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

Dr. WC Yam

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, radiographic, 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*

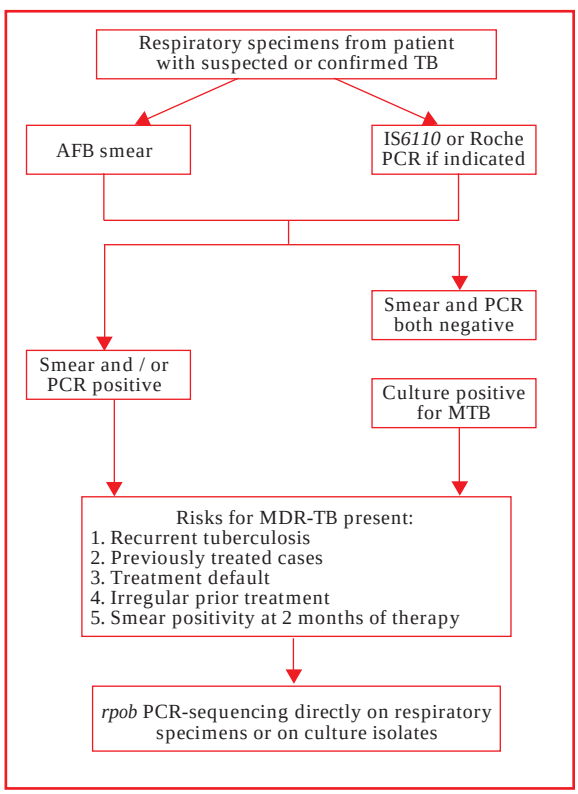
Since the introduction of nucleic acid amplification assays into the arena of diagnostic mycobacteriology, many publications have confirmed the sensitivity and specificity of fully manual in-house and commercial assays (BDProbeTec ET, Becton Dickinson; COBAS AMPLICOR, Roche; Amplified Mycobacterium Tuberculosis Direct Test AMTDT ; Gen Probe, USA). Our centre routinely performs in house IS*6110* and ROCHE 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 to 98% RIF resistance is caused by mutations in the *rpoB* gene encoding the RNA polymerase b-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 IS*6110* PCR or ROCHE 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*

Respiratory specimens from patients with suspected TB		Turn-around time	Sensitivity	
Molecular Diagnosis	Conventional Diagnosis			
	AFB smear	2 hours	< 50%	8-10 weeks
	LJ culture	2-8 weeks		
	MGIT 960 culture	1-6 weeks		
	Mycobacterial culture identification and drug susceptibility testing	2-3 weeks		
BDProbeTec™		4-5 hours	86-92%	4 days
GenProbe AMTDT™		4-6 hours	85-95%	
ROCHE COBAS™		5-6 hours	78-97%	
In-house *IS6110* PCR		18 hours	88-94%	
rpob PCR sequencing for RIF resistance		72 hours	92%	

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



References:

1. Bogard M, Vincelette J, Antinozzi R, Alonso R, Fenner T, Schirm J, Aubert D, Gaudreau C, Sala E, Ruiz-Serrano MJ, Petersen H, Oostendorp LA, Burkardt H. Multicenter study of a commercial, automated polymerase chain reaction system for the rapid detection of Mycobacterium tuberculosis in respiratory specimens in routine clinical practice. *Eur J Clin Microbiol Infect Dis.* 2001 Oct;20(10):724-31.
2. Goessens WH, de Man P, Koeleman JG, Luijendijk A, te Witt R, Endtz HP, van Belkum A. Comparison of the COBAS AMPLICOR MTB and BDProbeTec ET assays for detection of Mycobacterium tuberculosis in respiratory specimens.*J Clin Microbiol.* 2005 Jun;43(6):2563-6.
3. Lemaitre N, Armand S, Vachee A, Capilliez O, Dumoulin C, Courcol RJ. Comparison of the real-time PCR method and the Gen-Probe amplified Mycobacterium tuberculosis direct test for detection of Mycobacterium tuberculosis in pulmonary and nonpulmonary specimens. *J Clin Microbiol.* 2004 Sep;42(9):4307-9.
4. Levidiotou S, Vrioni G, Galanakis E, Gesouli E, Pappa C, Stefanou D. Four-year experience of use of the Cobas Amplicor system for rapid detection of Mycobacterium tuberculosis complex in respiratory and nonrespiratory specimens in Greece. *Eur J Clin Microbiol Infect Dis.* 2003 Jun;22(6):349-56. Epub 2003 Jun 3.
5. Yam WC, Cheng VC, Hui WT, Wang LN, Seto WH, Yuen KY. Direct detection of Mycobacterium tuberculosis in clinical specimens using single-tube biotinylated nested polymerase chain reaction-enzyme linked immunoassay (PCR-ELISA). *Diagn Microbiol Infect Dis.* 2004 Apr;48(4):271-5.
6. Yam WC, Tam CM, Leung CC, Tong HL, Chan KH, Leung ET, Wong KC, Yew WW, Seto WH, Yuen KY, Ho PL.Direct detection of rifampin-resistant mycobacterium tuberculosis in respiratory specimens by PCR-DNA sequencing. *J Clin Microbiol.* 2004 Oct;42(10):4438-43.