The role of the microbiology laboratory in diagnosing tuberculosis

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ESGMYC Post-graduate Course – Singapore 2019
Tuberculosis and mycobacterial Infections: an educational update

No conflicts of interest
Role of the clinical microbiology lab

1. Assess the patient has TB by demonstrating the presence of *Mycobacterium tuberculosis* complex

2. Detect rifampicin resistance in order to decide if a treatment different from the standard regimen HRZE should be given

3. If Rmp-R, determine the susceptibility to other anti-TB drugs

4. Compare isolates in order to demonstrate transmission
Technical problems related to *Mycobacterium tuberculosis*

- Highly pathogenic (BSL3)
- Slow growth (20h vs 20 min)
- In vitro growth requires rich medium and time (specific culturing after decontamination)
- High GC content of the genome and repeated sequences
Tools for microbiological TB diagnosis

- Smear microscopy of respiratory specimens (ME)
- Direct nucleic acid amplification (NAAT/PCR)
- Culture (liquid and solid media) and identification
- Drug (antibiotic) susceptibility testing (DST)
- Molecular detection of resistance
- Genotyping

Recommendations and guidelines

• ERS-ECDC, Eur Respir J 2018; 51: 1702678
• ATS-CDC-IDSA, Clin Infect Dis 2017; 64: e1–e33
• National Institute for Health and Care Excellence (NICE) 2016
• WHO Europe 2017
• Smear-first strategy followed as classical diagnosis in Europe
• PCR –first new WHO strategy recommended in endemic countries
Strategy « smear-first » for diagnosis of MDR-tuberculosis (ECDC – ERS)

Specimen

- Smear and microscopic exam
  - NAAT (PCR)
    - Pos = MTBC
      - mutation Rif (+ Inh)
      - other genes/ resistome
  - AFB-pos
    - mutation
      - 2ndline DST
  - AFBneg
    - Culture
      - C+
        - Quick Identification
          - Drug Susceptibility Testing
            - Genotyping if needed, or for surveillance
  - C0

Strategy « PCR-first » for bacteriological diagnosis of MDR-tuberculosis (WHO- FIND)

Specimen

NAAT (PCR)

POS

NEG

Microscopic examination

AFB-pos

Rif-mutation

Isolation culture and DST

Resistome

Mutation Rif + Inh

Rif-R Neg

Culture

Genotyping if needed

Adapted from WHO publications

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Detecting the presence of *Mycobacterium tuberculosis* complex

Appropriate sampling for CM lab

**Respiratory specimens**
- 2 to 3 sputum
- Early morning samples
- Same day if needed
- at least 3 ml good quality
- if unavailable:
  - sputum induction
  - Gastric lavages
  - Bronchoscopy
  - Post bronchoscopy

**If extra-pulmonary TB**
- Sample also respiratory specimen
- Sample various body sites: biopsies, aspirates, fluids…
- Specific blood culture

Jacob ST Plos one 2013, Corbett IJTLD 2013, IDSA 2017, NICE 2016, ERS 2018
Acid Fast bacilli smear examination: improvements

- Coated slides, light fixation step
- Light-emitting diodes (LED)
- Generalization of Auramine staining
- Automated stainers

Still: Sensitivity of 10,000 to 100,000 bacilli per ml
Primary culturing

<table>
<thead>
<tr>
<th>Media</th>
<th>Smear pos</th>
<th>Smear neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid</td>
<td>14 - 21 days</td>
<td>21- 42 days</td>
</tr>
<tr>
<td>LJ, Coletsos, Middlebrook</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid*</td>
<td>5 - 10 days</td>
<td>10 - 28 days</td>
</tr>
<tr>
<td>+automated systems</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Improvements
- Standardization of the decontamination procedure (NALC-NaOH)
- Automated detection in liquid media
- Indicators for decontamination
Sub-optimal sensitivity of diagnosis tools for TB diagnosis

