Diagnosis of Latent TB Infection (LTBI)

Diagnosis of Pulmonary Tuberculosis
ORGANIZATION
OF CASE-FINDING
The main objective of case-finding

• is to identify smear-positive pulmonary tuberculosis patients, who are the most potent sources of infection
• These patients are found among adults (individuals aged over 15 years), as tuberculosis in children is rarely smear-positive and smear-negative patients rarely transmit disease, even if they are positive on culture
Passive case detection

• The system used to evaluate patients presenting with symptoms suggestive of tuberculosis (suspects) is often likened to a funnel with a series of filters that identify smear-positive cases among symptomatic individuals
The first filter is the clinical examination

• among patients presenting with general symptoms, the staff working at the primary level of the health services must identify those with respiratory symptoms

• On average 10–15% of adults presenting to the general health services have respiratory symptoms
The second filter
is also a clinical examination

- This distinguishes patients who have symptoms of less than 3 weeks’ duration, who most probably have acute respiratory infection.
- Among those with longer duration of symptoms are not only tuberculosis patients but also patients with chronic lung disease.
- Tuberculosis patients most frequently have symptoms of at least 3 weeks (distinguishing them from those with acute respiratory infection) but usually of less than one year (distinguishing them from those with asthma or other chronic lung conditions). These patients are termed "tuberculosis suspects"
The third, bacteriological, filter

• is indispensable, as it is the only means by which the most potent sources of infection can be identified

• At least two smear microscopy examinations are performed to detect tuberculosis in all those individuals designated “tuberculosis suspects” after passing through the previous filters.
Active detection of cases and infected individuals among at-risk populations
Main groups at risk

• “Groups at risk” are population groups whose risk of contracting tuberculosis is 5–10 times higher than that of the general population, either because they have a greater risk of being infected, or because they have a greater likelihood of progressing to disease once infected.
Groups most exposed to sources of infection

• **The family circle of index cases.** Subjects living in contact with smear-positive cases have a risk that is directly proportional to their contact with the patient. The greatest risk is observed in individuals who live in the same household as a smear-positive pulmonary tuberculosis case.

• **Health institutions.** Immunosuppressed individuals hospitalized at the same time as untreated or drug-resistant tuberculosis patients, and health personnel working in tuberculosis services or in bacteriology laboratories where cultures are carried out, are more exposed to sources of infection than the general population.
Groups with lowered immunity

• This group mainly consists of individuals who are HIV-positive or who have AIDS

• Other diseases (such as silicosis, lymphoma, and diabetes) and immunosuppressive treatment, in particular among organ transplant patients, provoke a lowering of immunity that is much less significant

• Drug dependence and alcoholism favour reduction in defences
Underprivileged and marginalized groups

• individuals in precarious situations
• homeless
• who live in poor areas of big cities
• prisoners
• HIV infection may also be higher in underprivileged population groups
Main groups at risk

• Migrants and refugees from countries with a high prevalence of tuberculosis

• Individuals with extensive sequelae of untreated tuberculosis:
  ✓ These individuals have a higher risk of recurrence of tuberculosis through reactivation of bacilli that have remained latent after their disease has become quiescent
  ✓ This is principally the case if they have had inadequate or no treatment for their previous episode of tuberculosis
Danger group

- it is important to detect such patients for the sack of public protection those include school teachers, food servers, hair dressers, public transport workers, doctors, nurses and all hospital employees
Outline

• Diagnosis of latent tuberculosis infection (LTBI)
  – Tuberculin skin test TST
  – IGRAs - Interferon-γ Release Assays

• Diagnosis of pulmonary TB
  – Medical history
  – Physical examination
  – Chest radiograph
  – Bacteriologic exam
Diagnosis of tuberculosis

Latent Infection
- TST
- IFN-\(\gamma\) techniques

Active tuberculosis
- Smear examination
- Solid and liquid culture
- Identification
- Susceptibility testing methods

Molecular Epidemiology
- RFLP
- MIRU
- Spoligotyping

Molecular methods
- Detection
- Identification
- Detection of resistance
## LTBI vs. TB Disease

