Rapid Diagnosis and Detection of Drug Resistance in Tuberculosis

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Tuberculosis

- Re-emerging problem in industrialized countries
- Infections in immuno-compromised patients
- Multi-drug resistant strains (MDR-TB)
- *Mycobacterium tuberculosis* (Mtcb)
- Obligate aerobic, acid fast bacilli (AFB)
- Spread from person to person by aerosols - droplets infections
- Pulmonary tuberculosis
Diagnosis of Pulmonary Tuberculosis

- Chest X-ray
- Direct smear for AFB in sputum
  - Turn around time < 2hr
  - Low sensitivity (<50%)
- Sputum culture for *M. tuberculosis*
  - 1~4 wk (solid / liquid medium)
  - 1~2 wk (identification)
  - High sensitivity (gold standard)
Conventional Laboratory Diagnosis for Tuberculosis
Molecular Diagnosis of Tuberculosis
Roche COBAS Taqman

Abbott m2000TB
Chest X-ray

Sputum

Direct smear

Sensitivity <50%

6-8 week

Report
Culture positive for MTB

Gold Standard
Chest X-ray

Sputum

Direct smear

Sensitivity <50%

6-8 week

PCR for MTB

24 hr

Report

Culture positive for MTB

Gold Standard

Sensitivity 85% - 93%

Specificity 98% - 100%

EJCMI D 2015
DMID 2012
Int J Antimicro Ag 2010
DMID 2004
J Clin Micro 2004
J Clin Micro 1997
Clinical Impact

- Polymerase Chain Reaction (PCR) for *Mycobacterium tuberculosis* is a rapid and reliable method in the diagnosis of tuberculosis, which allows early initiation of anti-tuberculosis therapy and management of patients.

*J Clin Pathol. 2004*
Reporting format

Sample 1 (sputum):
- *M. tuberculosis* DNA detected (not detected) by PCR
- Not for monitoring treatment progress

Sample 2 (body fluids, tissues & wound swabs):
- Result indeterminate due to presence of PCR inhibitors

Sample 3 (histological sections):
- *M. tuberculosis* DNA is often truncated due to formalin fixation, leading to false negative PCR result
Multidrug Therapy for Tuberculosis

3 - 9 months treatment of 2-3 primary drugs:

- Rifampin
- Isoniazid
- Streptomycin
- Ethambutol
- Pyrazinamide
- Fluoroquinolone
Cepheid’s GeneXpert® System

Rapid Diagnosis of:

1) *M. tuberculosis*
2) Resistance to Rifampicin
3) $$$
Hain System (PCR-Reverse hybridization)
Rifampin

- an effective anti-tuberculosis agent
- a surrogate marker of Multidrug-resistant tuberculosis (MDR-TB)
- rapid detection is important for the treatment and control of tuberculosis
- Resistance caused by the mutation in \textit{rpoB} gene
RNA Polymerase β Subunit Gene

157bp (hot point mutations region)
Extensively Drug Resistant Tuberculosis (XDR-TB)

Definition by WHO (October 2006):

- Resistance to at least **isoniazid** and **rifampin** among first-line anti-TB drugs
- Resistance to any **fluoroquinolone**, and resistance to at least one second-line injectable drug (amikacin, capreomycin, or kanamycin)
- High mortality rate (USA)
- Highly associated with HIV+ patients (USA)
Fluoroquinolones resistance in MTB

- DNA gyrase (Quinolone resistance-determining region - QRDR) - Ofloxacin

- Most gyrase A missense mutations were found at positions 90, 91, and 94 that were located within QRDR.
  - Significant increase in MIC (>4.8µg/ml).
Direct detection of XDR-TB from **Sputum**
[culture confirmation : INH$^R$; Rif$^R$; OFX$^R$]
Direct detection of XDR-TB from Sputum
[culture confirmation: INH\textsuperscript{R}; Rif\textsuperscript{R}; OFX\textsuperscript{R}]

Wild type: Ala\textsubscript{90}, Asp\textsubscript{94}

Hotspot mutations: 90Val, 94Gly\textsubscript{e}

C+T\textsubscript{e}

Ala+Val\textsubscript{e}

90\textsubscript{e}

A+G\textsubscript{e}

Asp+Gly\textsubscript{e}

94\textsubscript{e}

PL215\textsubscript{e}

Direct specimen

OFX MIC: 16 \text{ug/ml}

Ala\textsubscript{90}Val\textsubscript{e}

Asp\textsubscript{94}Gly\textsubscript{e}
Sputum Direct smear

< 1 hr

Sensitivity <50%

6-8 week

Report
Culture positive for MTB with susceptibility test result

2 week

Gold Standard
Sputum

Direct smear

- katG MAS-PCR
- rpoB PCR sequencing
- gyrA PCR sequencing

Sensitivity <50%

6-8 week

~5 days

Report

Culture positive for MTB with susceptibility test result

J Antimicrob Chemother 2011 66(4)
Diagn Microbial Infect Dis 2011 69(1)
Antimicrob Agents Chemother 2011 55(2)
Int J Antimicrob Agents 2010 35(2)
Known mutations associated with Rifampin and Ofloxacin resistance

**Rifampacin**

- *rpoB (hot point mutations)*
- Mutations at positions 516, 526, and 531

**Fluoroquinolones**

- *gyrA (hot point mutations)*
- Mutations at positions 90, 91, and 94
Novel mutations associated with Rifampin and Ofloxacin resistance

Rifampin MIC = 16 - 64 ug/ml

*rpoB* (*hot point mutations*)

V146F  
I572F

Ofloxacin MIC = 8 ug/ml

*gyrA* (*hot point mutations*)

A74S

Clone the mutated *rpoB* gene into pOLYG and transformed in *M. tuberculosis* H37Ra.
Reporting format

- **Susceptible ?:**
  - Known mutation associated with resistance to Isoniazid/Rifampin/Fluoroquinolones NOT detected

- **Resistance :**
  - Mutation in *katG* gene associated with resistance to Isoniazid detected at *S315T*
  - Mutation in *rpoB* gene associated with resistance to Rifampin detected at *S531L*
  - Mutation in *gyrA* gene associated with resistance to Fluoroquinololones detected at *A94G*
Rapid Diagnosis of Tuberculosis

- Cepheid (TB + Rif) 3 hours ➢ $$$$$
- Hain (TB + Rif+INH+OFX+AMI+EMB) 1 day ➢ $$$$$$
- HKU /QMH (in-house protocol)
  - TB qPCR 1 day ➢ $
  - qPCR + (~15%) ➢ $
  - DNA sequencing (Rif+INH+OFX) ~3-5 days
Summary

- PCR provides rapid diagnosis of *M. tuberculosis*
  - Early initiation of anti-TB therapy
  - Effective public health control

- MAS-PCR, PCR-sequencing provide rapid diagnosis of Rifampin, Ofloxacin and Isoniazid resistant *M. tuberculosis* (MDR-TB)

- Molecular diagnosis cannot replace conventional TB Laboratory practice

- Massive parallel sequencing or next-generation sequencing (NGS) to improve sensitivity
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