Rapid Diagnosis and Detection of Drug Resistance in Tuberculosis

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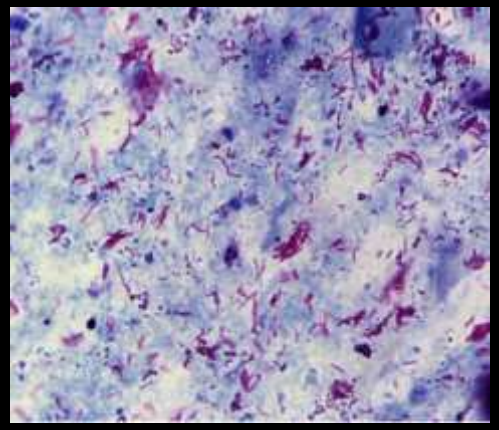
Tuberculosis

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

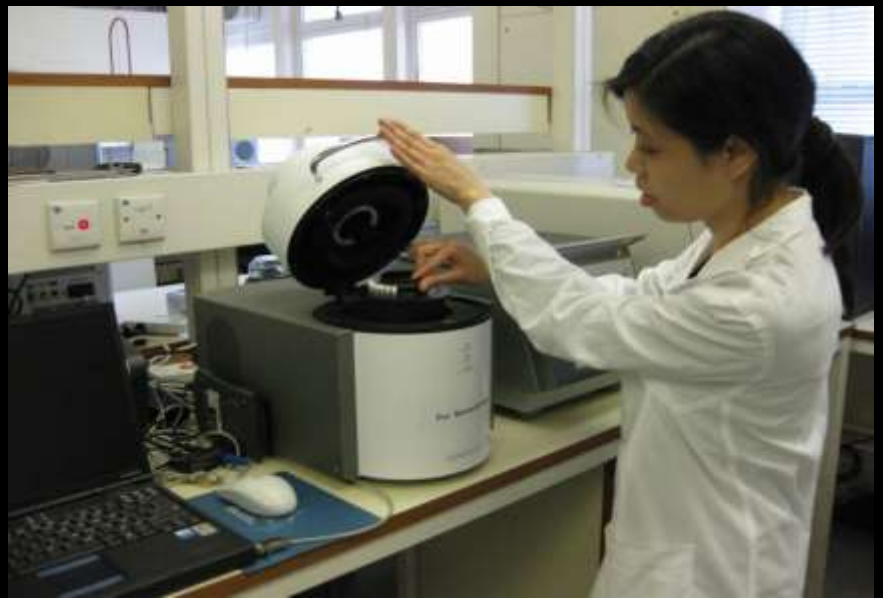
Diagnosis of Pulmonary Tuberculosis

- 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

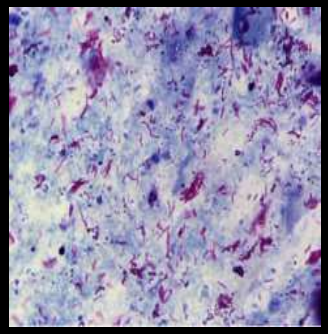
Abbott m2000TB

Chest X-ray

Sputum

< 2 hr

Direct smear



Sensitivity <50%

6-8 week

Gold Standard

Report

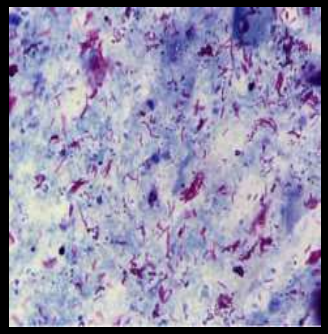
Culture positive for MTB

Chest X-ray

Sputum

< 2 hr

Direct smear



Sensitivity <50%

6-8 week

Gold Standard

PCR for MTB

24 hr

Sensitivity 85% - 93%

Specificity 98% - 100%

Report

Culture positive for MTB

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

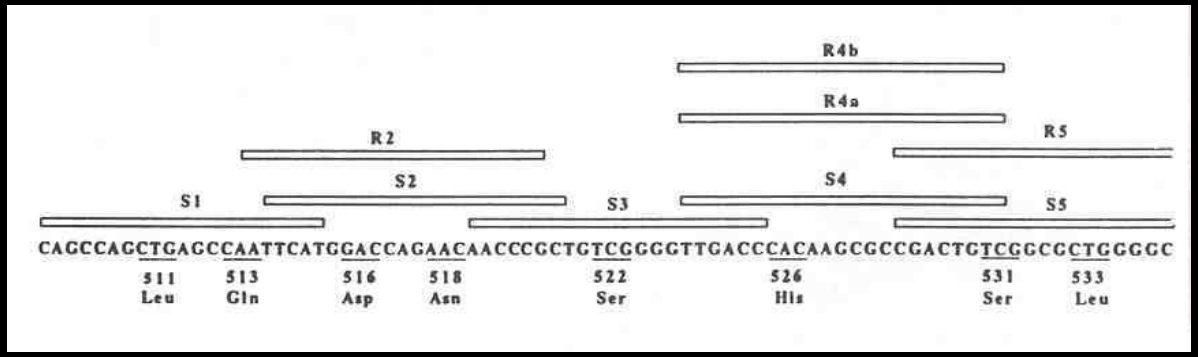

100
STR

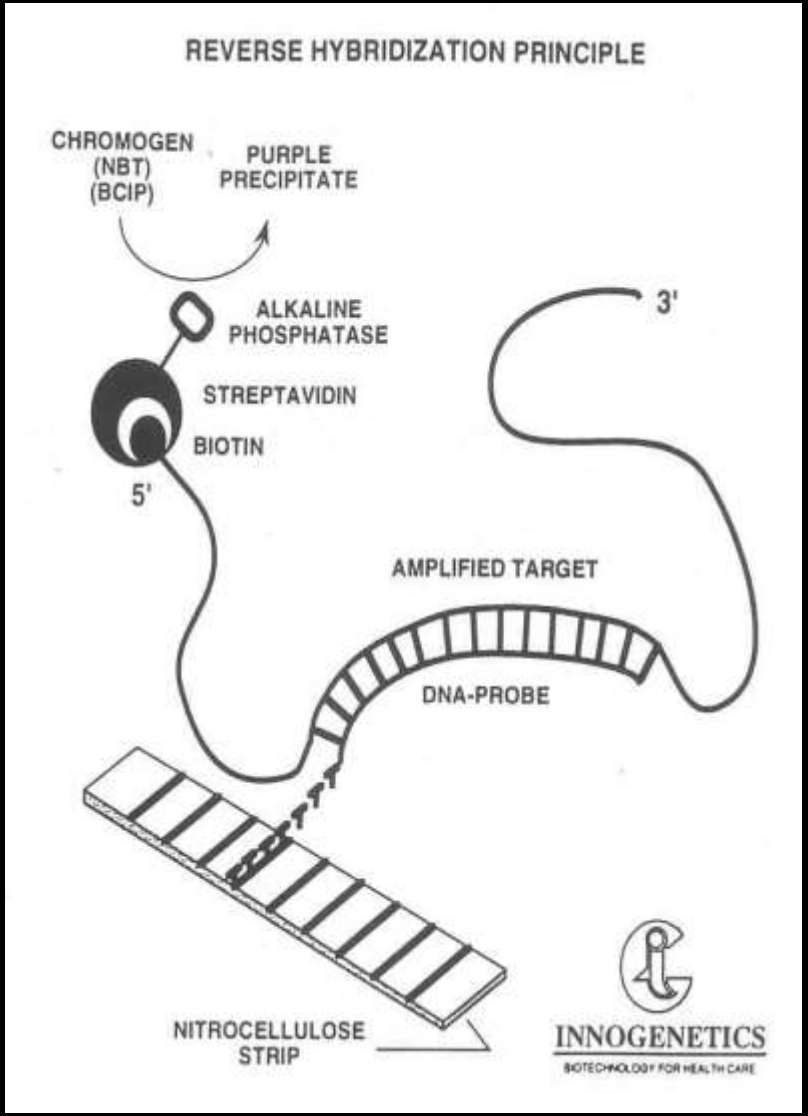
Cepheid's GeneXpert® System

Rapid Diagnosis of:

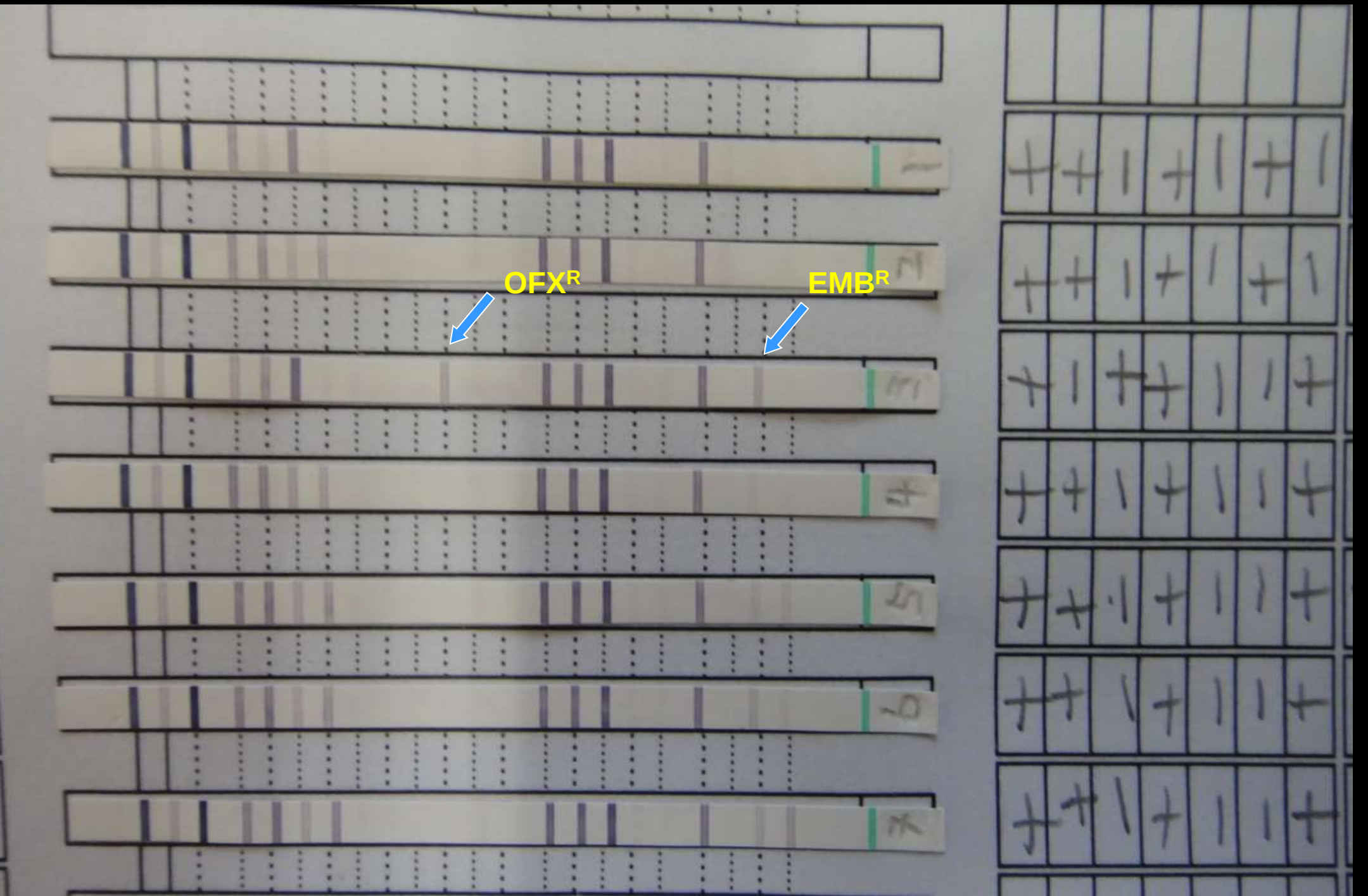
1) *M. tuberculosis*
2) Resistance to Rifampicin
3) $$$

Hain System (PCR-Reverse hybridization)