<table>
<thead>
<tr>
<th>Person with LTBI (Infected)</th>
<th>Person with TB Disease (Infectious)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has a small amount of TB bacteria in his/her body that are alive, but inactive</td>
<td>Has a large amount of active TB bacteria in his/her body</td>
</tr>
<tr>
<td>Cannot spread TB bacteria to others</td>
<td>May spread TB bacteria to others</td>
</tr>
<tr>
<td>Does not feel sick, but may become sick if the bacteria become active in his/her body</td>
<td>May feel sick and may have symptoms such as a cough, fever, and/or weight loss</td>
</tr>
<tr>
<td>Usually has a TB skin test or TB blood test reaction indicating TB infection</td>
<td>Usually has a TB skin test or TB blood test reaction indicating TB infection</td>
</tr>
<tr>
<td>Radiograph is typically normal</td>
<td>Radiograph may be abnormal</td>
</tr>
<tr>
<td>Sputum smears and cultures are negative</td>
<td>Sputum smears and cultures may be positive</td>
</tr>
<tr>
<td>Should consider treatment for LTBI to prevent TB disease</td>
<td>Needs treatment for TB disease</td>
</tr>
<tr>
<td>Does not require respiratory isolation</td>
<td>May require respiratory isolation</td>
</tr>
<tr>
<td>Not a TB case</td>
<td>A TB case</td>
</tr>
</tbody>
</table>
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 test (QFT-G)
    - QuantiFERON®-TB Gold In-Tube (QFT-GIT)
    - T-SPOT
Mantoux Tuberculin Skin Test
Mantoux Tuberculin Test

• Preferred method of testing for TB infection in adults and children

• Tuberculin skin testing useful for
  – Examining person who is not ill but may be infected
  – Determining how many people in group are infected
  – Examining person who has symptoms of TB
Tuberculin

• is obtained from a sterilized and concentrated *M. tuberculosis* culture filtrate

• PPD – purified protein derivative of tuberculin (antigenic)
  • PPD-S
  • PPD-RT21
  • PPD-RT23
  • PPD-CT68
Mantoux Tuberculin Skin Test

- Inject intradermally 0.1 ml of 2TU PPD tuberculin
- Produce wheal 6 mm to 10 mm in diameter
- Represent DTH (delayed type hypersensitivity)
- Most people who have TB infection will have a reaction at injection site
Applying the tuberculin skin test
Applying the tuberculin skin test
Reading of Mantoux Tuberculin Skin Test

• Forearm should be examined within 48 - 72 hours by HCW

• Reaction is an area of **induration** (swelling) around injection site

• Measure only induration
  
  – Induration is measured in millimeters
  
  – Erythema (redness) is not measured
Mantoux Tuberculin Skin Test
Interpreting the Reaction

- **negative:**
  - no induration
  - only erythema without induration
  - indurations till 5 mm at the no vaccinated BCG persons, indurations till 10 mm at the vaccinated BCG persons
Mantoux Tuberculin Skin Test
Interpreting the Reaction

- **positive:**
  induration from 5 mm at the no vaccinated BCG persons,
  indurations from 10 mm at the vaccinated BCG persons till 16 mm at the children and 20 mm at the adults
Mantoux Tuberculin Skin Test
Interpreting the Reaction

• hyperergical:
  ✓ A tuberculin reaction of 17 mm or greater of induration at the children, a tuberculin reaction of 21 mm or greater of induration
  ✓ Induration accompanied by vesiculation, necrosis
  ✓ lymphangitis and satellite adenopathies
Tuberculin conversion

- Tuberculin conversion is defined as a test response in an individual previously classified as a non-reactor.
Booster Phenomenon

An increase in the size of a tuberculin reaction (> 6 mm, or from < to > 10 mm) after a 2nd PPD skin test for TB, repeated at short time interval
Factors may affect TST

• False negative
  - Faulty application
  - Anergy
  - Acute TB (2-10 wks to convert)
  - PPD may not become “positive” until 3 months after exposure
  - Very young age (< 6 months old)
  - Recent live-virus (e.g., measles or smallpox) vaccination
  - HIV
  - Malnutrition
  - Steroid therapy

