can:**
 - **Help rule out possibility of pulmonary TB disease in persons who have a positive TST or IGRA result**
 - **Check for lung abnormalities**

4. Chest X-Ray (3)

- **Chest x-rays cannot confirm TB disease**
 - **Other diseases can cause lung abnormalities**
 - **Only bacteriologic culture can confirm patient has TB disease**
 - **Chest x-ray may appear unusual or even appear normal for persons living with HIV**

Chest X-Ray

Study Question 3.27

Name 2 purposes of the chest x-ray.

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

Chest X-Ray

Study Question 3.28

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

No. A variety of illnesses may produce abnormalities on chest x-ray. Only bacteriologic culture can prove whether or not a patient has TB disease.

Diagnosis of TB Disease

Medical Evaluation

5. Bacteriologic Examination

5. Bacteriologic Examination (1)

- **TB bacteriologic examination is done in a laboratory that specifically deals with *M. tuberculosis* and other mycobacteria**
 - **Clinical specimens (e.g., sputum, urine) are examined and cultured in laboratory**

5. Bacteriologic Examination (2)

- **Bacteriologic examination has 5 parts**
 - **Specimen collection**
 - **Examination of acid-fast bacilli (AFB) smears**
 - **Direct identification of specimen (nucleic acid amplification)**
 - **Specimen culturing and identification**
 - **Drug susceptibility testing**

5. Bacteriologic Examination (3)

Specimen Collection

- For pulmonary TB, specimens can be collected by:
 - Coughing up sputum sample
 - Inducing sputum sample
 - Bronchoscopy
 - Gastric washing



TB patient coughing up sputum in a sputum collection booth

5. Bacteriologic Examination (4)

Sputum Sample Specimen Collection

- **Easiest and least expensive method is to have patient cough into sterile container**
- **HCWs should coach and instruct patient**
- **Should have at least 3 sputum specimens examined**
 - **Collected in 8 to 24 hour intervals**
 - **At least one early morning specimen**

5. Bacteriologic Examination (5)

Induced Sputum Collection

- **Induced sputum collection should be used if patient cannot cough up sputum on their own**
- **Patient inhales saline mist, causing deep coughing**
- **Specimen often clear and watery, should be labeled “induced specimen”**

5. Bacteriologic Examination (6)

Bronchoscopy

- **Bronchoscopy may be used:**
 - If patient cannot cough up enough sputum
 - If an induced sputum cannot be obtained
- **Procedure: instrument is passed through the mouth into the diseased portion of the lung to obtain sputum or lung tissue**



Bronchoscopy being performed on a patient

5. Bacteriologic Examination (7)

Gastric Washing

- **Usually only used if sample cannot be obtained from other procedures**
- **Often used with children**
- **Tube is inserted through nose and into stomach to obtain gastric secretions that may contain sputum**

5. Bacteriologic Examination (8)

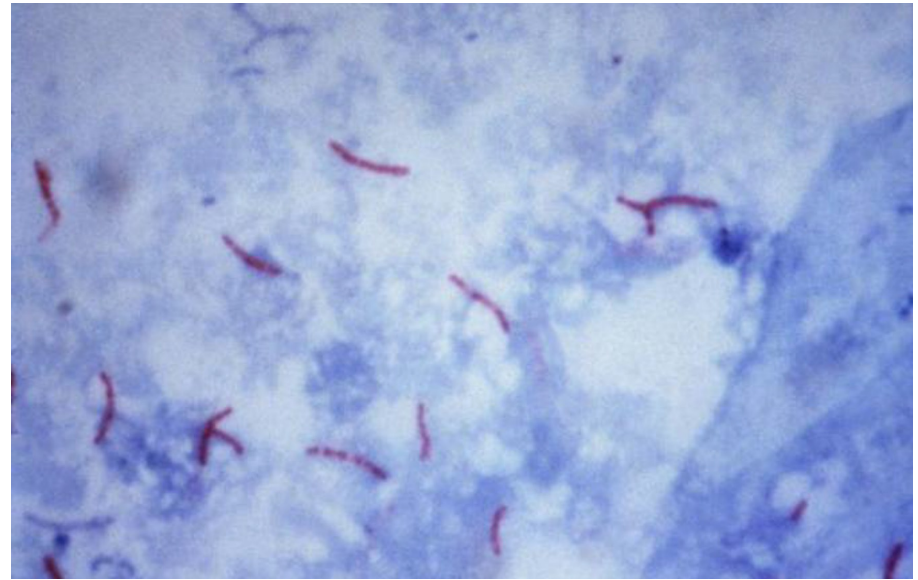
Extrapulmonary TB

- **Specimens other than sputum may be obtained**
- **Depends on part of body affected**
- **For example:**
 - **Urine samples for TB disease of kidneys**
 - **Fluid samples from area around spine for TB meningitis**

5. Bacteriologic Examination (9)

Examination of AFB Smears

- **Specimens are smeared onto glass slide and stained**
- **AFB are mycobacteria that remain stained after being washed in acid solution**



AFB smear

5. Bacteriologic Examination (10)

Examination of AFB Smears

- Number of AFB on smear are counted
- According to number of AFB seen, smears are classified as 4+, 3+, 2+, or 1+
 - For example, 4+ smear has 10 times as many AFB than 3+ smear
- If very few AFB are seen, the smear is classified by the actual number of AFB seen
- A negative smear does not rule out the possibility of TB

5. Bacteriologic Examination (11)

Examination of AFB Smears

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

Bacteriologic Examination

Study Questions 3.29

What are the 4 ways to collect sputum specimens? Indicate which procedure is the least expensive and easiest to perform.

- Patient simply coughs up sputum and the sputum is collected in a sterile container. This is the least expensive and easiest procedure.
- Induced sputum
- Bronchoscopy
- Gastric washing

Bacteriologic Examination

Study Question 3.30

What do laboratory personnel look for in a smear?

Acid-fast bacilli (AFB)

Bacteriologic Examination

Study Question 3.31

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

Patients who have many tubercle bacilli seen 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.

Diagnosis of TB Disease

Medical Evaluation

5. **Bacteriologic Examination (continued)**

5. Bacteriologic Examination (12)

Nucleic Acid Amplification Tests (NAA)

- **NAA tests directly identify *M. tuberculosis* from sputum specimens by:**
 - **Amplifying (copying) DNA and RNA segments**
- **Can help guide clinician's decision for patient therapy and isolation**
- **Does not replace need for AFB smear, culture, or clinical judgment**

5. Bacteriologic Examination (13)

Nucleic Acid Amplification Tests (NAA)

- **If NAA test and AFB smears are positive:**
 - **Patient is presumed to have TB and should begin treatment**

- **If NAA test is negative and AFB smears are positive:**
 - **Patient may have nontuberculous mycobacteria infection (NTM)**

5. Bacteriologic Examination (14)

Xpert MTB/RIF Assay

- Xpert MTB/RIF assay is a NAA test that simultaneously detects *Mycobacterium tuberculosis* complex (MTBC) and resistance to rifampin
- To conduct this test, a sputum sample is mixed with the reagent that is provided with the assay
- A cartridge containing the mixture is placed in the GeneXpert machine
- Results are available in less than 2 hours

5. Bacteriologic Examination (15)

Xpert MTB/RIF Assay

- **Results that are positive for MTBC and for rifampin resistance indicate that the bacteria have a high probability of resistance to rifampin**
 - **Should be confirmed by additional rapid testing**
- **If rifampin resistance is confirmed, rapid molecular testing for drug resistance to both first-line and second-line drugs should be performed so an effective treatment regimen can be selected**

5. Bacteriologic Examination (16)

Culturing and Identifying Specimen

- **Culturing:**
 - **Determines if specimen contains *M. tuberculosis***
 - **Confirms diagnosis of TB disease**
- **All specimens should be cultured**



