Molecular diagnosis of TB and drug resistant TB: In-house development

Angkana Chaiprasert, Dr.rer.nat.
Therdsak Prammananan, Dr.rer.human.biol.
Manoon Leechawengwong, M.D.
Department of Microbiology
Faculty of Medicine Siriraj Hospital
Mahidol University
Objectives

- To share our past experience in developing one-tube nested PCR for direct detection of *M. tuberculosis* complex in clinical samples
- Developing multiplex PCR for identification of *M. tuberculosis* and *M. tuberculosis*
- Developing multiplex allele-specific (MAS) PCR for detection of rifampicin resistant *M. tuberculosis*
One-tube nested PCR kit
M. tuberculosis 16S rDNA

PCR products

16SOL/OR 555 bp
16SIL/IR 306 bp
In House PCR for Rapid Detection of *Mycobacterium tuberculosis* complex

**PRINCIPLE:**

1. *dNTP*
2. PCR buffer
4. Primers

DNA extraction → Physical rupture tech. → rDNA → DNA Thermal-cycler

1 cycle

$2^n$ (n = number of cycle)

Denaturation step
Annealing step
Extension step
5 *M. bovis* and 11 *M. tuberculosis* isolates
### DETECTION LIMIT

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>91.9 %</td>
<td>95.0 %</td>
</tr>
<tr>
<td>Bronchoalveolar lavage</td>
<td>70.0 %</td>
<td>84.0 %</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>&lt;10.0 %</td>
<td>&gt;95.0 %</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>~20.0 %</td>
<td>&gt;85.0 %</td>
</tr>
</tbody>
</table>

In House PCR 16S rRNA          Turnaround time 1 wk

Reference:
Training Workshop sponsored by Biotec, Thailand
The test is used at Fact. of Medicine Siriraj and ISO 15189 accredited by Bureau of Laboratory Quality Standards MOPH

- **Retrospective analysis from 27.12.2006-12.9.2008**  4,972 samples tested

- **Smear + Culture + PCR +**  266 sample
- **PCR -**  18 sample
- **Smear - Culture + PCR +**  290 sample
- **PCR -**  279 sample
- **Smear - Culture - PCR -**  4,101 sample
- **PCR +**  18 sample

- **sensitivity for sm+**  92.6%  sm-  51%
- **specificity for all**  99.6%
Multiplex PCR kit
Molecular Identification of Mycobacteria by “home brew” kit or tests

Identification of *M. tuberculosis* and TB complex by multiplex PCR based on KS₄ and *mtp₄₀*

**Positive culture**

- Crude DNA
  - Boil in TE for 20 min.
  - multiplex PCR
  - Gel electrophoresis

- AFB +ve
Using In-house PCR with specific primer

- **Specific primers from:**
  - KS4, and *mtp40*

- **Able to identify:**
  - *M. tuberculosis* (1,3,4)
  - *M. tuberculosis* complex (2)
  - NTM (5)

- **Detect DNA band by electrophoresis**

**Q.C. with known species (+/- control)**  
**TAT: 1-2 d**

Sensitivity 98%, Specificity 99%

Reference:
The test is used at Fact. of Medicine Siriraj and ISO 15189 accredited by Bureau of Laboratory Quality Standards MOPH

- Retrospective analysis from 01.12.2005-01.05.2007: 3,654 LJ/M7H10 cultures tested:
  - *M. tuberculosis* 2997 isolates
  - Tuberculosis complex 381 isolates
  - Nontuberculous mycobacteria 276 isolates

- Turn around time (TAT) 1 working day
- Price per test (reagent cost) 1 US$
Press release in 2003
Development of a multiplex nested PCR for detection of rifampicin resistant *M. tuberculosis*
Amplified products of the simplex PCR with the primer, RPOR-1
The MAS-PCR of the strains with the known mutations

Sensitivity 94.2%, Specificity 100%, PPV 100%, NPV 95%
Acknowledgements:

National Center for Genetic Engineering and Biotechnology, Thailand

Faculty of Medicine Siriraj Hospital, Mahidol University

Drug - Resistant Tuberculosis Fund under the Patronage to the late Her Royal Highness Princess Galyani Vadhana Krom Luang Naradhiwas Rajanagarindra
Thank you for your kind attention

This is our team