• False positive
  - BCG vaccination (usually <10mm by adulthood)
  - Nontuberculous mycobacteria infection
  - Administration of incorrect antigen
  - Incorrect measuring or interpretation of TST reaction
Diagnosis of Latent TB Infection (LTBI)

Interferon-Gamma Release Assays (IGRAs)
Types of IGRAs

• QuantiFERON®-TB Gold (QFT-G)
  – CDC guidelines published in 2005

• QuantiFERON®-TB Gold In-Tube (QFT-GIT)
  – Approved 10/2007

• T-Spot®.TB test (T-SPOT)
  – Type of ELISpot assay
  – Approved 7/2008

• CDC guidelines for IGRAs are under development
QFT-G and QFT-GIT

• Measures person’s immune reactivity to *M. tuberculosis*

• Used to help diagnose *M. tuberculosis* infection in persons suspected of having either LTBI or TB disease
Quantiferon Testing

• Whole blood *in vitro* test:
  – Lymphocytes release IFN gamma in presence of 2 TB antigens

• Will be positive in latent or active TB

• Advantages:
  – No error in interpretation
  – No follow-up in 48-72 hours
  – No boosting
  – Not affected by BCG
QFT-G and QFT-GIT

Conducting the Test

• Follow manufacturer’s instructions

  – Confirm arrangements for delivery and testing of blood within 12 hours of collection

  – Draw sample of blood into tube with heparin

  – Schedule appointment for patient to receive test results

• If needed, medical evaluation and treatment for LTBI or TB disease
QFT-G and QFT-GIT

How it Works

• Blood samples are mixed with antigens and incubated for 16 - 24 hours

• If infected with *M. tuberculosis*, blood cells will recognize antigens and release interferon gamma (IFN-γ) in response

• Results are based on the amount of IFN-γ released in response to antigens and control substances
QFT-G and QFT-GIT
Interpreting Results

- Test results are based on IFN-γ concentrations
- Laboratories can use software provided by manufacturer to calculate results
- Results are sent to requesting clinician
### QFT-G and QFT-GIT Report of Results

<table>
<thead>
<tr>
<th>Result</th>
<th>Report/Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive</strong></td>
<td><em>M. tuberculosis</em> infection likely</td>
</tr>
<tr>
<td></td>
<td><em>M. tuberculosis</em> infection unlikely, but cannot be excluded especially if:</td>
</tr>
<tr>
<td></td>
<td>1. Patient has TB signs and symptoms</td>
</tr>
<tr>
<td></td>
<td>2. Patient has a high risk for developing TB disease once infected with <em>M. tuberculosis</em></td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td>Test did not provide useful information about the likelihood of <em>M. tuberculosis</em> infection. Options are to repeat test, administer a TST, or do no additional testing</td>
</tr>
<tr>
<td><strong>Indeterminate</strong></td>
<td></td>
</tr>
</tbody>
</table>
T-SPOT

• Type of ELISpot assay

• Interferon gamma is presented as spots from T cells sensitized to *M. tuberculosis*

• Results are interpreted by subtracting the spot count of the control from the spot count of the sample
T-SPOT™.TB (Oxford Immunotec)

Figure 1: Diagram illustrating the main steps of the T-SPOT.TB assay.

1. Collect peripheral venous blood
2. Centrifuge
3. Incubate overnight
4. Wash, develop, and dry plate
5. Pre-coated wells
6. Add cells and antigens to 4 wells
7. Wash and count
8. Count the coloured spots in each well
IGRA Advantages

• Requires single patient visit to conduct test
• Results can be available in 24 hours
• Does not cause booster phenomenon
• Less likely to have incorrect reading of results as compared to TST
• BCG vaccination does not affect results
IGRA Disadvantages and Limitations

• Blood samples must be processed within 12 hours for some IGRAs

• Errors in running and interpreting test can decrease accuracy

• Limited data on its use in certain populations

• Limited data on its use to determine who is at risk for developing TB disease
Diagnosis of TB Disease