N per ml specimen

- 100 000 000 = 10^8
- 10 000 000 = 10^7
- 1 000 000 = 10^6
- 100 000 = 10^5
- 10 000 = 10^4
- 1 000 = 10^3
- 100 = 10^2
- 10

Microscopy

AFB-pos

AFB-neg

PCR-pos

PCR-neg

Culture-pos

Culture

C-neg

Clinics

TB case

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Low sensitivity of PCR in smear-negative but culture positive specimens

- Cochrane review
  - Sensitivity: 67% (95% CrI 60%-74%)

- LID Review Sensitivity: 75% (range: 47%-83%)
  - Extrapulmonary TB: 77% (range 25%-97%)

- WHO expert group review
  - Sensitivity: 68% (95% CrI 61%-74%)

Needs for a diagnosis tool for culture-negative TB cases

Gene Xpert® MTB/RIF Cepheid (USA) => Gene Xpert® MTB/RIF Ultra 2018

Two targets: 
*rpoB* gene (monocopy) 
+ *IS 6110* (multicopy sequence) 

=> + 10-15% in sensitivity
29 studies, 3774 specimens
Sensitivity: 71.1% (60.9% to 80.4%)
Specificity: 98.0% (97.0% to 98.8%)
Moderate-certainty evidence

Authors’ conclusions
For people with presumed TB meningitis, treatment should be based on clinical judgement, and not withheld solely on an Xpert result, as is common practice when culture results are negative.
Low positive predictive value (PPV) in extra-pulmonary TB because of 98-99% specificity

<table>
<thead>
<tr>
<th>TB forms</th>
<th>PV</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pulmonary smear-positive</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTM infection also smear-positive</td>
<td>98%</td>
<td>98%</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>to 100%</td>
<td>to 99.5%</td>
<td></td>
</tr>
<tr>
<td><strong>Pulmonary smear-negative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in occidental countries</td>
<td>2 – 5%</td>
<td>34</td>
<td>97</td>
</tr>
<tr>
<td>endemic countries</td>
<td>10%</td>
<td>to 57%</td>
<td>to 99%</td>
</tr>
<tr>
<td>screened with XRay or other test</td>
<td>30%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Extra-pulmonary specimen</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>as CSF even if screened on the basis of Leucocytes&gt;10/mm3</td>
<td>0.5%</td>
<td>8%</td>
<td>99.7%</td>
</tr>
</tbody>
</table>

PV, prevalence; NPV, negative predictive value

Tricky to PCR testing extra-pulmonary smear-negative specimens
Xpert® MTB/RIF assay for direct diagnosis of tuberculous meningitis
Kholi et al. Cochrane Database Syst Rev. 2018, issue 8

- 29 studies, 3774 specimens
- Sensitivity: 71.1% (95% CrI 60.9% - 80.4%)
- Specificity: 98.0% (95% CrI 97.0% - 98.8%)

- For a population of 1000 people where 100 have TB meningitis on culture (10% prevalence)
  - 89 would be Xpert-positive: of these, 18 (20%) would not have TB (false-positives)
  - 911 would be Xpert-negative: of these, 29 (3%) would have TB (false-negatives).
Rapid Identification of positive cultures by immunochromatography

How to use

15 min incubation

100μl of liquid culture
100μl of suspension of colonies

Control band only
Control band and Additional band for *M. tuberculosis*

BD MGIT® TBc

BIOLINE TB Ag. MPT64®

15 minutes, easy, safe and cheap

Said JCM 2011

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Speciation within the *M. tuberculosis* complex is rarely done

1. *M. tuberculosis* or *M. canetti* or *M. africanum II*

2. *M. africanum I*

3. *M. microti*

4. *M. bovis*

5. *M. bovis BCG*

6. *M. bovis caprae*
Mycobacterium tuberculosis complex

Genomics
4 411 529 bases

Species, lineages and variants

Orgeur and Brosch 2018

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Lipoarabinomannane detection in urines (antigenuria)

