Original Article

Comparison of Two Molecular Assays For Detecting Smear Negative Pulmonary Tuberculosis

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Abstract

Objective To compare the performance of MTBDRplus V2 and Xpert MTB/RIF for detecting smear negative pulmonary tuberculosis (PTB).

Methods Clinical PTB suspects were enrolled consecutively in Anhui Chest Hospital and Xi'an Chest Hospital from January to December in 2014. The sputum samples of smear negative PTB suspects were collected and decontaminated. The sediment was used to conduct MTBDRplus V2, Xpert MTB/RIF and drug susceptibility test (DST). All the samples with discrepant drug susceptibility result between molecular methods and phenotypic method were confirmed by DNA sequencing.

Results A total of 1973 cases were enrolled in this study. The detection rates of *Mycobacterium tuberculosis* complex (MTBC) by MTBDRplus V2 and Xpert MTB/RIF were 27.67% and 27.98%, respectively. When setting MGIT culture result as a gold standard, the sensitivity and specificity of MTBDRplus V2 were 86.74% and 93.84%, and the sensitivity and specificity of Xpert MTB/RIF were 86.55% and 93.43%, respectively. For the detection of the resistance to rifampin, the sensitivity and specificity of MTBDRplus V2 were 94.34% and 96.62%, and the sensitivity and specificity of Xpert MTB/RIF were 88.68% and 95.96%, respectively. For the detection of the resistance to isoniazid, the sensitivity and specificity of MTBDRplus V2 were 77.38% and 98.02%, respectively.

Conclusion MTBDRplus V2 and Xpert MTB/RIF can be used to detect MTBC in smear negative samples with satisfactory performance.

Key words: Smear negative pulmonary tuberculosis, Diagnosis; Drug resistance

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INTRODUCTION

Tuberculosis (TB) remains a major global public health problem, affecting millions of people each year, and TB is the second leading cause of death among infectious diseases worldwide[1]. China ranks third in the countries with heavy TB burden in the world. In recent years, the proportion of smear positive TB cases declined, while the prevalence of active PTB showed no significant decrease according to the fifth national TB epidemiology survey in China in 2010[2]. Smear negative PTB patients, especially drug resistant patients, cannot receive timely and effective diagnosis and treatment due to the lack of sensitive laboratory test.

Because of its rapid detections for MTBC and the resistance to rifampin, Xpert MTB/RIF (Cepheid, USA) is widely used in the world[3-4]. Multicenter studies have demonstrated that Xpert MTB/RIF can be used for the detection of MTBC with high sensitivity and specificity[5-6], and it was recommended by World Health Organization (WHO) for the diagnosis of TB in 2010[7]. MTBDRplus V1 (Hain, Germany) can be used to detect MTBC and the resistance to rifampin and

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isoniazid from smear positive sputum with high accuracy[8-9] in 1 work day, and it was recommended by WHO for screening of multidrug resistance tuberculosis (MDR TB) in countries with heavy TB burden[10]. MTBDRplus V2 has significantly improved sensitivity of detection for MTBC compared with MTBDRplus V1, which can be directly applied to test the sputum samples collected from TB suspects[11]. However, there is limited data of MTBDRplus V2 in the clinical practice.

In this study, the performance of MTBDRplus V2 and Xpert MTB/RIF were compared among smear negative PTB suspects to provide scientific evidence for the diagnosis of smear negative PTB.

MATERIALS AND METHODS

Clinical Sample

PTB suspects were enrolled at outpatient departments in Anhui Chest Hospital and Xi'an Chest Hospital from January to December in 2014. One sputum sample was collected from each patient for different laboratory tests. After smear tests at laboratory, a total of 1993 smear negative sputum samples were collected for other laboratory tests.

Sample Processing

A 2 mL sputum sample of each suspect was processed by using N-acetyl-Lcysteine-sodium citrate-NaOH (NALC-NaOH) method[12]. The supernate was discarded following centrifugation, and the sediments were resuspended in 2 mL of phosphate buffer solution. Three aliquots were prepared to perform MTBDRplus V2, Xpert MTB/RIF and MGIT 960 culture.

MTBDRplus V2 test

The assay was performed according to manufacturer's protocol (Hain, germany)[13]. The test has three steps: DNA extraction, PCR amplification and hybridization.

Xpert MTB/RIF test

The test was conducted according to manufacturer's protocol (Cepheid USA). 0.5 mL aliquot was mixed with sample reagent buffer at a ratio of 3:1, followed by incubation at room temperature for 15 min. Two mL sample was transferred to Xpert MTB/RIF cartridge and the cartridge was loaded into the instrument.

BACTEC MGIT 960 Culture and Drug Susceptibility Test

A 0.5 mL aliquot sample was inoculated in Bactec-MGIT 960 tube. After the culture flashed positive, the susceptibility test to rifampin and isoniazid was performed according to the manufacturer's protocol[14].

Sequencing

All the culture positive strains were collected for DNA sequencing to identify TB related gene (*16S rRNA*) and drug resistance related gene mutation for rifampin (*rpoB*) and isoniazid (*katG* and *inhA*) at national TB reference laboratory (Table 1). The sequencing results were entered into the Basic Local Alignment Search Tool (BLAST), an international database (http://www.ncbi.nlm.nih.gov/BLAST), for the alignment with reference strain H37Rv. The mutations of *rpoB, katG*, and *inhA* gene were compared with H37Rv.

Data Analysis

SPSS 22.0 was used for data analysis. χ^2 test was used for comparison of detection rate of different methods.

Table 1. Primer used for Sequencing to Identify MTBC and Detection of Drug Resistance Related Genes

Gene	Primer Pairs (5'-3')	Amplification Length (bp)
16S rRNA	F: GGCCTAACCCTCGGGAGGGAG	440
	R: CCCGAGGCATATCGCAGCCTC	
rpoB	F: ACCGACGACATCGACCACTT	430
	R: GTACGGCGTTTCGATGAACC	
katG	F: AATCGATGGGCTTCAAGACG	500
	R: CTCGTAGCCGTACAGGATCTCG	
inhA	F: CCTCGCTGCCCAGAAAGGGA	248
	R: ATCCCCCGGTTTCCTCCGGT	