Colonies of *M. tuberculosis* growing on media

5. Bacteriologic Examination (17)

Culturing and Identifying Specimen

- **Step 1: Detect growth of mycobacteria**
 - **Solid media: 3 to 6 weeks**
 - **Liquid media: 4 to 14 days**
- **Step 2: Identify organism that has grown**
 - **Nucleic acid probes: 2 to 4 hours**

5. Bacteriologic Examination (18)

Culturing and Identifying Specimen

- **Positive culture: *M. tuberculosis* identified in patient's culture**
 - **Called *M. tuberculosis* isolate**
 - **Confirms diagnosis of TB disease**

5. Bacteriologic Examination (19)

Culturing and Identifying Specimen

- **Negative culture: *M. tuberculosis* NOT identified in patient's culture**
 - **Does not rule out TB disease**
 - **Some patients with negative cultures are diagnosed with TB based on signs and symptoms**

5. Bacteriologic Examination (20)

Culturing and Identifying Specimen

- **Bacteriological examinations are important for assessing infectiousness and response to treatment**
- **Specimens should be obtained monthly until 2 consecutive cultures are negative**
- **Culture conversion is the most important objective measure of response to treatment**

5. Bacteriologic Examination (21)

Drug Susceptibility Testing

- **Conducted when patient is first found to have positive culture for TB**
- **Determines which drugs kill tubercle bacilli**
- **Tubercle bacilli killed by a particular drug are susceptible to that drug**
- **Tubercle bacilli that grow in presence of a particular drug are resistant to that drug**

5. Bacteriologic Examination (22)

Drug Susceptibility Testing

- **Tests should be repeated if:**
 - **Patient has positive culture after 3 months of treatment; or**
 - **Patient does not get better**



Drug susceptibility testing on solid media

5. Bacteriologic Examination (23)

Types of Drug-Resistant TB

Mono-resistant	Resistant to any one TB treatment drug
Poly-resistant	Resistant to at least any two TB drugs (but not both isoniazid and rifampin)
Multidrug-resistant (MDR TB)	Resistant to at least isoniazid and rifampin, the two best first-line TB treatment drugs
Extensively drug-resistant (XDR TB)	Resistant to isoniazid and rifampin, PLUS resistant to any fluoroquinolone AND at least 1 of the 3 injectable second-line drugs (e.g., amikacin, kanamycin, or capreomycin)

5. Bacteriologic Examination (24)

Growth-Based Drug Susceptibility Testing

- **Growth-based susceptibility testing can be done using a liquid or solid medium method**
- **Organisms that grow in media containing a specific drug are considered resistant to that drug**
- **Liquid medium methods are faster than solid media methods for determining susceptibility to first-line TB medications**
- **Results can be obtained within 7 to 14 days for liquid medium method and up to 21 days for solid medium method**

5. Bacteriologic Examination (25)

Molecular Detection of Drug Resistance

- **Molecular tests provide preliminary guidance on effective therapy for TB patients**
- **These tests should be considered for patients with the following characteristics:**
 - **High risk of rifampin resistance, including MDR TB;**
 - **First-line drug susceptibility results are available and show resistance to rifampin;**
 - **Infectiousness poses a risk to vulnerable contacts; and**
 - **Contraindications to essential first-line medications**

Culture Specimen

Study Question 3.32

Why is it necessary to culture a specimen?

It is necessary to culture a specimen to determine whether the specimen contains *M. tuberculosis* and to confirm diagnosis of TB disease. Additionally, culture is needed for genotyping and for performing drug susceptibility testing.

Culture Specimen

Study Question 3.33

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

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

Drug Susceptibility

Study Question 3.34

Why are drug susceptibility tests done?

To determine which drugs will kill the tubercle bacilli that are causing disease in a particular patient. Test results can help clinicians choose the appropriate drugs for each patient.

