TB DIAGNOSIS
• Pedro presents with cough in your consulting room.

• HOW DO YOU PROCEED?
HISTORY
Signs & symptoms of PTB

• Cough – Chronic or more than 2 weeks
• Weight loss (unintentional) and anorexia
• Fever and night sweats
• Pyrexia of unknown origin
• Chest pain
• Dyspnea
• Haemoptysis
• Malaise and unusual tiredness
Medical history of the patient

Important questions to ask
- Is there a history of previous TB treatment. When and for how long
- Are there family members, co-workers, friends with TB or TB symptoms (Contact to TB/MDR/XDR TB)
- What do you know about TB

History of other medical conditions
- e.g. diabetes, steroid dependent medication, HIV

Employment history?
- Mineworker/ ex-mineworker, Health care worker

Habitat
Geographical area, congregate setting NB; prison
Physical Examination

- Wasted
- Pale
- clubbing
- Chest abnormalities etc....

Source: Lung Health Image Library)
Anexo 5 – Algoritmo de testagem dos pacientes suspeitos de TB pela técnica de GeneXpert® MTB/RIF

Critérios para testagem com o GeneXpert

- Pacientes de TB-MDR (considerado suspeito);
- Reinadmissões;
- Caso novo, BCG+ que depois de dois meses de tratamento a baciloscopia não manteve;
- Coberturas positivas em um paciente com MRK;
- Prófetados de risco, internos, prisioneiros;
- Indivíduos com infecção por HIV ou outras imunodeficiências, diabêtes e mulheres gravides;
- Doações de processar (1º ou 2º geração), (anti-microbianos)
- Doações de processar (2º ou 3º geração), (anti-microbianos);
- Diagnóstico de TB não confirmado (leitos e sistemas);
- Doações de processar (1º ou 2º geração), (antimicrobianos)

- LPA, Line Probe Assay; Rif, Rifampicina; ESAT-6, 6.5-10.8(kg/m²) de Sensibilidade aos antibióticos;
- (+) positivo; (-) negativo; KX: PankX

Para os resultados:
- Genexpert (+)
  - Rif (+)
    - Iniciar Tratamento para MDR e pedir Cultura/LSA/PA
  - Rif (-)
    - Iniciar Tratamento de 1º linha
  - Rif Indeterminado
    - Repetir GeneXpert
- Genexpert (-)
  - Forte suspeita clínica de TB e/ou Rário R compatible
  - Análise outras causas e se alguma específica
  - Instruir tratamento de 1º linha e solicitar cultura/LSA/PA
- Indeterminado ou erro
  - Repetir GeneXpert

Local com Genexpert deverá usar como teste inicial de diagnóstico sempre que estejam presentes os critérios de testagem.

Local sem Genexpert que entrem nestas Unidades Sanitárias terão de realizar a baciloscopia como teste inicial.
- Suspeito de TB-MDR, incluindo crianças e um outro deve ser referenciado para GeneXpert, independentemente do resultado da baciloscopia, para permitir a avaliação da resistência à rifampicina, um forte indicativo de TB-MDR.
- Indivíduos com infecção por HIV ou outras imunodeficiências, diabéticos e mulheres gravides, incluindo crianças e adolescentes devem ser referenciados para GeneXpert, a menos de baciloscopia a negativa.
- Suspeito de TB meningite, o mesmo deve ser referenciado de imediato para GeneXpert, sem passar pela baciloscopia devido à baixa sensibilidade da baciloscopia para o diagnóstico de LCR e a alta variabilidade para o diagnóstico.
General Laboratory Diagnosis of Tuberculosis

1. Microscopy:
   • Mainstay of NTP (id transmitters of TB, posing a ↑ infection risk)
   • Essential for diagnosis and/or management of drug susceptible and resistant strains of TB

2. Culture:
   • Adds sensitivity to diagnosis of TB in sputum specimens with lower bacillary load (e.g. extra-pulmonary TB, HIV co-infected patients), regardless of drug susceptibility

3. Culture and drug susceptibility testing (DST)
   • Essential for diagnosis and surveillance of drug resistance (e.g. M(X)DR-TB)

4. Molecular techniques (e.g. Line probe assay, GeneXpert)
   • Rapid diagnosis of DR TB
   • Identification of mycobacterial species
AFB Smear Microscopy: Advantages

- Identifies patients most likely to transmit TB (i.e., with high pulmonary bacillary load, 5,000-10,000 bacilli per milliliter of sputum)

- Can be done at point-of-care, with short TAT of 24-48 hr (no need for advanced infrastructure)

- It is accessible to most patients (even in resource-limited settings)

- Instrumental in monitoring treatment success (patient follow-up/smear conversions)
AFB Smear Microscopy
Limitations