Positive test associated with:
Positive blood culture for MTB: OR 6.1 [3.4-11.11]
CD4 < 100/mm3: OR 7.1 [2.7-19.4]


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Molecular detection of resistance to antituberculous drugs (resistome)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Main genes involved</th>
</tr>
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<tbody>
<tr>
<td>Rifampicin</td>
<td>rpoB</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>inhA, katg codon 315</td>
</tr>
<tr>
<td>Ethionamid</td>
<td>inhA, ethA, ethR</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>embB codon 306</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>pncA</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>rpsL (43, 88), rrs region 530, gidB</td>
</tr>
<tr>
<td>Kanamycin, Amikacin</td>
<td>rrs region 1401 and 1490, eis</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>rrs region 1401 and 1490, tlyA</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>gyrA (codons 88 to 94), gyrB</td>
</tr>
<tr>
<td>PAS</td>
<td>thyA?folC? folP, dfrA</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>??</td>
</tr>
<tr>
<td>Linezolid</td>
<td>rrl</td>
</tr>
<tr>
<td>Bedaquiline</td>
<td>atpE, Rv0678</td>
</tr>
</tbody>
</table>

Data bases
Web sites, kits
https://tbdreamdb.ki.se/
www.broadinstitute.org
MUBII-TB-DB
PhyResSE
Deeplex kit
GenoType MDR plus and MDR sl

Beware of false detection of resistance where resistance prevalence is low

For 1000 patients, sensitivity of 95% and specificity of 98%

<table>
<thead>
<tr>
<th>Prevalence of resistance</th>
<th>30%</th>
<th>2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nb of resistant isolates</td>
<td>300</td>
<td>20</td>
</tr>
<tr>
<td>Nb of false resistant test</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>PPV of detection of rifampicin resistance</td>
<td>96%</td>
<td>49%</td>
</tr>
</tbody>
</table>

Confirmation with another molecular test and with phenotypic determination is mandatory in countries with prevalence MDR < 20-30%

Being able to detect heteroresistance

- heteroresistance = not 100% resistant mutant
- Genotypic methods less sensitive than phenotypic methods (1% proportion)
  - wild type allele if resistant mutant < 10%
  - Mixed genotypes between 10% and 100%
- DNA strip methods: wt and mut bands
- Sequencing: two peaks
- WGS: 50X to 100X coverage is necessary
Phenomenon of selection of resistant mutant during treatment

Start Treatment of cavitary tuberculosis

Log_{10}

Susceptible Population

Resistant mutant

Month1 Month2 Month3 Month4 Month5 Month6 Month7 Month8

0.001% R/S

0.1% R/S

1% R/S

50% R/S

99.9% R/S

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Be aware of levels of resistance

Schon 2009, Angeby 2012

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7H10, IC100 McF 0.5
Super-phenotypic drug susceptibility testing (MGIT-TBeXiST protocol)

Diagnosis of MDR-TB

Diagnosis of XDR-TB

Bottger E 2013, Cambau E 2015; Schon T. 2017, Guglielmetti L submitted
EUCAST subcommittee on Antimycobacterial susceptibility testing (AMST)

- Define a reference method for MIC determination on *M. tuberculosis*
- public consultation and EUCAST endorsement
- Release in July 2019
- 4 to 6 AMST labs comparing results
- Rationale documents on new anti-TB drugs

http://www.eucastr.org/mycobacteria/
Points for improvement of DST in tuberculosis

- Susceptibility should be predictive for cure
- Resistance should be predictive for failure

Limiting factors
- Combination therapy (2, 3, 4, 5, 9 drugs together!)
- PK/PD missing data for many antituberculous drugs
- High workload on this bacteria
- Techniques used in routine conditions need to be calibrated with regard to one reference
Conclusions

• Detection of the tubercle bacillus: possible in a well equipped microbiology lab but still some clinical cases are negative

• Detection of resistance: possible with molecular and phenotypic methods (some tricks)

• Assessment of susceptibility predictive of cure: not so easy, requires standardization for CM and also for clinical studies