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

• Clinicians should ask patients if they have:
  - Symptoms of TB disease
  - History of TB exposure, infection, or disease
  - Any risk factors for developing TB disease
  - Had LTBI or TB disease before
  - Past TB treatment
  - Medical conditions that increase risk for TB disease
Medical History

- Have you had close contact with someone with TB?
- Do you have a cough? How long, dry, productive, colour?
- Is blood present in your sputum?
- Do you have chest pain? When & where?
- Do you have shortness of breath? How long?
- Do you sweat profusely at night?
- Have you lost weight?
- When did you start losing weight?
- When did you lose your appetite?
- How long have you been feeling weak and tired?
- Do you smoke?
- Have you previously been tested for TB?
- Do you know your HIV status?

Source: Chiang C et al., 2007.
Medical History

General Symptoms of TB Disease

- Fever
- Chills
- Night sweats
- Weight loss

- Appetite loss
- Fatigue
- Malaise
Medical History
Symptoms of Pulmonary TB Disease

• Cough lasting 3 or more weeks

• Chest pain

• Coughing up sputum or blood
“Any person who presents with symptoms or signs suggestive of TB, in particular cough of long duration (more than 3 weeks).”

Source: WHO, 2003
Mycobacterium tuberculosis
Signs & Symptoms of TB Disease

- Coughing for 2 weeks or longer
- Coughing up Blood
- Feeling Tired or Weak
- Chest Pain
- Night Sweats
- Weight Loss
- Fever
- Weight Loss
Medical History

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
Tuberculosis

• Commonly affects the lungs/pleura

• Extrapulmonary sites:
  • Lymph nodes – cervical most common - scrofula
  • Bones/joints – spine most common – Pott’s
  • GU system – sterile pyuria
  • CNS – Elevated CSF WBC (lymphocytes predominant), low glucose, and high protein in TB meningitis (insidious onset)
  • Abdomen
  • Pericardium

• Practically any organ can be involved
Physical Examination
Physical Examination

• Crackling rales in the infraclavicular space or in the interscapular-vertebral zone, in relation to exudative and cavitary lesions

• Uni- or bilateral bronchial rales (rhonchus, subcrepitations) in cases of bronchogenic disease dissemination

• In cases of pleural involvement: dull percussion, absence or reduction of vesicular murmur
Physical Examination

• Evidence of extrathoracic locations:
  – Erythema nodosum
  – Cervical and submaxillary fistulas and adenopathies, anal fistulas, osteoarticular involvement
Physical Examination

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

- Types of tests available for diagnosing TB infection:
  - TST
  - IGRAs
    - QFT-G
    - QFT-GIT
    - T-SPOT
Test for TB Infection

- 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 Active TB

• Acid fast stain of sputum
• Sputum AFB culture (culture needed for drug susceptibility)
• Radiographic imaging (CXR, CT)
• PCR
• Fluid Aspiration
• Tissue biopsy – higher yield than fluid
Diagnosis of Pulmonary TB

Cough 3 weeks

If 1 positive, X-ray and evaluation

Broad-spectrum antibiotic 10-14 days

If symptoms persist, repeat AFB smears, X-ray

If consistent with TB

Anti-TB Treatment

If 2/3 positive: Anti-TB Rx
Diagnosis of TB Disease

Bacteriologic Examination
Bacteriologic Examination

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

  - Clinical specimens (e.g., sputum and urine) are examined and cultured in laboratory
Bacteriologic Examination

- 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
Primary Isolation

Smear

Culture: gold standard liquid and solid media

Löwenstein-Jensen
Stonebrink
Middlebrook

MGIT 960

MB Bact
Sampling for diagnosis

• **For pulmonary tuberculosis:** the specimen that should be collected for examination is sputum obtained from the patient after coughing (more rarely the sample is obtained by gastric aspiration or bronchoscopy)

• **For extrapulmonary tuberculosis:** fluid from serous effusion, cerebrospinal fluid (CSF) or biopsied fragments can be sent to the laboratory for culture, urine samples for TB disease of kidneys
Sputum Smears

