ERS Annual Congress Vienna
1–5 September 2012

Postgraduate Course 7
TB and MDR-/XDR-TB: what is new in diagnosis, treatment and follow-up

Saturday, 1 September 2012
09:30–13:00
Room: C6
**From traditional bacteriology to rapid molecular methods: the revolution is going on**

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**Aims**

This presentation is relevant to policy makers who are working or interacting with patients with drug-resistant tuberculosis. By the end of this presentation, attendees should be familiar with the various point-of-care approaches for the diagnosis of tuberculosis, including accuracy and performance outcome data on the Gene Xpert MTB RIF and HAIN MDR PLUS and second line assays. The attendees should also be familiar with the strengths and drawbacks of these approaches and how these tests should be interpreted. They should have a basic understanding of novel approaches to point-of-care diagnosis of drug-resistant TB.

**Summary**

Power point slide hand-outs: This summary should be read in conjunction with the comprehensive power point slide hand-outs that have been provided. The first part of the talk will focus on specific aspects, including sampling and analytical strategies relevant to novel and new diagnostics in individuals with drug-resistant TB. The second part of the talk will focus on how newer platforms for the diagnosis of drug-resistant TB work, their strengths and drawbacks, and how to interpret these tests. Specific applications of the test are discussed in detail. In the third part of the talk novel approaches for the point-of-care diagnosis of drug-resistant TB are discussed.

**References**


Evaluation

1. A key drawback of the Gene Xpert MTB RIF assay for determination of rapid drug susceptibility testing is:
   a. It cannot be cited at point-of-care
   b. It requires a permanent electrical supply
   c. The positive predictive value for Rifampicin resistance is suboptimal, even in many high burden countries
   d. Maintenance cost are high

2. Response rates (rate of culture conversion) in XDR-TB hospitalised patients treated with intensive therapy and appropriate drugs in high burden countries like South Africa is approximately:
   a. 50%
   b. 60%
   c. 40%
   d. 20%

3. Please mark which of the following statements is false:
   The HAIN MTB DR second line assay
   a. Has a good sensitivity and specificity when using clinical isolates
   b. Works well using smear positive clinical samples
   c. Works well using smear negative clinical samples
   d. Interrogates for resistance to fluoroquinolones and second line injectable drugs

4. Which of the following novel technologies are currently being developed for potential point-of-care use for drug-resistant TB:
   a. Point-of-care nucleic acid amplification test platforms
   b. Electrochemical detection platforms
   c. Lateral flow assay formats
   d. All of the above

Please find all answers at the back of your handout materials.
From traditional bacteriology to rapid molecular methods: the revolution is going on (September 2012; Vienna)

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Conflict of interest: none

“If TB and AIDS are a snake, then the head is in South Africa while the tail is quickly moving through other African countries… And if the head of the snake is in South Africa then the teeth are in Durban”

Dr Aaron Motsoaledi (SA minister of Health)
World TB Day, 24 March 2011

Overview

- Epidemiology of DR-TB, TB control, and the unmet need for diagnostics
- Existing reference standards, analytical strategies, and their drawbacks
- Appreciating sampling error and its implications
- NAATS: Gene Xpert MTB-RIF
  - Hain MDR TB plus (including new version)
  - Hain MDR TB sl
  - Plex ID (combination of mass spec and NAAT)
- Newer and novel approaches: HRM assay
  - MDR on a chip (array and microfluidics)
  - Sequencing
  - Lateral flow assays
- Summary and conclusions
What is the size of the problem globally?

