GeneXpert for the diagnosis of Tuberculosis

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Approach to diagnosis

- Microscopy \rightarrow Culture \rightarrow susceptibility
- Molecular methods for rapid diagnosis…
- Newer Direct identification and susceptibility…
Microscopy

- ZN smear
- Fluorescent microscopy

Reported as per RNTCP guidelines.

2/18/2014
Microscopy

Rapid test

Inexpensive

* Does not allow species identification
* Not applicable to all samples

- Specificity: >95%
- Sensitivity: 25-65% (90% of infectious cases)
- Positive Predictive Value for TB depends on epidemiological situation

Excellent diagnostic test for TB control in high-incidence settings

<table>
<thead>
<tr>
<th></th>
<th>Fluorescence</th>
<th>Ziehl-Neelsen staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st AFB smear</td>
<td>80-82 %</td>
<td></td>
</tr>
<tr>
<td>2nd AFB smear</td>
<td>10-14 %</td>
<td></td>
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<tr>
<td>3rd AFB smear</td>
<td>5-8 %</td>
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TB Culture*

Advantages
• Definitive diagnosis of TB
• Increases case finding of 30-50%
• Early detection of cases
• Provide strains for DST and epidemiological studies

Disadvantages
• Complex and expensive compared to microscopy
• Requires complex handling of specimens
• Skilled technicians
• Appropriate infrastructure and biosafety levels

LIMITATIONS: need for decontamination and identification

*coverage 500.000/1000000
## Culture: solid/liquid

<table>
<thead>
<tr>
<th>solid</th>
<th>liquid</th>
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<tbody>
<tr>
<td>Low cost for reagents, not automated</td>
<td>Complex and expensive can be automated (MGIT)</td>
</tr>
<tr>
<td>Culture level infrastructure</td>
<td>Highest infrastructure and biosafety levels</td>
</tr>
<tr>
<td>Low contamination rate</td>
<td>Case finding increased 10% over solid</td>
</tr>
<tr>
<td>Long time to positivity</td>
<td>Diagnostic delay reduced to days</td>
</tr>
<tr>
<td>Colony morphology</td>
<td>ID required</td>
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<tr>
<td>ID required</td>
<td>ID required</td>
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</table>

Strip speciation tests needed for fast ID of Tbcomplex
Molecular test for speciation of most common mycobacteria
New

- MGIT 960 is a fully automated, nonradiometric liquid culture system. Based on Fluorescent principles, it is the Newer Gold standard in TB diagnosis.
Facts about TB

• Drug-resistant TB, including multidrug-resistant TB (MDR-TB, defined as resistance to at least isoniazid and rifampicin, the two most important first-line anti-TB drugs) and extensively drug-resistant TB (XDR-TB, defined as MDR-TB plus resistance to any fluoroquinolone (such as ofloxacin or moxifloxacin) and to at least one of three injectable second-line drugs (amikacin, capreomycin, or kanamycin)) has emerged as a serious threat to global health

• Worldwide, a substantial percentage (~35%) of patients with drug-susceptible TB remain undiagnosed and a staggering percentage (~85%) of patients with MDR-TB remain undiagnosed

• Accurate and rapid detection of TB, including smear-negative TB and drug-resistant TB, are critical for improving patient outcomes (increased cure and decreased mortality, additional drug resistance, treatment failure, and relapse) and decreasing TB transmission.

2/18/2014
What is GeneXpert?
• A fully automated molecular test for tuberculosis case detection and drug resistance testing, developed through collaboration in a public–private partnership

• Xpert MTB/ RIF, an automated molecular test for *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF)

• Uses heminested real-time polymerase chain reaction (PCR) assay to amplify an MTB-specific sequence of the *rpoB* gene

• Testing is carried out on the MTB/RIF test platform (GeneXpert, Cepheid), which integrates sample processing and PCR in a disposable plastic cartridge containing all reagents required for bacterial lysis, nucleic acid extraction, amplification, and amplicon detection

• The MTB/RIF cartridge is then inserted into the GeneXpert device, which provides results within 2 hours.
• A single Xpert run will provide both detection of TB and detection of rifampicin resistance

Limits of detection

• The lowest number of colony forming units per sample that can be reproducibly distinguished from negative samples with 95% confidence" (Cepheid 2009), is 5 genome copies of purified DNA per reaction or 131 colony forming units per mL in *M. tuberculosis* spiked sputum (Helb 2010).

• In comparison, to see TB bacilli by microscopic examination requires at least 10,000 bacilli per mL of sputum (Toman 2004)

• Xpert detects both live and dead bacteria
Sample Requirement

The test procedure may be used directly on clinical specimens, either raw sputum samples or sputum pellets (also called sputum sediment), samples created after decontaminating and concentrating the sputum.
Sensitivity and Specificity

Controlled Clinical Trials

1730 patients with suspected drug-sensitive or multidrug-resistant pulmonary tuberculosis were assessed

• Among culture-positive patients, a single, direct MTB/RIF test identified 551 of 561 patients with smear-positive tuberculosis (98.2%) and 124 of 171 with smear-negative tuberculosis (72.5%). The sensitivity increased to 90.2% when 3 samples were tested

• The test was specific in 604 of 609 patients without tuberculosis (Specificity 99.2%)

• As compared with phenotypic drug-susceptibility testing, MTB/RIF testing correctly identified 200 of 205 patients (97.6%) with rifampin-resistant bacteria and 504 of 514 (98.1%) with rifampin-sensitive bacteria

2/18/2014
Sensitivity and Specificity

Field Demonstration Studies

6,673 individuals prospectively enrolled in six distinctly different settings

- Single Xpert MTB/RIF test directly from sputum detecting 99% of smear-positive patients and >80% of patients with smear-negative disease

- Rifampicin resistance was detected with 95.1% sensitivity and 98.4% specificity

- Mean time to detection was <1 day for Xpert MTB/RIF, 1 day for microscopy, 17 days for liquid culture and >30 days for solid culture. Rifampicin resistance was detected in <1 day with Xpert MTB/RIF vs an average of 75 days for phenotypic DST
WHO Recommendations

The WHO evidence synthesis process confirmed a solid evidence base to support widespread use of Xpert MTB-RIF for detection of TB and rifampicin resistance. It is therefore recommended that:

1) Xpert MTB/RIF should be used as the initial diagnostic test in individuals suspected of MDR-TB or HIV-associated TB (strong recommendation);

2) Xpert MTB/RIF may be used as a follow-on test to microscopy in settings where MDR and/or HIV is of lesser concern, especially in smear-negative specimens (conditional recommendation, recognising major resource implications).
Algorithm for diagnosis of tuberculosis

Sample Type

Pulmonary

- AFB Smear
  - Positive
    - AFB- Identification and MDR screen by molecular genotyping.
      - Positive
      - Negative
  - Negative

- Resistance pattern (RIF & INH)
- May be MOTT

  If MDR

  - AFB- Identification and XDR screen by molecular genotyping

Extra Pulmonary

- AFB Smear
  - Positive
  - Negative

- Culture + TMA (rRNA testing)

  - Positive
    - AFB- Identification and MDR screen by molecular genotyping.
  - Negative
    - Negative

- Resistance pattern (RIF & INH)
  - If MDR
    - AFB- Identification and XDR screen by molecular genotyping
THANK YOU
• Email:
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