• low sensitivity
  – requires presence of at least 5,000 or more AFB/ml of sputum
  – worsened by non-cavitary disease as in HIV co-infected patients

• does not distinguish tubercle bacilli from other mycobacteria (most mycobacteria are acid-fast)

• does not distinguish live bacilli from dead bacilli

• cannot detect drug resistance
Culture Advantages

• more sensitive than microscopy (can detect as few as 10 bacilli per milliliter of sputum vs >5,000 required for AFB smear microscopy

➢ very useful in diagnosis of HIV infected TB patients and children, with normally low sputum bacillary load

• Allows for further identification to distinguish between tubercle bacilli and other mycobacteria (species identification)

• allows for drug susceptibility testing (diagnosis of mono-, poly-, multi-, and extensively-drug resistant TB)

• allows for epidemiological studies (e.g community outbreaks, nosocomial infections, etc)
• Live vs. dead bacilli

• **Culture is the gold-standard for the diagnosis of TB**
Culture Limitations

- long TAT due to slow growth of tubercle bacilli - Culture takes 2-6 weeks
- requires advanced infrastructure and highly trained personnel
- reagents are expensive
- limited facilities (culture coverage) in the country
- poses higher biohazard risk than microscopy to lab personnel, hence need for higher level safety measures and quality management.
# Identification of M. tuberculosis

**M. Tuberculosis complex**
- majority of mycobacterial isolates in high-TB burden countries
- niacin and nitrate tests positive

**Non-Tuberculous Mycobacteria**
- prevalence varies from country to country
- can be more common in HIV patients
- isolates may appear phenotypically resistant to first-line drugs.
- treatment is entirely different from MDR-TB
Culture results interpretation

• A **positive culture** means that Mycobacteria are present, and the patient needs TB treatment if MTB cultured.

• A **negative** culture means that MTB is absent in the sample

• **False negative cultures** may occur when MTB is killed by decontamination or if a poor specimen is sent

• A contaminated culture means that normal bacterial flora present in the sputum over-grew in the MGIT tube. Even if MTB was present, it could not be detected

• **False positive** cultures are uncommon but can occur
Drug susceptibility testing (DST)

• DST is required to make a definitive diagnosis of drug-resistant TB (DR-TB)

• It is the Gold standard in the diagnosis of drug resistant TB
DST is required to make a definitive diagnosis of DR-TB

1\textsuperscript{st} line DST

- well studied and established
- reliable and reproducible (INH & Rif)
- high correlation with clinical outcome

- Drugs currently tested (NHLS): STP, INH, RMP, EMB, PZA*

* On request

2\textsuperscript{nd} line DST

- inadequate knowledge on mechanisms of resistance
- lower reproducibility (exceptions: aminoglycosides, fluoroquinolones)
- correlation of laboratory resistance to clinical outcome not well studied

- Drugs currently tested (NHLS): ETH, OFLX, KM, CAP*

* On request
Limitations of DST

- Intrinsic accuracy of DST varies with the drug tested - or first line drugs, DST (FLD) is most accurate for rifampicin and isoniazid and less so for streptomycin and ethambutol.

- Testing of second-line drugs is not as simple as DST for the first-line drugs.

- Proficiency testing results similar to those obtained for first-line drugs are not available for any of the second-line agents.

- SLD has not been standardized internationally due to in vitro drug instability.

- Good reproducibility are for aminoglycosides, fluoroquinolones and polypeptides.

- Reproducibility and reliability of DST is much less reliable for PAS, terizidone, ethionamide and cycloserine.
What is Line Probe Assay (LPA)?

• It is a test that diagnoses TB and simultaneously detects resistance to **RIF and INH**.

• It does this by detecting the presence of the DNA of *Mycobacterium tuberculosis* in the sputum and also identifies any changes/mutations in the DNA that may cause rifampicin and/or isoniazid resistance.

• Presence of mutations is interpreted as resistance to the antibiotic in question (e.g. RIF and/or INH)
  - The test is called Genotype MTBDRplus
Instruments required for LPA

- Heating block
- Ultrasonic bath
- Thermocycler (PCR machine)
- GT Block 48
- Genoscan
Advantages of LPA (Genotype MTBDR\textit{plus})

• It detects MTB and resistance to RIF & INH at the same time
  – From the same specimen, within the same test.

• Short turnaround time for diagnosis of MDR-TB
  – Processing time for the test itself is approx. 8 hours.
  – minimum TAT is expected to be a week.