• Smear microscopy is widely available and accessible for diagnosis

• When pulmonary TB is suspected, two sputum specimens must be collected for examination
  – *Outpatient*: “spot-early morning”
  – *Inpatient*: two early morning specimens over two consecutive days
Sputum Smears

- Inpatient: two early morning specimens over two consecutive days

Day 1       Day 2       Day 3
Two Specimens

- Two specimens optimal
  - Spot specimen on first visit; sputum container given to patient
  - Early morning collection by patient on next day
Specimen Collection

• Obtain 2 sputum specimens for smear examination and culture
  • Persons unable to cough up sputum
    – induce sputum
    – bronchoscopy
    – gastric aspiration

• Follow infection control precautions during specimen collection
Sputum Collection Techniques

• Sputum collection should be done outside or in an empty room with very good ventilation
• If above not possible, try for best possible ventilation
• Use sterile glass or plastic containers, 5-6 cm deep, with screw cap
Sputum Collection Techniques

• The health worker should explain and demonstrate procedure

• The health worker should supervise, but should NOT stand in front of the patient
  – Collect away from other people

• Only sputum (2-5 ml) should be accepted as a good specimen

• Saliva (white, watery, frothy) should not be accepted because it will yield useless and misleading results
Specimen Quality

Poor quality sputum

Better quality

Source: CDC, 2007
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”
Bronchoscopy

• Bronchoscopy may be used:
  – If patient cannot cough up enough sputum
  – If an induced sputum cannot be obtained

• Procedure: instrument is passed through nose or mouth into lung to obtain pulmonary secretions or lung tissue
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
Smear Examination

- Strongly consider TB in patients with smears containing acid-fast bacilli (AFB)
- Results should be available within 24 hours of specimen collection
- Presumptive diagnosis of TB
- Not specific for M. tuberculosis
Why the Emphasis on Sputum Smears?

Direct Microscopy is the most reliable and cost effective way to identify persons who are most likely to transmit TB to others.
AFB Smear

- Sensitivity: 40-70%
- Specificity: 90%
- Ziehl-Neelsen staining
- Fluorescent auramine staining
Ziehl-Neelsen staining
## Reporting on AFB Microscopy

<table>
<thead>
<tr>
<th>Number of bacilli seen</th>
<th>Result reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>None per 100 oil immersion fields</td>
<td>Negative</td>
</tr>
<tr>
<td>1-9 per 100 oil immersion fields</td>
<td>Scanty. report exact number</td>
</tr>
<tr>
<td>10-99 per 100 oil immersion fields</td>
<td>1+</td>
</tr>
<tr>
<td>1-10 per oil immersion field</td>
<td>2+</td>
</tr>
<tr>
<td>&gt; 10 per oil immersion field</td>
<td>3+</td>
</tr>
</tbody>
</table>
Proportion of patients with pulmonary TB who have positive AFB smears

AFB positivity in TB patients

HIV Negative

Early HIV

Late HIV
What does a Positive Sputum Smear Mean?

• Positive smear predicts higher contagiousness to others

• Smears may be positive and not mean TB
  – Due to laboratory error or MOTT

• Sensitivity and specificity of a positive smear depends on prevalence of MOTT and HIV in a population
LED based fluorescence microscopy

**Advantages of fluorescence microscopy**

a) 10% more sensitive than conventional light microscopy.
b) Comparable specificity.
c) Reading 75% faster than with conventional light microscopy.

**Disadvantages of fluorescence microscopy**

a) Microscopes very expensive & not robust.
b) Dark room required.