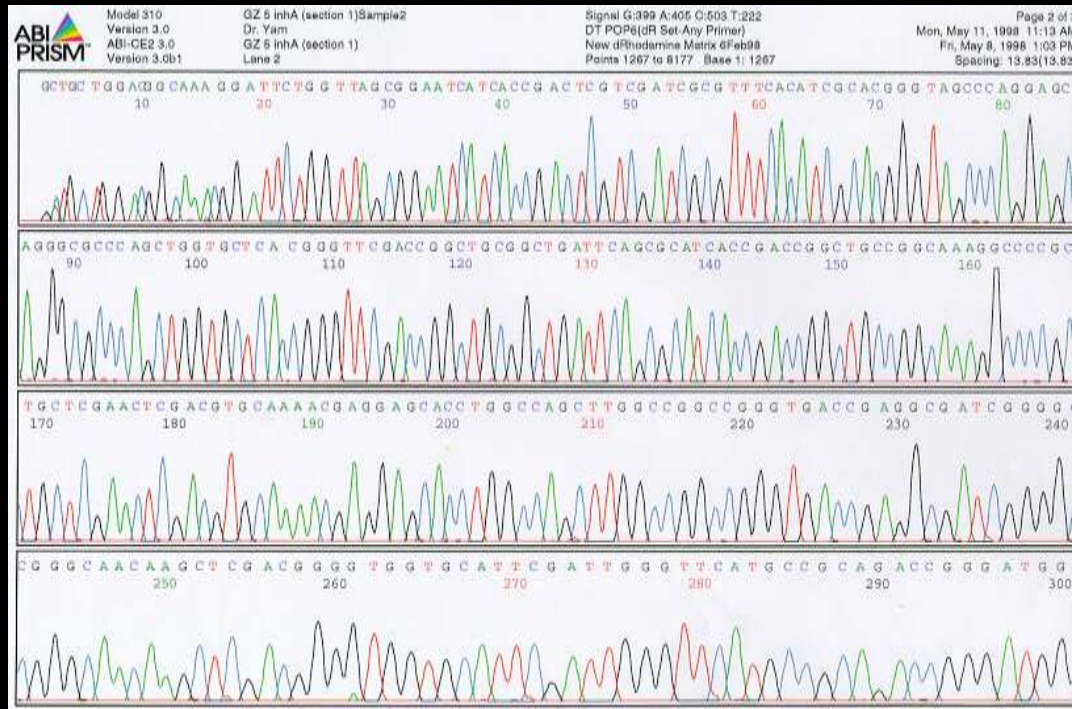




CC
AC
TUB
rpoB WT1
rpoB WT2
rpoB WT3
rpoB WT4
rpoB WT5
rpoB WT6
rpoB WT7
rpoB WT8
rpoB MUT1
rpoB MUT2A
rpoB MUT2B
rpoB MUT3
katG WT
katG MUT1
katG MUT2
inhA WT1
inhA WT2
inhA MUT1
inhA MUT2
inhA MUT3A
inhA MUT3B
M
INH^R
Rif^R