Drug Susceptibility

Study Question 3.35

How often should drug susceptibility tests be done?

- **Should be done when the patient is first found to have a positive culture for *M. tuberculosis***
- **Tests should be repeated if a patient has a positive culture for *M. tuberculosis* after 3 months of treatment or if a patient is not getting better**

Reporting TB Cases

Criteria for Reporting TB Cases (1)

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

- 1. Patient has positive culture for *M. tuberculosis***
- 2. Patient has positive NAA test for *M. tuberculosis***
 - NAA test must be accompanied by culture for mycobacteria species**

Criteria for Reporting TB Cases (2)

- 3. Patient has positive AFB smear, but culture has not been obtained or is falsely negative or contaminated**

- 4. In the absence of laboratory confirmation, patient meets all of the following criteria:**
 - Positive TST or IGRA,**
 - Other signs and symptoms of TB disease,**
 - Treatment with 2 or more TB drugs, and**
 - A completed diagnostic evaluation.**

Criteria for Reporting TB Cases (3)

- **Cases that do not meet any of these sets of criteria may be counted as a verified TB case if health care provider has reported the case and decided to treat the patient for TB disease**

Case Studies

Module 3: Case Study 3.1

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

a. Mr. West, 36 yrs. old, HIV infected, 8 mm induration

b. Ms. Hernandez, 26 yrs. old, native of Mexico, 7 mm induration

c. Ms. Jones, 56 yrs. old, diabetic, 12 mm induration

d. Mr. Sung, 79 yrs. old, nursing home resident, 11 mm induration

e. Mr. Williams, 21 yrs. old, no known risk factors, 13 mm induration

f. Ms. Marcos, 42 yrs. old, chest x-rays findings suggestive of previous TB, 6 mm induration

g. Ms. Rayle, 50 yrs. old, husband has pulmonary TB, 9 mm of induration

Module 3: Case Study 3.2 (1)

A 30 year-old man who recently immigrated to the United States from India is given a TST and found to have 14 mm 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.

Module 3: Case Study 3.2 (2)

How should this man's results be interpreted?

- **Positive reaction to TST**
- **Should be further evaluated for LTBI or TB disease**

What factors make it more likely that this man's positive reaction is due to TB infection?

- **From area of the world where TB is common**
- **Wife had pulmonary TB**

Module 3: Case Study 3.3 (1)

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 mm of induration.

Module 3: Case Study 3.3 (2)

What are 3 ways to interpret this result?

- **May not have TB infection**
- **May be anergic**
- **It may be less than 8 to 10 weeks since he was exposed to TB**

Module 3: Case Study 3.4 (1)

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 mm 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 mm of induration.

Module 3: Case Study 3.4 (2)

What are 2 ways to interpret this result?

- **She was exposed to TB sometime in the 6 months between her first and second skin tests**
- **She had a boosted reaction**

Module 3: Case Study 3.5 (1)

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.

Module 3: Case Study 3.5 (2)

What should be done?

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

Module 3: Case Study 3.6 (1)

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+.

Module 3: Case Study 3.6 (2)

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

- **Results show that Ms. Thompson's sputum specimens contain many acid-fast bacilli**
- **Clinicians should suspect that she has TB disease and should consider her infectious since her smears are positive**
- **It is possible that the AFB are mycobacteria other than tubercle bacilli**
- **Diagnosis cannot be confirmed until culture results are available**

Module 3: Case Study 3.7 (1)

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.

Module 3: Case Study 3.7 (2)

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 has pulmonary TB disease.**

Module 3: Case Study 3.8 (1)

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.

Module 3: Case Study 3.8 (2)

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

- **Symptoms of TB disease (weight loss, fever, persistent cough)**
- **Past treatment for TB disease**
- **History of injecting illegal drugs**

What diagnostic tests should be done?

- **Chest x-ray**
- **Sputum smear and culture**
- **Drug susceptibility testing**