- Worldwide 650,000 prevalent cases of MDR-TB in 2010 (5.4% of the 12 million prevalent cases)
  (490,000 new cases; 3.4% of new cases and 20% of retreatment cases)

- XDR-TB: globally ~ 25,000 XDR-TB cases annually

MDR-TB in SA

- 80% of MDR-TB results from ongoing transmission
  Streicher and Warren, Infect Gen Evol, 2011

- In Khayelitsha, Cape Town, 81% of MDR-TB likely due to primary transmission
  Cox HS, PLoS One, 2010

- Therefore any cost saving strategy that targets testing, only to those with risk factors, will miss a substantial number of cases and fail to suppress ongoing transmission

Early treatment outcomes and HIV status of patients with extensively drug-resistant tuberculosis in South Africa: a retrospective cohort study

Lancet 2010; 375: 1798-807
Published Online
May 19, 2010
DOI:10.1016/S0140-6736(10)60492-8
Dheda et. Al.
*Lancet* 2010
375: 1798-807

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Major unmet diagnostic need

- Only 7% MDR-TB reported and 1-2% actually treated to WHO standards
- Less than 2% of cases have available DST result
- Very few reference laboratories in the 22 high burden countries
- Need user-friendly rapid tools, preferably at point of care, given decentralised MDR treatment programmes

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Approach to diagnosis

Visualise the bug - LED microscopy, various techniques to concentrate the bugs (AB, magnets, spin filters)

Approach to diagnosis and new technologies


Grow the bug - liquid culture, MODS

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Diagnostic test impact

Diagnostic only one element of DR-TB control

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TB diagnosis and control in HIV-infected persons

- ~ 20% are sputum scarce
- 20% have extrapulmonary TB (paucibacillary)
- ~ 30 to 40% are smear negative - often poor quality
- Smaller volume of sputum and lower concentration of *M. tb*
- Pulmonary infiltrates atypical or chest x-ray may be normal

LOW CD4 count < 200 cells/ ml

Theron and Dheda, AJRCCM, 2011
Problem of sample acquisition

- Even with tests like Xpert about 1/3 of TB will probably remain undiagnosed without additional interventions (sputum scarce PTB, undiagnosed fraction of smear negative TB, and EPTB)

Sputum induction and bronchoscopy- only available in tertiary care facilities

What is Xpert MTB/RIF?

- Xpert is an automated real-time PCR for the diagnosis of TB and genotypic rifampicin resistance
  - WHO approved: frontline dx for individuals suspected of TB-HIV co-infection
  - SA DoH has initiated the replacement of smear by Xpert for TB suspects


Boehme C, NEJM, 2010
Gene Xpert (WHO endorsed)

- **Cost:** R1003 (Path Care)- Jan 2012
- **How good is it:** Sensitivity = 97%; Specificity = 99%
  
  User-friendly and quick. Closed system.
  
  Low inconclusive rate = 2%

How does Xpert MTB/RIF perform?

Boehme et al, NEJM, 2010 (N= 1730); Boehme et al, Lancet, 2011 (n= 5000)
Gene Xpert (WHO endorsed)

- **Interpretation:** +ve test: treat for TB. Negative test high rule-out value in uninfected but not HIV-infected persons. Theron and Dheda, AJRCCM, 2011
- **Drawbacks:** (i) PPV for DR-TB locally is sub-optimal so may be overcalling DR-TB- new cartridge being trialled (G5).
  (ii) Expensive.

Role in monitoring treatment?

GeneXpert Diagnostic Algorithm

HIV-infected persons: Studies from ARV clinic in CT (n= 468 patients)


- If no sputum- 2 induced samples
- Otherwise 1 spot and 1 induced sample
- 10% of cases no samples could be obtained
**Studies from primary care TB clinics- Cape Town**  
(n=480 with suspected TB)