OFX^{R}
EMB^{R}


DANGER

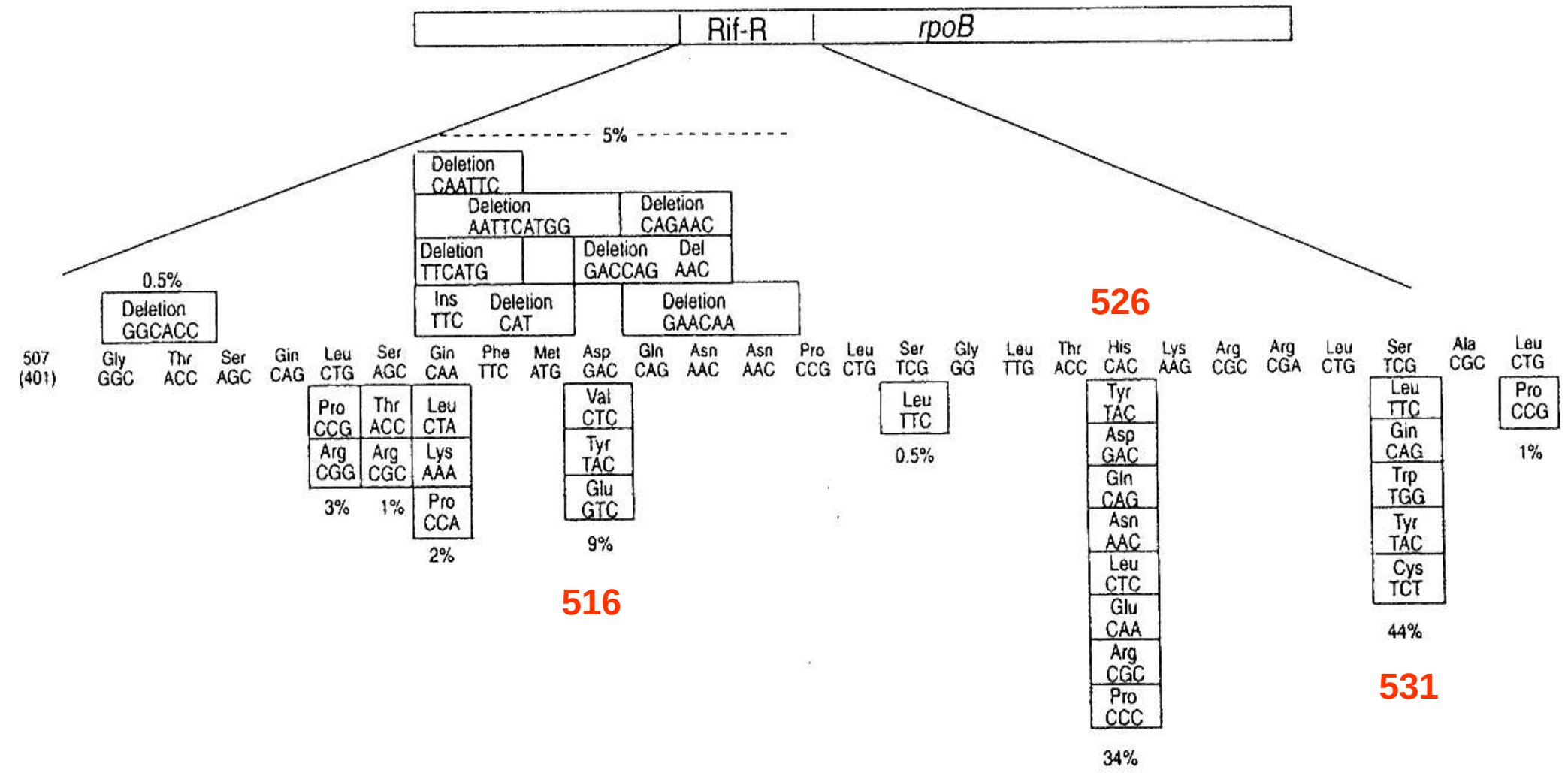

ABI PRISM
Model 310
Version 3.0
ABI-CE2 3.0
Version 3.0b1
GZ 5 inhA (section 1)Sample2
Dr. Yam
GZ 5 inhA (section 1)
Lane 2
Signal G:399 A:405 C:503 T:222
DT POP6(dR Set-Any Primer)
New dRhodamine Matrix 6Feb98
Points 1267 to 8177 Base 1: 1267
Page 2 of
Mon, May 11, 1998 11:13 AM
Fri, May 8, 1998 1:03 PM
Spacing: 13.83(13.83

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 *rpoB* gene

RNA Polymerase β Subunit Gene

157bp (hot point mutations region)



Extensively Drug Resistant Tuberculosis (XDR-TB)