• It is specific for MTB complex, (i.e. it can differentiate MTB from other mycobacteria).
  – The test is designed to specifically detect MTB complex DNA, and not other mycobacteria
Limitations of LPA (Genotype MTBDR\textit{plus})

- Cannot be used for monitoring treatment

- It is dependent on smear results (hence smear TAT)
  - The test is done on smear positive specimens or smear-negative culture-positive samples
  - NB 2\textsuperscript{ND} generation tests able to detect MTB DNA even in smear negative samples

- Prone to contamination and human error
  - The test is multi-stepped and only partially automated (labor-intensive)
  - it requires at least 3 separate rooms for different steps

- False positive RIF / INH resistance
  - a small fraction of resistance detected may not correlate with physiological resistance (leading to discordance between LPA and conventional DST results or clinical outcome)
Interpretation of LPA results

• MTB complex positive = positive for MTB
  – RIF sensitive OR resistant
  – INH sensitive OR resistant
    • If both RIF and INH are sensitive → susceptible MTB
    • If only RIF or INH is resistant → mono-resistant MTB
    • If both RIF and INH are resistant → MDR-TB

• MTB not detected = negative for MTB - This result does not exclude TB
What is GeneXpert?

• It is an instrument that is used to conduct rapid diagnosis of tuberculosis and detection of rifampicin resistance.

• It does this by detecting the presence of the DNA of *Mycobacterium tuberculosis* in the sputum and also identifies any changes in the DNA that may cause rifampicin resistance.

  – The test is called Xpert MTB/RIF

  • *This test shares fundamental principles with the LPA (both are PCR-based; detect presence of MTB complex DNA; detect changes in the DNA that may cause RIF resistance).*
Gene Xpert MTB/RIF:

Total processing time = \[2\text{ hours}\]

Reportable result:

- Positive/negative TB
- Resistance yes/no to Rifampicin
GeneXpert Instruments

Xpert MTB/RIF

GX4

GX16

GX48 (Infinity)

16  64  200  throughput/ 8hr day

Slide adapted from FIND, 2010
Advantages of Gene Xpert

• It detects MTB and RIF resistance at the same time.
  – From the same specimen, within the same test.

• Short turnaround time.
  – Processing time for the test itself is approx. 2 hours.
  – minimum TAT is expected to be the same or less than that of smear microscopy.

• It is specific for MTB complex, (i.e. it can differentiate MTB from other mycobacteria).

• Can be used on: CSF, aspirates (gastric and lymph nodes) and tissue (e.g. pleural biopsy)

• Less prone to contamination and human error.
  – The test for each specimen is carried out in a closed system (cartridge), so there is a reduced risk of cross-contamination from other specimens.
Limitations of GeneXpert

• Cannot be used for monitoring treatment (limited to diagnosis largely)

• False positive RIF resistant
  – a small fraction of resistance detected may not correlate with physiological resistance (leading to discordance between Gene-Xpert and DST results or clinical outcome)
GXP POSITIVE/ RIF NEGATIVE

MTB is present, and sensitive to Rifampicin

• XPert MTB/RIF is sensitive and specific for detection of TB and Rifampicin resistance
• However this result does not exclude possibility of resistance to other drugs.
GXP POSITIVE/ RIF POSITIVE (RESISTANT)

MTB present

• Rifampicin resistance may be falsely positive (10%)
• Second sputum specimen must be sent for confirmatory culture and DST
GXP POSITIVE/ RIF INDETERMINATE

MTB present

• Rifampicin resistance could not be assessed

• Repeat GXP

• A second sputum may be sent for TB culture and DST to confirm susceptibility/ LPA

• Treat the patient as if they have drug-sensitive TB
ERROR

• The test failed

• Caused by problem with the cartridge, e.g. food particles

• Submit a second specimen for Xpert MTB/RIF
Turn Around Time for Culture DST vs LPA & GXP

**Culture DST**
- Raw Sputum
- Processing
- **Culture** positive
  - 7 to 42 days (6 weeks)
- **DST**
  - 6 to 10 weeks

**LPA**
- Raw Sputum
- Processing
- **LPA**
  - Smear (+): ±3 days
  - Smear (-): >7 days*

**GXP**
- Raw Sputum
- **GXP**
  - ±48 hours
Anexo 5 – Algoritmo de testagem dos pacientes suspeitos de TB pela técnica de GeneXpert® MTB/RIF

Criterios para testagem com o GeneXpert

- Suspeita de TB MR (ou evidência de resistência):
  - Tratamentos anteriores
  - Caso novo, DRC que depois de dois meses de tratamento o bacilo persiste inativo
  - Convém prosseguir em um paciente com MDR
  - Presença de síndrome rodante de TB NR
  - Profissionais de saúde, familiares, profissionais de saúde suicidas.
- Indivíduos com infecção por HIV ou outras imunodeficiências.
- Infecção em crianças menores de 6 meses.
- Tudo o que esteja em massa de TB em TBM NR, sugestões de esporos e bacilos de esporos (sistema respiratório e sistema digestivo).
- Sugestão de TB meningite, sugerindo seguimento de pacientes (LPA, Line Probe Assay; Rif, Rifampicina; EIA, Teste de Sensibilidade aos Antibioticos; - teste positivo; + teste negativo; XR, PazirK).