<table>
<thead>
<tr>
<th></th>
<th>Patients Uninfected with HIV (n = 266)</th>
<th>Patients Infected with HIV (n = 112)</th>
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<tbody>
<tr>
<td><strong>Sputum smear</strong></td>
<td>Sens. (95% CI)</td>
<td>Spec. (95% CI)</td>
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<td></td>
<td>73.2 (62.7–81.6)</td>
<td>98.2 (90.2–100)</td>
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<td>100 (98.2–100)</td>
<td>204 of 204</td>
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<td>204 of 204</td>
<td>98.2 (94.4–99.8)</td>
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<td>69 of 82</td>
<td>100 (98.2–100)</td>
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<tr>
<td><strong>Xpert MTB/RIF</strong></td>
<td>Sens. (95% CI)</td>
<td>Spec. (95% CI)</td>
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<td>91.8 (90.4–93.2)</td>
<td>99.8 (98.3–100)</td>
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<tr>
<td></td>
<td>91.8 (90.4–93.2)</td>
<td>69 of 68</td>
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<td>68 of 68</td>
<td>96 (94.4–97.9)</td>
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<tr>
<td></td>
<td>195 of 204</td>
<td>83 (80.0–85.8)</td>
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<td>32 of 46 (P = 0.09)</td>
<td>77 of 84</td>
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NPV was 91·3% (388/426; 95% CI 88·3–93·6) in HIV-infected versus 96·0% (748/779; 94·4–97·2; P=0·001) in the uninfected; the respective negative LRs were 0·18 (0·17–0·19) versus 0·09 (0·08–0·10).

Thus, about one in ten people that have active tuberculosis will have a negative test result.

**Boehme C, Lancet, 2011**  
Multisite evaluation study  
Comment by Theron, Lancet, 2011 (letter)

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**Studies from primary care TB clinic- Johannesburg (n= 311 TB suspects)**

Scott and Stevens,  
*PLoS Med, 2011*  
Sensitivity in HIV-infected persons not significantly different from the CT study
Xpert MTB/RIF research gaps
Beyond diagnostic accuracy to patient outcomes

Early proof of concept studies

Phased demonstration and implementation studies: What is the technical feasibility? What are the short-term patient outcomes?

Diagnostic RCTs addressing long-term patient outcomes (morbidity, mortality, etc.)

Xpert RCT: Patient flow

Hypothesis: One sputum Xpert MTB/RIF performed at the point-of-treatment is feasible and will improve TB-related morbidity and patient-level costs in individuals suspected of TB who present to primary level clinics in Africa.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>HIV-uninfected</th>
<th>HIV-infected</th>
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<tbody>
<tr>
<td>Sensitivity microscopy</td>
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<tr>
<td>Xpert MTB/RIF</td>
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<tr>
<td>0.996 (95% CI)</td>
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<td>0.905 (95% CI)</td>
<td>0.953 (95% CI)</td>
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<tr>
<td>NPV 97% (95% CI)</td>
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<td>0.897 (95% CI)</td>
<td>0.956 (95% CI)</td>
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<tr>
<td>PPV 99% (95% CI)</td>
<td></td>
<td>0.930 (95% CI)</td>
<td>0.997 (95% CI)</td>
</tr>
<tr>
<td>Xpert MTB/RIF</td>
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<td></td>
</tr>
<tr>
<td>Sensitivity microscopy</td>
<td>0.999 (95% CI)</td>
<td>0.920 (95% CI)</td>
<td>0.970 (95% CI)</td>
</tr>
<tr>
<td>NPV 95% (95% CI)</td>
<td>0.914 (95% CI)</td>
<td>0.949 (95% CI)</td>
<td>0.975 (95% CI)</td>
</tr>
<tr>
<td>PPV 98% (95% CI)</td>
<td>0.950 (95% CI)</td>
<td>0.970 (95% CI)</td>
<td>0.990 (95% CI)</td>
</tr>
</tbody>
</table>

TB-NEAT study - N= 508 participants in Durban and CT (of 600 participants)
Xpert MTB/RIF sensitivity is significantly diminished in HIV infected vs. uninfected patients (p=0.04327)
### What currently available approaches can be used if Xpert is negative?

- Perform CXR (rule out test)
- Await culture
- Perform a second Xpert
- Refer for further investigation
- Hain not an option in smear negative persons
- Treat empirically for TB (commonest approach)

### How should Xpert be integrated with existing diagnostic algorithms?

- Assessed the diagnostic accuracy and/or cost-effectiveness of smear-microscopy, chest-radiography, IGRAs combined with a single Xpert-MTB/RIF assay in 480 patients with suspected TB
- Xpert negative- although CXR has poor rule-in value, it can reliably rule-out TB in approximately 1 in 4 of such cases.