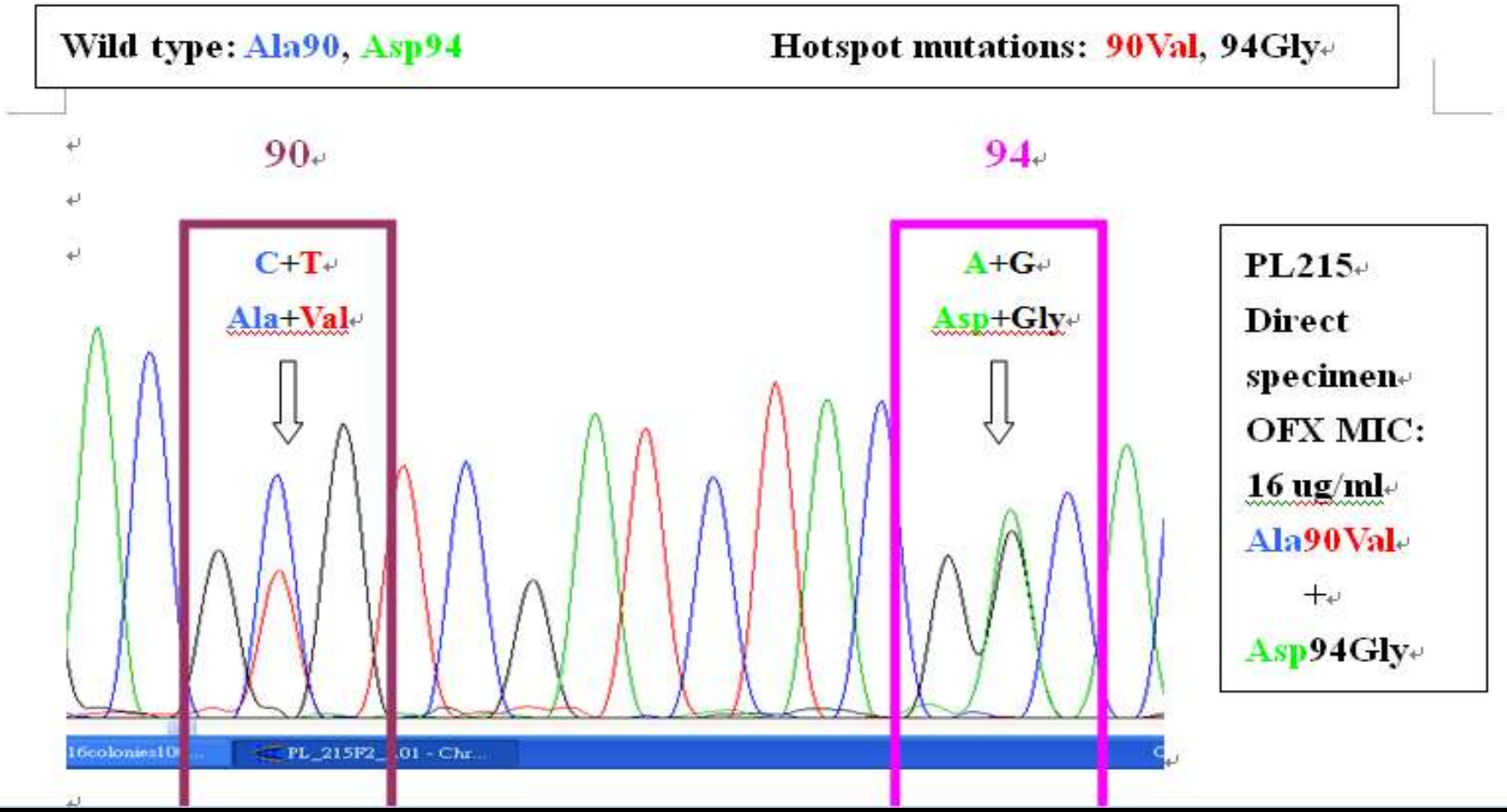
Definition by WHO (October 2006):

- 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]