Existing microscopes not used.
Acid-fast stains → auramine-rhodamine
Bacteriologic Examination Culturing and Identifying Specimen

• Culturing:
  
  – Determines if specimen contains *M. tuberculosis*

  – Confirms diagnosis of TB disease

• All specimens should be cultured
Bacteriologic Examination
Culturing and Identifying Specimen

• Step 1: Detect growth of mycobacteria
  – Solid media: 3 - 6 weeks
  – Liquid media: 4 - 14 days

• Step 2: Identify organism that has grown
  – Nucleic acid probes: 2 - 4 hours
  – Biochemical tests: 6 - 12 weeks
Laboratory Diagnosis of Tuberculosis

Culture of acid-fast bacilli

- **Solid medium:**
  - Egg based medium (Lowenstein-Jensen)
  - Agar and broth based medium (Middlebrook)

- **Liquid medium:**
  - Using culture on radioactive (Bactec) or non-radioactive (MGIT) media, the bacilli can be detected in 8–14 days
Culture: Solid Media

• Solid media have the advantage that organisms (colonies) can be seen on the surface of the medium

• Types most commonly used are:
  – Lowenstein-Jensen: egg-based
  – Middlebrook 7H 10 or 7H11: agar-based
  – Ogawa
Culture: Liquid Media

- More sophisticated equipment
- Faster detection of growth
- Higher sensitivity than solid media
- Can also be used for drug-susceptibility testing
- Two examples:
  - BACTEC
  - MGIT
Liquid media compared to solid media

Advantages compared to solid media:
- more rapid
- high quality of media
- fully automated system
- testing of 1st, 2nd, and new drugs
- safety: plastic tubes

Disadvantages:
- expensive
- higher contamination rate
- dependency on a company
- no DST for Cycloserine
Culture: Identification of Mycobacteria
Culture: Identification of Mycobacteria

Visual assessment of colony morphology:

Smooth, buff-colored colonies suggestive of *Mycobacterium avium* complex

Rough, buff-colored colonies suggestive of *Mycobacterium tuberculosis*
Bacteriologic Examination
Culturing and Identifying Specimen

• Positive culture: *M. tuberculosis* identified in patient’s culture
  
  – Called *M. tuberculosis* isolate

  – Confirms diagnosis of TB disease
Bacteriologic Examination
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
Bacteriologic Examination
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
Bacteriologic Examination
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
Culture: Drug Susceptibility Testing

Methods for susceptibility testing

• Agar proportion method: Compares growth on solid agar media with and without one of the four primary drugs (on discs)

• Broth based (BACTEC, MGIT): Liquid broth is inoculated with each test drug; growth in vial indicates resistance to that drug
Bacteriologic Examination
Drug Susceptibility Testing

• Tests should be repeated if:
  
  – Patient has positive culture after 3 months of treatment; or
  
  – Patient does not get better
### Bacteriologic Examination

#### Types of Drug-Resistant TB

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono-resistant</td>
<td>Resistant to any one TB treatment drug</td>
</tr>
<tr>
<td>Poly-resistant</td>
<td>Resistant to at least any two TB drugs (but not both isoniazid and rifampin)</td>
</tr>
<tr>
<td>Multidrug-resistant (MDR TB)</td>
<td>Resistant to at least isoniazid and rifampin, the two best first-line TB treatment drugs</td>
</tr>
<tr>
<td>Extensively drug-resistant (XDR TB)</td>
<td>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)</td>
</tr>
</tbody>
</table>
Methods for primary isolation and DST for all drugs

Löwenstein-Jensen medium

BACTEC 460TB

MGIT 960
Methods for Drug Susceptibility Testing

Proportion method on Löwenstein-Jensen medium
H, R, E, S, PTH, CM, OFL, CS, NSA (instead of P)

BACTEC 460TB
All drugs except cycloserine

MGIT 960
For all drugs, except CS
AFB smear vs. Cultures

• AFB smear
  – Rapid diagnosis

• Cultures
  – More sensitive
  – Allows drug susceptivity test
Laboratory Diagnosis of Tuberculosis

• Polymerase chain reaction (PCR) (Roche AMPLICOR) of first-time smear positive respiratory specimens
IS6110 DNA Fingerprint Pattern
Detection of resistant TB

- Solid media: 3-4 weeks
- Liquid media: 1-2 weeks
- Real-time PCR with fluorogenic probes: 5-6 hours
- Sequencing: 2 days
Nucleic Acid Amplification Tests (NAA)