Theron and Dheda, Eur Resp J, 2011

### What currently available approaches can be used if Xpert is negative?

- Perform CXR (rule out test)
- Await culture
- Perform a second Xpert (10% increase yield in SM-ve)
- Refer for further investigation
- Hain not an option in smear negative persons
- Blood culture
- Treat empirically for TB (commonest approach)
**Other applications of Xpert: Bacterial burden and infectiousness**

- Evaluated CT values in 496 patients with suspected TB
- Xpert CT values have poor rule-in
  - [cut-point ≤20.2; sensitivity 32.3%; specificity 97.1%]
- Moderately good rule-out value for smear positivity
  - [NPV 80.0%]. Thus, 20% of individuals with CT values >31.8 were smear-positive patients erroneously ruled out as smear-negatives.
- But smear status a crude proxy of infectiousness and Xpert may also detect intact but dead bugs!
- Same considerations apply to disease monitoring in MDR patients

**Bacterial burden and infectiousness**

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**Urine-orientated approach in HIV-infected sputum-scarce persons?**

- Sputum-based diagnostic unhelpful in 20-30% HIV-infected patients
- 116/242 culture +ve patients (54/116 i.e. 48% SS or SN)
- 18% (20/116) of all culture +ve were sputum scarce
- In this group (using urine) sensitivity:
  - Xpert 40% (95%CI: 22-61)
  - LAM 58% (95%CI: 49-67)
  - LAM and Xpert 68% (95%CI: 60-77) [better than either; p= 0.003]
- In 6/14 +ve urine cases, LAM was exclusively positive

Peter and Dheda, PLoS One, 2012
Urine diagnostics to target HIV-infected sputum-scarce persons

- Urine centrifugation and pelleting improved performance of Xpert in paired samples compared to unprocessed urine (42 vs 8%; p= 0.003)

Urine-orientated approach feasible when a sputum-based tests are not feasible

Peter and Dheda, PLoS One, 2012

DST: Line probe assay (Hain, InnoLip A)

- Hain MTBDRplus (version 2.0)
- In 104 smear-ve culture +ve sputum samples combined sensitivity (80%) and specificity (99%)

- Hain Lifescience GenoType® MTBDRplus
- Clinical samples (sens, spec):
  - Rif (99; 99%) INH (85; 99%)
  - Smear positive samples

Crudu V, J Clin Micro, 2012

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- Hain Lifescience GenoType® MTBDRplus
- Clinical samples (sens, spec):
  - Rif (99; 99%) INH (85; 99%)
  - Smear positive samples

Morgan M, BMC Infect Dis, 2005;
Ling D, Eur Resp J, 2008
Barnard M, AJRCCM, 2008
Hain MDR+ sl version - to be used when R resistance is noted. Rapid evaluation of drug-resistance for FQ, AG + capreomycin [SLID] and ethambutol

Total 64 sputum samples (26 DR-TB)
FQ (89%; 8/9), AG/capreo (87%; 7/8), and ethambutol (39%; 10/26); 100% specificity

Hilleman D, J Clin Micro, 2009

Miotto P, ERJ, 2012 (10 clinical samples resistant to SLIDS)

Lancoma A, JCM, 2012 (52 clinical samples resistant to SLIDS)

-N= 158 sputum samples
-In smear+ve 12% of SL indeterminate.
- In smear-ve 37% of SL were indeterminate

Thus, only useful in smear +ve samples

Here sensitivity for 2nd line agents is good. Culture DST still required to clarify type of SLID resistance
Combined approaches: PCR and mass spec (alternative to Hain and Xpert for isolate ID and DST)

- PCR for gene-specific mutations followed by R, I, E and FQ resistance; already commercially available

Why do we need new tests?