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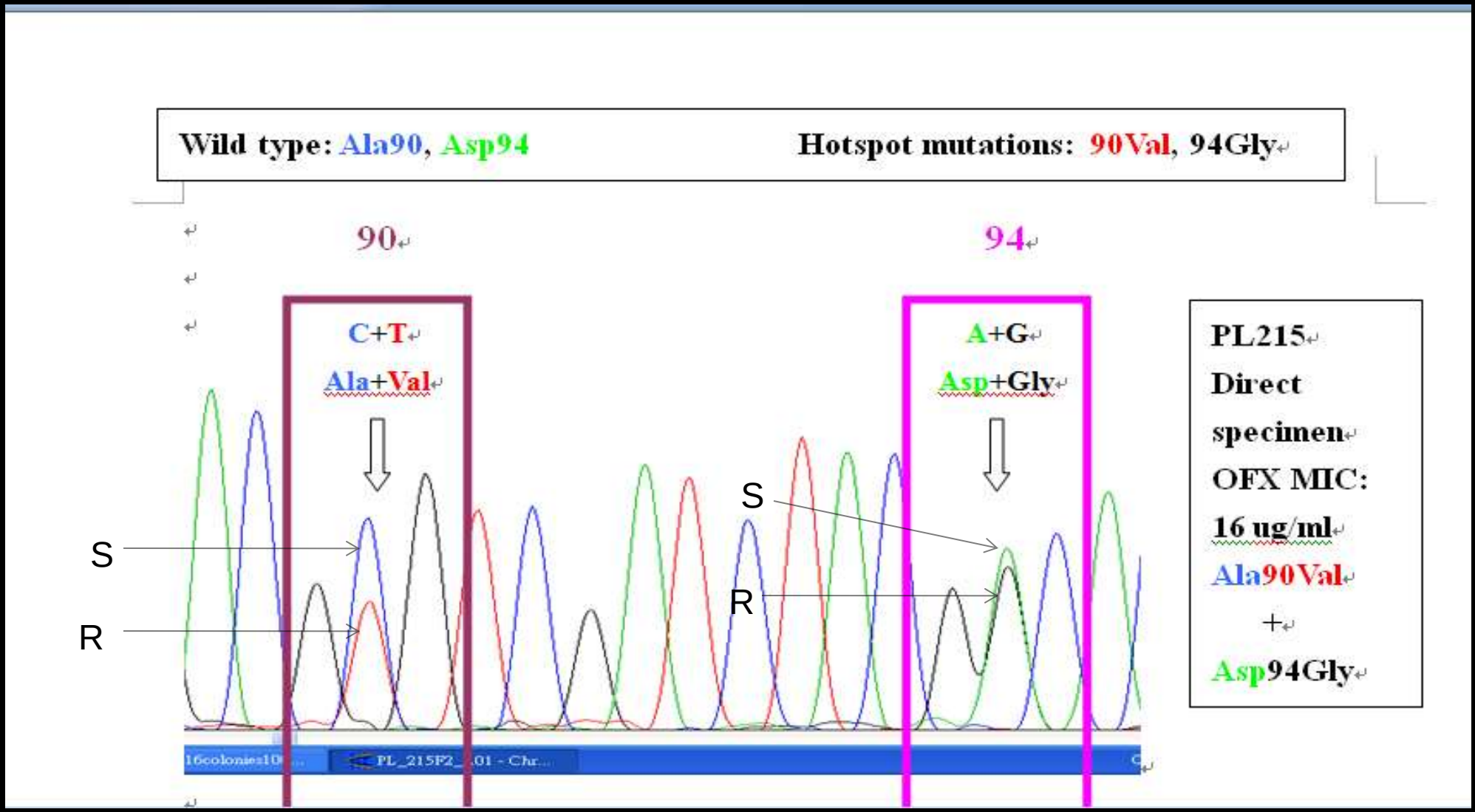
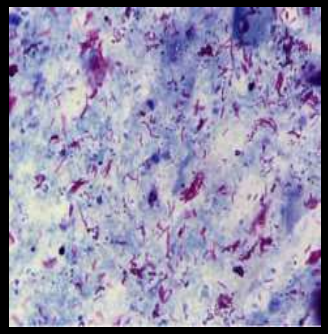