GeneXpert (+)

- Rif (+)
  - Iniciar Tratamento para MDR e pedir cultura/TSA ALPA
- Rif (-)
  - Iniciar tratamento de 1º linha
- Rif Indeterminado
  - Repetir GeneXpert mantendo-se determinado: iniciar tratamento de 1ª linha ou cultura/TSA/ALPA

GeneXpert (-)

- Forte suspeita clínica de TB e/ou Raro X compatível
  - Avaliar outras causas, ver algoritmos específicos
- Indeterminado ou erro
  - Repetir GeneXpert

- Locais com GeneXpert deverão usá-lo como teste inicial de diagnóstico sempre que estejam presentes os critérios de testagem.
- Locais sem GeneXpert que usam outra Unidade Sanitária para referências, deve realizar a baciloscopia como o teste inicial.
- Suspeita de TB MR, incluindo crianças, e outros deverão ser referenciados para GeneXpert independente do resultado da baciloscopia, para podermos avaliar a resistência à rifampicina, um forte indicativo de TB MR.
- Indivíduos com infecção por HIV ou outras imunodeficiências, diabéticos e mulheres gravíssimas, incluindo crianças, e outros deverão ser referenciados para GeneXpert, no caso de baciloscopia negativa.
- Suspeita de TB meningite, em outras deverão ser referenciadas de imediato para GeneXpert, sem fazer baciloscopia devido a baixa sensibilidade da baciloscopia para amostras de LCR e a forte verossimilhança para o diagnóstico.
CHEST X RAYS

- May result in over diagnosis
- Old TB?
- Depends on the skill of the reader!

- **Indications for CXR:**
  - Complications If GXP negative/ cant produce sputum and HIV positive
  - If EPTB is suspected
  - If complications of TB are suspected (pneumothorax, pleural effusions)
  - Diagnose concomitant lung disease (cancer, lung abscess, bronchiectasis, pneumoconiosis)
  - **Always interpret CXR in light of history and clinical examination**
## TB
### Regimen 1

<table>
<thead>
<tr>
<th>Pre treatment body weight</th>
<th>Intensive Phase Treatment given 7 days a week for 2 months</th>
<th>Continuation Phase Treatment given 7 days a week for 4 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RHZE (150,75,400,275)</td>
<td>RH (150,75)</td>
</tr>
<tr>
<td></td>
<td>RH (300,150)</td>
<td></td>
</tr>
<tr>
<td>30 – 37 kg</td>
<td>2 tabs</td>
<td>2 tabs</td>
</tr>
<tr>
<td>38 – 54 kg</td>
<td>3 tabs</td>
<td>3 tabs</td>
</tr>
<tr>
<td>55 – 70 kg</td>
<td>4 tabs</td>
<td></td>
</tr>
<tr>
<td>&gt; 70 kg</td>
<td>5 tabs</td>
<td>2 tabs</td>
</tr>
</tbody>
</table>
Regimen 2: For Retreatment patients

<table>
<thead>
<tr>
<th>Pre treatment body weight</th>
<th>Intensive Phase</th>
<th>Continuation Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment given 7 days a week for 2 months</td>
<td>Treatment given 7 days a week for 4 months</td>
</tr>
<tr>
<td></td>
<td>RHZE 150,75,400,275</td>
<td>RH 150,75</td>
</tr>
<tr>
<td></td>
<td>Streptomycin (g)</td>
<td>RH 300,150</td>
</tr>
<tr>
<td></td>
<td>RHZE 150,75,400,275</td>
<td>Ethambutol 400</td>
</tr>
<tr>
<td>30 – 37 kg</td>
<td>2 tabs</td>
<td>0.5</td>
</tr>
<tr>
<td>38 – 54 kg</td>
<td>3 tabs</td>
<td>0.75</td>
</tr>
<tr>
<td>55 – 70 kg</td>
<td>4 tabs</td>
<td>1.0</td>
</tr>
<tr>
<td>&gt; 70 kg</td>
<td>5 tabs</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Note: The table does not include Ethambutol dosages for body weights of 30-37 kg and 38-54 kg.*
Regimen 2 cont’d

• The basis for this regimen was that the previous treatment failures/ defaulters/ relapses have high rates of MDR-TB.

• Retreatment regimen using first line drugs is not supported by evidence derived from clinical trials

• The regimen was designed primarily for use in:
  – Settings with low prevalence of primary drug resistance
  – Patients who were previously treated with a regimen that included rifampicin for the first two months of treatment not throughout