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

- If NAA test is negative and AFB smears are positive:
  - Patients may have nontuberculous mycobacteria infection (NTM)
Bacteriologic Examination
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
The “magic” Gene Xpert

1. Sputum liquefaction and inactivation with 2:1 sample reagent
2. Transfer of 2 ml material into test cartridge (end of hands-on work)
3. Cartridge inserted into MTB-RIF test platform
4. Sample automatically filtered and washed
5. Ultrasonic lysis of filter-captured organisms to release DNA
6. DNA molecules mixed with dry PCR reagents
7. Seminested real-time amplification and detection in integrated reaction tube
8. Printable test result

Time to result, 1 hour 45 minutes
GeneXpert

• Detects DNA sequences specific for \textit{M. tuberculosis} and Rifampicin resistance by PCR

• Based on Nucleic Acid Amplification Test (NAAT). The Xpert® MTB/RIF
  – purifies
  – concentrates
  – amplifies (by real-time PCR) and
  – identifies targeted nucleic acid sequences in the \textit{Mycobacterium tuberculosis} genome
TB point of care testing

- Simple
- Minimum 3 steps for sample preparation

- Rapid
- 2 hours result availability

- Accurate
  - Sensitivity: adult PTB Smear+Culture+ = 95%
  - Smear- Culture+ = 80%
  - Specificity: adult PTB = 95%
Xpert MTB/RIF assay & GeneXpert instrument

1. Sputum liquefaction and inactivation with 2:1 sample reagent
2. Transfer of 2 ml material into test cartridge
3. Cartridge inserted into MTB-RIF test platform (end of hands-on work)
4. Sample automatically filtered and washed
5. Ultrasonic lysis of filter-captured organisms to release DNA
6. DNA molecules mixed with dry PCR reagents
7. Seminested real-time amplification and detection in integrated reaction tube
8. Printable test result

Time to result, 1 hour 45 minutes

Figure 2. Assay Procedure for the MTB/RIF Test. Two volumes of sample treatment reagent are added to each volume of sputum. The mixture is shaken, incubated at room temperature for 15 minutes, and shaken again. Next, a sample of 2 to 3 ml is transferred to the test cartridge, which is then loaded into the instrument. All subsequent steps occur automatically. The user is provided with a printable test result, such as “MTB detected; RIF resistance not detected.” PCR denotes polymerase chain reaction.
Xpert MTB/RIF assay & GeneXpert instrument
Xpert MTB/RIF assay & GeneXpert instrument
GeneXpert
Xpert MTB/RIF assay & GeneXpert instrument
GeneXpert Performance Con’t

- Mean time for Detection of MTB
  - GeneXpert = < day
  - Microscopy = 1 day,
  - Liquid culture - MGIT = 17 days
  - Solid Culture = > 30 days

- Mean time for Detection of Rifampicin Resistance
  - GeneXpert = < 1day
  - Liquid DST = 30 days
  - Conventional DST (Solid proportional Method) = 75 days
Chest radiography
Chest X-Ray

• 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
Chest Radiograph

• With pulmonary TB being the most common form of disease, the chest radiograph is useful for diagnosis of TB disease

• Chest abnormalities can suggest pulmonary TB disease

• A posterior-anterior radiograph of the chest is the standard view used for the detection of TB-related chest abnormalities

• In some cases, especially in children, a lateral view may be helpful
Chest radiography

• **Classical radiograph appearance**
  - Nodules
  - Infiltrates
  - Cavities (hollow spaces within lung)
  - Fibrosis with traction
  - Enlargement of hilar and mediastinal lymph node
  - Pleural effusion

• **In reactivity TB**
  - Classically fibrocavitary apical disease

• **Primary TB**
  - Middle or lower lobe consolidation
CXR Findings

• Primary TB:
  • Lower or middle lobe infiltrates

• Reactivated TB:
  • Apical infiltrates/cavitation

• Latent TB:
  • Usually normal
Chest radiography

• Abnormalities often seen in apical or posterior segments of upper lobe or superior segments of lower lobe
• However, lesions may appear anywhere in the lungs and may differ in size, shape, density, and cavitation
• May have unusual appearance in HIV-positive persons
• **Cannot confirm diagnosis of TB!!**

[cavity in patient’s RUL classic" for adult-type, reactivation tuberculosis]
Can this be TB?

“Typical Pattern”:

Secondary TB

• Distribution
  – Apical / posterior segments of upper lobes
  – Superior segments of lower lobes
  – Isolated anterior segment involvement unusual for *M. tb* (think *M. avium* complex)
Whenever you see an area of increased density within the lung, it must be the result of one of these four patterns:

- **Consolidation** - any pathologic process that fills the alveoli with fluid, pus, blood, cells (including tumor cells) or other substances resulting in lobar, diffuse or multifocal ill-defined opacities
- **Interstitial** - involvement of the supporting tissue of the lung parenchyma resulting in fine or coarse reticular opacities or small nodules
- **Nodule or mass** - any space occupying lesion either solitary or multiple
- **Atelectasis** - collapse of a part of the lung due to a decrease in the amount of air in the alveoli resulting in volume loss and increased density
Imaging of Tuberculosis

• Lymphadenopathy
  – More common in children
  – Usually unilateral, right-sided
  – Necrotic nodes
  – May be sole radiographic finding
  – Ranke complex—Ghon focus plus calcified hilar lymph nodes
  – Prolonged resolution
Enlargement of lymph nodes
Enlargement of lymph nodes
Nodules

• Nodules are round shadows (or “densities”) with clearly defined borders; their size varies from a micronodule (less than 3mm in diameter), to a nodule (more than 3mm and less than 1cm in diameter), to a round shadow (more than 1cm in diameter)
Patchy shadows, or infiltrates, have irregular borders that are not as clearly defined. They are of varying size, sometimes extending to large parts of the lungs.
Classic adult TB CXR

- PA view
  - diffuse parenchymal disease with multiple cavities and bulla formation on the left
  - Sputum smear was positive for AFB
Imaging of Tuberculosis

- **Miliary Disease**
  - Clinically significant in small percentage of patients
  - Infants, elderly and immunocompromised
  - Randomly distributed small 2-3 nodules
  - Slow resolution with therapy
Can this be TB? Miliary TB
# Radiographic Patterns: Pulmonary TB

<table>
<thead>
<tr>
<th>TB Pattern</th>
<th>Secondary</th>
<th>Primary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infiltrate</td>
<td>85% upper</td>
<td>Upper : Lower 60 : 40 Usually upper in children</td>
</tr>
<tr>
<td>Cavitation</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Adenopathy</td>
<td>Uncommon</td>
<td>Children common Adults ~30% Unilateral &gt; bilateral</td>
</tr>
<tr>
<td>Effusion</td>
<td>May be present</td>
<td>May be present</td>
</tr>
</tbody>
</table>
Can this be TB?

“Old / Healed” TB

- Ca\textsuperscript{++} granuloma–Ghon lesion
- Ca\textsuperscript{++} granuloma and hilar node calcification–Ranke complex
- Apical pleural thickening
- Fibrosis and volume loss
Fibro-cavitary pulmonary tuberculosis
Tuberculous effusion
Chest X-Ray

- Chest x-rays cannot confirm TB disease
- Other diseases can cause lung abnormalities
- Only bacteriologic culture can prove patient has TB disease
- Chest x-ray may appear unusual or even appear normal for persons living with HIV
In some instances, a computerized tomography (CT) scan may provide additional information. A CT scan provides more detailed images of parts of the body that cannot easily be seen on a standard chest radiograph. However, CT scans can be substantially more expensive.
Bronchoscopy
General laboratory tests

- Moderate anaemia and hypoproteinaemia in long-evolving cases
- An increased erythrocyte sedimentation rate, which usually does not exceed 50 to 60 mm in the first hour
- Altered coagulation test results
- Acute and febrile presentations can show leucocytosis with neutrophilia, although lymphocytopenia is more common in the subacute and chronic forms of the disease
Thanks for your attention!