Sputum

< 1 hr

Direct smear



Sensitivity <50%

6-8 week

Gold Standard

2 week

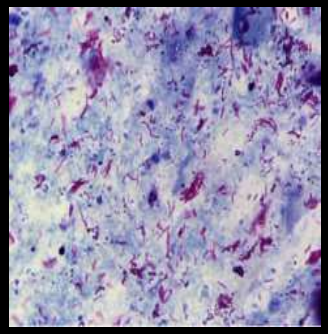
Report

Culture positive for MTB with susceptibility test result

Sputum

< 2 hr

Direct smear



Sensitivity <50%

6-8 week

Gold Standard

2 week

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

~ *5 days*

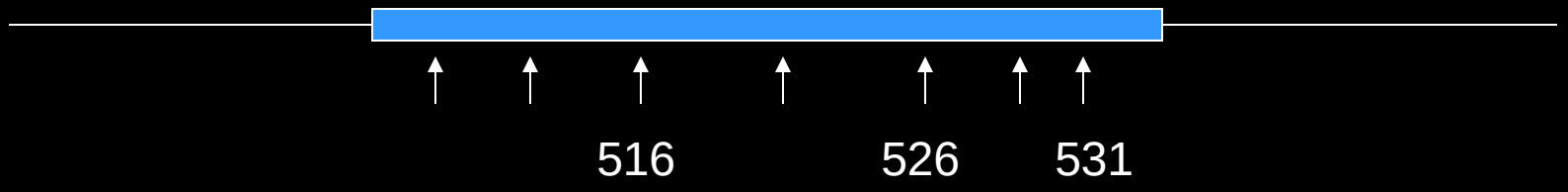
J Antimicrob Chemother 2011 66(4)
Diagn Microbial Infect Dis 2011 69(1)
Antimicrob Agents Chemother 2011 55(2)
Int J Antimicrob Agents 2010 35(2)

Report

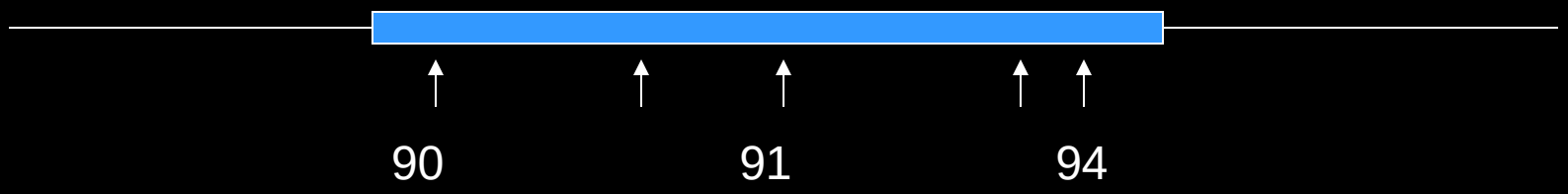
Culture positive for MTB with susceptibility test result

Known mutations associated with Rifampin and Ofloxacin resistance

Rifampacin *rpoB (hot point mutations)*



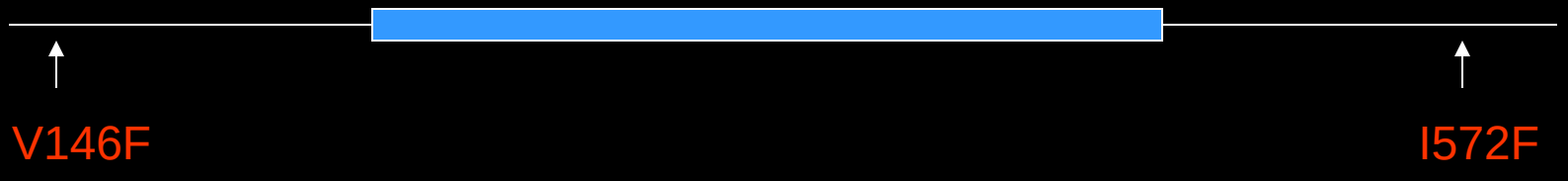
Fluoroquinolones *gyrA (hot point mutations)*



Novel mutations associated with Rifampin and Ofloxacin resistance

Rifampin MIC = 16 - 64 ug/ml

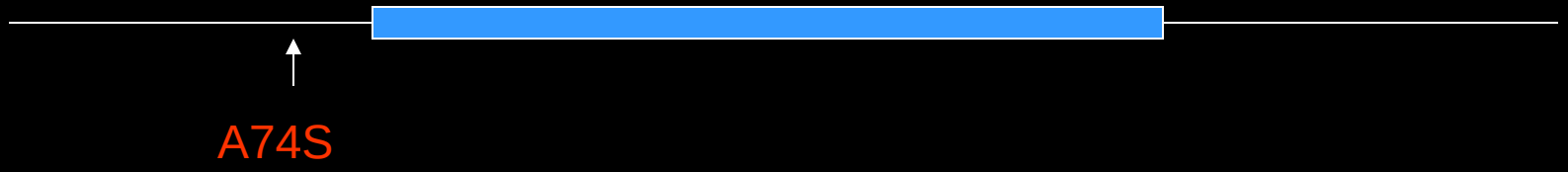
rpoB (hot point mutations)



J Antimicrob Chemother 2011 66(4)

Ofloxacin MIC = 8 ug/ml

gyrA (hot point mutations)



Antimicrob Agents Chemother 2011 55(2)

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 Fluoroquinolones 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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