- Xpert is accurate & cost-effective but very costly (even at $10 per cartridge consume about 25% of SA NTP budget)
  - stable power supply
  - suited to centralised rather than decentralised use
  - in up to a third of cases diagnosis cannot be made (sputum scarce, EPTB, smear negative TB undiagnosed)
- Large burden of undiagnosed TB (cost and access)
- Lack of a cheap same day test (human aspect and poverty)

POC detection technologies

- NAAT with visual real-time readout including HRM
- NAAT with lateral flow readout
- NAAT with calorimetric readout (including LAMP)
- NAAT with biosensor readout (electro-chemical detection, piezoelectric quartz crystal biosensors, magnetoeleastic biosensors)
- NAAT with SERS readout
- Biochip readouts for DR TB (currently not automated and uses DNA from isolates)
  
  Park H, JCM, 2006
Several new commercial platforms that lend themselves to POC NAAT detection are now available


Bio


Integrates all the diagnostic steps needed to provide true sample-in to result-out functionality, providing a significantly shorter turnaround time than current MDx technologies while requiring minimal hands-on time and training.

Others including Enigma, Idahotech,

TWISTDX

http://www.twistdx.co.uk/products/twista/
High resolution melt and PCR

<table>
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<tr>
<th>Species-specific DNA</th>
<th>rpoB-specific DNA mutations</th>
</tr>
</thead>
</table>

- NAAT with real-time readout using melting curves of amplified DNA amplicons – high sensitivity and specificity for MDR-TB

Gold nanoparticles for detection of SNPs for rifampicin resistance using calorimetric readout

Veigas B, Nanotech, 2010

Simplified NAAT: LAMP (loop mediated isothermal amplification)

- Immediate (1 hour), isothermal, high throughput, clinical samples
- Feasible in high burden settings (hospital or central laboratories)
- Sens in sputum smear negative TB 49%
- Limited data in HIV+
- Cheaper
- Cannot multiplex

Boehme CC, J Clin Micro, 2007
USTAR- RPA


Lateral flow readout for NAAT products


Best Cassette- detection platform
EC detection platform for NAAT products

Gonzales- Diaz M, *Biosens & Bioelec*, 2005

Alternative detection technologies: Aptamers

- Aptamers, in contrast to protein-based antibodies, are simply ‘chemical’ or NA antibodies.

Aptamers and a SERS detection platform

- We have generated aptamers to TB-specific antigens (CSIR-Shooz Kathi)
- Grand Challenges Canada (J Blackburn) to develop a platform using antigen-specific aptamers and a SERS detection platform
- Surface enhanced resonance spectroscopy
Technological innovation is not enough- other challenges

- Lack of private investment because of perceived lack of return (changing rapidly)
- Need better regulatory standards for approval, and these need international harmonisation
- Variable quality of diagnostic services, need to improve quality control
- More innovation in developing countries (ANDI, Gates etc) including involvement from EDCTP, Wellcome etc
- New ways to deal with IP and patent fees
- Translation from research to policy- need streamlining of approval process and guidance for high burden settings
- Robust health care systems with good supply chain management
- Better representation in medical and nursing curricula

Summary

- Xpert- good test but expensive and suboptimal PPV for R resistance (no H, FQ, SLID readout)
- Hain MTBDRplus- main drawback is poor performance in smear negative TB (await data version 2.0)
- Hain SL- only useful in smear pos TB and does not distinguish SLIDs
- Revolution in the development of new and POC diagnostic platforms
- Major challenge is to include DR-TB readouts
- Need platforms that will give multiplex readouts – TB, HIV, pneumonia, other OI, malaria etc
- Challenge of bringing these to market and incorporating them into clinical algorithms and niche areas taking into account clinical context
### Funding Agencies:

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<thead>
<tr>
<th>EUFP7</th>
<th>Discovery</th>
<th>NIH Fogerty</th>
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<td>South African National Research Foundation</td>
<td>EDCTP</td>
<td>South African MRC</td>
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