Recent Advances in Rapid Laboratory Diagnosis of Tuberculosis

Dr. WC Yam  MRCPath (UK), PhD (HK), FIBMS (UK), CIBiol (UK)
Clinical Bacteriologist, Department of Microbiology, Queen Mary Hospital, The University of Hong Kong

Introduction

Despite continuous effort in monitoring and treatment of tuberculosis, the disease remains a major public health issue. According to the WHO, there were 8.8 million new cases of tuberculosis globally in 2003 with an expected 1% increase annually. Rapid diagnosis and appropriate therapies become the first priorities in controlling the growing epidemics. In Hong Kong, despite universal BCG vaccination of all infants, and routine use of DOTS for patients receiving antituberculosis treatment, more than 6,000 cases of tuberculosis were reported annually. The bedside decision on the initiation of anti-tuberculous drug therapy are based on epidemiologic, clinical, radiolographic, and/or histological findings, which can generally be supported by a rapid microbiologic test, commonly a positive acid-fast bacilli (AFB) smear result. However, AFB smear is positive in only half of patients with subsequently culture positive for Mycobacterium tuberculosis. Although the sensitivity of the smear is improved by fluorescent staining, the test fails to distinguish between tuberculous and nontuberculous mycobacteria. Mycobacterial culture with the Lowenstein-Jensen (LJ) medium after decontamination and concentration is the traditional method for identification, but it takes at least 3 weeks to allow for sufficient growth for biochemical or genotypic confirmation. The BACTEC MGIT 960 culture system, which uses the modified Middlebrook 7H9 broth and a fluorescent signalling system, allows for earlier detection of growth, but it still takes at least 10 days.

Recent surveys also reveal that drug-resistant tuberculosis is still ubiquitous and alarmingly high in several countries. The situation is further complicated by the emergence of multi-drug resistant tuberculosis (MDR-TB). MDR-TB results from improper administration of antibiotics in chemotherapies of TB patients and is recognised as Mycobacterium tuberculosis resistant to at least isoniazid (INH) and rifampin (RIF), the two most common first-line anti-tuberculosis drugs. Anti-mycobacterial susceptibility testing requires an additional 2 weeks before reports are issued.

Rapid Diagnosis of Mycobacterium tuberculosis

Since the introduction of nucleic acid amplification assays into the arena of diagnostic mycobacteriology, many publications have confirmed the sensitivity and specificity of fully manual in-house and commercial assays (BDProbeTec ET, Becton Dickinson; COBAS AMPLICOR, Roche; Amplified Mycobacterium Tuberculosis Direct Test AMTDT; Gen Probe, USA). Our centre routinely performs in house IS6110 and ROCHIE Polymerase Chain Reaction (PCR) assays for molecular diagnosis and monitoring therapy of pulmonary tuberculosis. The diagnosis of extrapulmonary TB is complicated by the difficulty in obtaining adequate material for examination. Tuberculous pleuritis, pericarditis, and meningitis have been associated with low number of organisms but high mortality. Microscopic examination of fluid or tissue is rarely positive and culture yield is also low. Therefore, a sensitive, rapid and accurate test would be of tremendous benefit in the diagnosis of extrapulmonary TB. Recently, our PCR assays have been modified and extended to detect Mycobacterium tuberculosis in extra-pulmonary specimens with satisfactory results.

In MTB, monoresistance to RIF is rare and at least 90% of all RIF-resistant clinical isolates are also resistant to INH. Hence, a positive result for RIF resistance would be useful as a strong surrogate of MDR-TB. In resistant isolates, it has been shown that up to 95% RIF resistance is caused by mutations in the rpoB gene encoding the RNA polymerase β-subunit. A rapid assay based on PCR and sequencing of the rpoB gene for direct detection of RIF-resistant MTB in clinical isolates and respiratory specimens was developed in our centre. Turn-around-time for conventional and molecular diagnosis of Mycobacterium tuberculosis is compared in Table 1. Due to the significant costs of PCR and sequencing, a molecular strategy based on identification of Mycobacterium tuberculosis by IS6110 PCR or ROCHIE PCR, followed by rpoB PCR-DNA sequencing for direct detection of RIF resistance in respiratory specimens is depicted in Figure 1.
Table 1. Comparison of turnaround time and sensitivity for laboratory diagnosis of *Mycobacterium tuberculosis*

<table>
<thead>
<tr>
<th></th>
<th>Turn-around time</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular Diagnosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFB smear</td>
<td>2 hours</td>
<td>&lt;50%</td>
</tr>
<tr>
<td>LJ culture</td>
<td>2-8 weeks</td>
<td></td>
</tr>
<tr>
<td>MGIT 960 culture</td>
<td>1-6 weeks</td>
<td></td>
</tr>
<tr>
<td>Mycobacterial culture</td>
<td>2-3 weeks</td>
<td></td>
</tr>
<tr>
<td>identification and drug</td>
<td></td>
<td></td>
</tr>
<tr>
<td>susceptibility testing</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Conventional Diagnosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LJ culture</td>
<td>4-5 hours</td>
<td>86-92%</td>
</tr>
<tr>
<td>MGIT 960 culture</td>
<td>4-6 hours</td>
<td>85-95%</td>
</tr>
<tr>
<td>BD ProbeTec™</td>
<td>5-6 hours</td>
<td>78-97%</td>
</tr>
<tr>
<td>Gen Probe AMTDT™</td>
<td>18 hours</td>
<td>88-94%</td>
</tr>
<tr>
<td>ROCHE COBAS™</td>
<td>72 hours</td>
<td>92%</td>
</tr>
<tr>
<td>In-house IS6110 PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>rpol</em> PCR sequencing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Model for the implementation of PCR-based detection of rifampin and isoniazid resistant *Mycobacterium tuberculosis* in respiratory specimens

- Respiratory specimens from patients with suspected TB
  - Molecular Diagnosis
  - Conventional Diagnosis
  - AFB smear
  - LJ culture
  - MGIT 960 culture
  - Mycobacterial culture identification and drug susceptibility testing
  - BD ProbeTec™
  - Gen Probe AMTDT™
  - ROCHE COBAS™
  - In-house IS6110 PCR
  - *rpol* PCR sequencing for RIF resistance

- Respiratory specimens from patient with suspected or confirmed TB
  - AFB smear
  - IS6110 or Roche PCR if indicated
  - Smear and PCR both negative
  - Culture positive for MTB
  - Risks for MDR-TB present:
    1. Recurrent tuberculosis
    2. Previously treated cases
    3. Treatment default
    4. Irregular prior treatment
    5. Smear positivity at 2 months of therapy
  - *rpol* PCR-sequencing directly on respiratory specimens or on culture isolates

References: