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# Rapid diagnosis of pulmonary tuberculosis and detection of drug resistance by combined simultaneous amplification testing and reverse dot blot

Yiwen Chen,<sup>1</sup> Lahong Zhang,<sup>2</sup> Liquan Hong,<sup>2</sup> Xian Luo,<sup>2</sup> Juping Chen,<sup>3</sup> Leiming Tang,<sup>3</sup> Jiahuan Chen,<sup>3</sup> Xia Liu,<sup>2</sup> Zhaojun Chen<sup>2</sup>

<sup>1</sup>Hangzhou Normal University Affiliated Hospital (Clinical College), Hangzhou, China  
<sup>2</sup>Clinical Laboratory Department, Hangzhou Normal University Affiliated Hospital, Hangzhou, China  
<sup>3</sup>Tuberculosis Laboratory Department, Hangzhou Normal University Affiliated Hospital, Hangzhou, China

## Correspondence to

Professor Zhaojun Chen, Department of Clinical Laboratory, Hangzhou Normal University Affiliated Hospital, Hangzhou, Zhejiang Province 310015, China; hzcj2006@163.com

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## ABSTRACT

**Aims** Making a correct and rapid diagnosis is essential for managing pulmonary tuberculosis (PTB), particularly multidrug-resistant tuberculosis. We aimed to evaluate the efficacy of the combination of simultaneous amplification testing (SAT) and reverse dot blot (RDB) for the rapid detection of *Mycobacterium tuberculosis* (MTB) and drug-resistant mutants in respiratory samples.

**Methods** 225 suspected PTB and 32 non-TB pulmonary disease samples were collected. All sputum samples were sent for acid-fast bacilli smear, SAT, culture and drug susceptibility testing (DST) by the BACTEC™ MGIT™ 960 system. 53 PTB samples were tested by both RDB and DNA sequencing to identify drug resistance genes and mutated sites.

**Results** The SAT positive rate (64.9%) was higher than the culture positive rate (55.1%), with a coincidence rate of 83.7%. The sensitivity and specificity of SAT for diagnosing PTB were 66.7% and 100%, respectively, while those for culture were 53.9% and 84.2%, respectively. RDB has high sensitivity and specificity in identifying drug resistance genes and mutated sites. The results of RDB correlated well with those of DST and DNA sequencing, with coincidence rates of 92.5% and 98.1%, respectively.

**Conclusions** The combination of SAT and RDB is promising for rapidly detecting PTB and monitoring drug resistance in clinical laboratories.

## INTRODUCTION

The *Mycobacterium tuberculosis* (MTB) epidemic is a major health concern worldwide. The new global tuberculosis (TB) report from the WHO stated that approximately one-third of the world's population was infected with MTB. Furthermore, in 2015 there were an estimated 10.4 million new TB cases worldwide and approximately 1.4 million deaths, with China having the third highest number of TB patients in the world.<sup>1</sup> With the widespread use of anti-TB drugs, multidrug-resistant tuberculosis (MDR-TB) and even extensively drug-resistant tuberculosis (XDR-TB) are now emerging and pose a considerable challenge to current TB prevention and control programs.<sup>2-4</sup> Moreover, the number of inaccurate diagnoses and inappropriate treatments for TB patients are increasing, which encourages continued transmission of TB. Therefore, the

rapid detection of TB and drug resistance both optimises treatment and improves outcomes and is also critical for reducing overall morbidity and mortality rates, which would greatly benefit public health.

Traditionally, MTB has been detected by acid-fast bacilli (AFB) smear and the gold standard microbial culture and identification.<sup>5,6</sup> However, these methods can have low sensitivity, are time consuming in routine clinical practice and require large amounts of bacteria. Developments in the field of molecular biology mean that nucleic acid amplification methods have better sensitivity and specificity for diagnosing TB than traditional diagnostic methods.<sup>7</sup> In recent years, simultaneous amplification testing (SAT), which is a new-generation technology that uses isothermal RNA amplification and real-time fluorescence detection, has shown markedly higher sensitivity and specificity than other methods and has been widely used in various fields for detecting pathogens.<sup>8,9</sup> Its excellent detection rate of smear-negative samples can decrease the number of inaccurate diagnoses.

Conventional drug susceptibility testing (DST) requires 3–8 weeks before the results are available, while rapid culture still takes an average of 7–9 days for smear-positive samples. However, this is sometimes too long in clinical practice. DNA sequencing for the identification of drug-resistant mutants is expensive and inconvenient in routine laboratory settings. In contrast, reverse dot blot (RDB) is rapid, sensitive, and has a high throughput, particularly for detecting gene mutations. RDB costs approximately US\$40 and includes four first-line anti-TB drugs. It can be used as a conventional method to replace sequencing, can simultaneously test for multidrug resistance and is widely applied in the field of molecular diagnostics.<sup>10,11</sup>

SAT has a higher sensitivity and is more accurate and rapid than traditional methods.<sup>12,13</sup> RDB can precisely and rapidly identify drug-resistance genes and mutated sites. In the present study, we aimed to evaluate the clinical value of combined SAT and RDB to rapidly diagnose pulmonary TB (PTB) and monitor drug resistance.

## MATERIALS AND METHODS

### Sample collection

Sputum samples were collected from patients screened at the Tuberculosis Department of



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Hangzhou Normal University Affiliated Hospital between October 2015 and October 2016. A total of 225 sputum samples were obtained from 225 different patients with suspected TB, including newly diagnosed and relapsed patients. In addition, 32 sputum samples were randomly collected from patients with respiratory disease in whom TB had been excluded (15 cases of community-acquired pneumonia, 10 of bronchial pneumonia and seven of bronchiectasis). All sputum samples were collected from patients in the early morning, and suspected TB sputum samples were consecutively collected before TB treatment.

#### AFB smear and culture assays

All sputum samples were routinely tested by AFB smear (Zhuhai BASO Biotechnology, China), culture and DST according to the WHO guidelines.<sup>14</sup> Culture and DST were tested using the BACTEC™ MGIT™ 960 system (Becton Dickinson Diagnostic Systems, Sparks, MD). Mycobacterial identification was performed at the Center for Disease Control (CDC) in Hangzhou. All tests were carried out at the TB reference laboratory of Hangzhou Normal University Affiliated Hospital, according to the manufacturer's instructions and in accordance with the Chinese Laboratory Science Procedure of Diagnostic Bacteriology in Tuberculosis guidelines,<sup>15</sup> with quality control routinely performed. Sputum samples were dissolved in 4% sodium hydroxide for 15–20 min at room temperature before they were tested using the BACTEC™ MGIT™ 960 system.

#### SAT for TB

SAT (Shanghai Rendu Biotechnology, China) was performed for all 225 sputum samples, according to the manufacturer's instructions. Sputum samples were processed after they had been dissolved in 4% sodium hydroxide for 15–20 min at room temperature. All processed samples were centrifuged at 13 000×g for 5 min, and the supernatant was discarded. A 50 µL dilution solution was added for re-suspension. The *M. tuberculosis* inactivated strain H37Ra (ATCC 25177) was used as a positive control, and double-distilled water as the negative control. Each sample, positive and negative control were placed in a 300 W water bath sonicator (Shanghai Shengyan Ultrasound Machines, China) for 15 min at room temperature. This was followed by centrifugation, and the supernatant was used as a template for SAT. For simultaneous RNA isothermal amplification, 2 µL processed supernatant and 30 µL reaction solution were prepared in a PCR tube. The mixture was pre-incubated at 60°C for 10 min and at 42°C for 5 min. A 10 µL aliquot containing 2000 units of Moloney murine leukaemia virus (M-MLV) reverse transcriptase and 2000 units of T7 RNA polymerase was then added. The solution was gently mixed and was immediately placed in a 7500 real-time PCR system (Applied Biosystems, Foster City, CA). Amplification was conducted at 42°C for 1 min for 40 cycles. FAM fluorescence data were collected after each amplification cycle. Samples with a cycle threshold ( $C_T$ ) of  $\leq 35$  were classified as TB positive, while samples with  $35 < C_T < 40$  were rechecked and were classified as TB positive if  $C_T$  was  $< 40$ , and as TB negative if  $C_T$  was  $\geq 40$ .

#### RDB

RDB (Shenzhen Yaneng Biotechnology, China) was performed on 53 samples obtained from clinically diagnosed PTB patients, according to the manufacturer's instructions. All processed samples were centrifuged at 13 000×g for 5 min, and the supernatant was discarded. A 50 µL lysis buffer was added to the sediment, which was followed by incubation at 100°C for 10 min

**Table 1** Primers used for drug-resistant gene amplification

Genes	Primers (sequences 5'→3')	Product size (bp)
rpoB	F (5'-ACGGTCGGCGAGCTGATCC-3') R (5'-CAGACCCGATGTTGGGCCCT-3')	351
katG	F (5'-GACATTCGCGAGACGTTTCGG-3') R (5'-GCTCTAAGGCTGGCAATCTCG-3')	469
inhA	F (5'-CTATATCTCCGGTGGCGTCA-3') R (5'-CTTGGCCATCGAAGCATA-3')	469
rpsL	F (5'-AGGTACGCGGTACATTC-3') R (5'-GCCCTTCTCTTCTAGCG-3')	188
embB	F (5'-TGATATTCGGCTTCTGCTC-3') R (5'-ACCGCTCGATCAGCACATAG-3')	417

embB, EMB mutant gene; katG and inhA, INH mutant gene; rpoB, RFP mutant gene; rpsL, SM mutant gene.

and centrifugation at 10 000×g for 2 min. The supernatant was used as a template for RDB.

The PCR amplification programme comprised DNA amplification to yield a PCR mixture with a final volume of 25 µL containing 4 µL processed supernatant and 21 µL reaction solution. PCR conditions were as follows: 50°C for 2 min, 95°C for 10 min, 30 cycles of denaturation at 95°C for 45 s and annealing and extension at 68°C for 1 min, 30 cycles of denaturation at 95°C for 30 s and annealing at 54°C for 30 s and extension at 68°C for 1 min, and final extension at 68°C for 10 min.

PCR products and membranes of immobilised probes were added to a 5 mL hybridization buffer A (1×SSC, 0.1% SDS), followed by denaturation at 100°C for 10 min and hybridization at 59°C for 1.5 hours. The membranes were washed by gentle shaking in 40 mL buffer B (0.5×SSC, 0.1% SDS) for 15 min at 49°C. The membranes were then incubated in an 8 mL streptavidin–peroxidase dilution (1:2000) at room temperature for 30 min. After being washed twice with the same buffer A for 5 min, the membranes were then washed with buffer C (0.1 M sodium citrate) for 2 min. They were visualised by adding a colour substrate solution (tetramethyl benzidine) and kept in the dark for 5–10 min. The presence of clearly visible purple–blue spots on the membrane was considered to indicate a positive hybridization reaction.

#### DNA sequencing

Based on the study by Spinato *et al*,<sup>16</sup> we designed five pairs of primers (table 1) to amplify regions of five genes associated with resistance to four anti-TB drugs: rifampicin (RFP), isonicotinic hydrazide (INH), streptomycin (SM) and ethambutol (EMB). The DNA template was purified using the TIANamp Virus DNA/RNA Kit (Beijing Tiangen Biotech, China). The remainder of the supernatant that was used as a template in RDB was added to a solution containing 20 µL proteinase K and 200 µL carrier RNA before incubation at 56°C for 15 min. Thereafter, the following steps were performed: addition of 200 µL absolute ethanol, gentle mixing of the liquid solution, transfer to an absorbing column and centrifugation at 8000×g for 1 min, discarding of the waste liquid, addition of 500 µL buffer GD and centrifugation for 1 min, discarding of the waste liquid, addition of 600 µL washing buffer PW and centrifugation for 1 min after letting the solution stand for 2 min, discarding of the waste liquid, addition of 500 µL absolute ethanol and centrifugation for 3 min, discarding of the waste liquor, and elution of DNA into 30 µL RNase-free water.

Purified DNA was amplified in a 10 µL reaction mixture containing 1×buffer (Mg2+ plus), 200 µM dNTP mixture,

0.25 units DNA polymerase (PrimeSTAR HS), 0.2  $\mu$ M primer-F, 0.2  $\mu$ M primer-R and 3  $\mu$ L DNA template. PCR conditions were as follows: 50°C for 2 min and 95°C for 10 min, 30 cycles of denaturation at 95°C for 45 s and annealing with extension at 68°C for 60 s, 30 cycles of denaturation at 95°C for 30 s and annealing at 58°C for 30 s and extension at 68°C for 60 s, and final extension at 68°C for 10 min.

Agarose gel electrophoresis was performed, and the particular gene band was excised according to the required size of the gene. Purified PCR products were recovered by a DNA gel extraction kit (Beijing Tiangen Biotech, China). The resistance-determining region (*rpoB*, *katG*, *inhA*, *rpsL*, *embB*) was directly sequenced in an automated DNA sequencer by Hangzhou Qinke Biotech in China.

### Statistical analysis

Statistical analysis was conducted using SPSS (version 18.0, SPSS, Chicago, IL). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of SAT and culture were calculated. Categorical variables were analysed using the  $\chi^2$  test. A P value of  $\leq 0.05$  was considered statistically significant. Coincidence between SAT and culture data was assessed by Cohen's kappa test, with  $\kappa \geq 0.75$  designated as excellent agreement,  $0.4 < \kappa < 0.75$  as moderate agreement, and  $\kappa < 0.4$  as poor agreement. DST and DNA sequencing were used as standards to estimate the accuracy of RDB.

## RESULTS

### Patient diagnoses

Of the 225 patients with suspected TB, 219 were confirmed as having PTB and the remaining six as having non-TB mycobacteria (NTM) during their clinical follow-up. The diagnosis of PTB and NTM including bacteriology and radiography was based on guidelines for the treatment of TB by the WHO issued in 2010.<sup>17</sup>

### Comparison between SAT and culture

Among the 225 sputum samples from the suspected TB patients, the SAT positive rate was significantly higher than the culture positive rate (64.9%, 146/225 vs 55.1%, 124/225,  $\chi^2=11.52$ ,  $P < 0.05$ ). The coincidence rate of SAT and culture was 83.7%. A moderate level of agreement was found between the two methods ( $\kappa=0.75$ ), indicating suboptimal agreement (table 2).

The sensitivity, specificity, PPV and NPV of SAT for diagnosing PTB were 66.7% (146/219), 100% (38/38), 100% (146/146) and 34.2% (38/111), respectively, compared with corresponding values of 53.9% (118/219), 84.2% (32/38), 95.2% (118/124) and 24.1% (32/133) for 960 culture. The sensitivity of SAT for

**Table 3** Performance of SAT and BACTEC™ MGIT™ 960 culture compared with clinical assessment of patients

Method and group	Result	No. of patients with a clinical diagnosis of MTB		Mean (%)			
		+	-	Se	Sp	PPV	NPV
<b>SAT</b>							
All	+	146	0	66.7	100	100	34.2
	-	73	38				
Smear-positive	+	103	0	93.6	100	100	36.4
	-	7	4				
Smear-negative	+	43	0	39.4	100	100	34.0
	-	66	34				
<b>BACTEC™ MGIT™ 960 culture</b>							
All	+	118	6	53.9	84.2	95.2	24.1
	-	101	32				
Smear-positive	+	100	4	90.9	0	96.2	0
	-	10	0				
Smear-negative	+	18	2	16.5	94.1	90.0	26.0
	-	91	32				

MTB, Mycobacterium tuberculosis; NPV, negative predictive value; PPV, positive predictive value; SAT, simultaneous amplification and testing; Se, sensitivity; Sp, specificity.

diagnosing PTB was significantly higher than that of culture ( $\chi^2=20.25$ ,  $P < 0.05$ ).

For smear-positive samples, the sensitivity of SAT was similar to that of culture (93.6%, 103/110 vs 90.9%, 100/110,  $\chi^2=0.36$ ,  $P > 0.05$ ). For smear-negative samples, the sensitivity of SAT was significantly higher than that of culture (39.4%, 43/109 vs 16.5%, 18/109,  $\chi^2=23.04$ ,  $P < 0.05$ ) (table 3).

### Performance of RDB, DST and DNA sequencing for the detection of the TB drug-resistance gene

DST was performed on the sputum samples of 53 patients confirmed as having PTB. Nine isolates were drug-resistant strains, six of which were mono-resistant to RFP (n=2), INH (n=3) or EMB (n=1), while three were MDR-TB to at least RFP and INH. In total, resistance was identified to RFP in five isolates, INH in six, SM in three, and EMB in two.

RDB was performed on the sputum samples of 53 patients confirmed as having PTB. All five (100%) RFP-resistant isolates contained a mutation in the target region of the *rpoB* gene, four of the six (66.7%) INH-resistant isolates contained mutations in the target region of the *katG* gene, all three (100%) SM-resistant isolates contained mutations in the target region of the *rpsL* gene, and one of the two (50%) EMB-resistant isolates had mutation in the *embB* gene. The mutations are showed in table 4. Using DST as the gold standard, RDB had a sensitivity of 77.8% (7/9) for the nine drug-resistant strains and a specificity of 95.5% (42/44) for the 44 drug-sensitive strains. The rate of agreement between RDB and DST was 92.5% (49/53).

Using DNA sequencing as the gold standard, RDB had a sensitivity of 87.5% (7/8) and a specificity of 100% (45/45). RDB and DNA sequencing results had an agreement rate of 98.1% (52/53) (table 4).

## DISCUSSION

TB infection is a major public health problem worldwide. The traditional culture method to detect TB often takes 3–8 weeks, and while a rapid culture system, such as BACTEC™ MGIT™

**Table 2** Comparison of the coincidence rate between SAT and BACTEC™ MGIT™ 960 culture for the detection of MTB

	SAT	Culture		Positive coincidence rate	Negative coincidence rate	Total coincidence rate
		+	-			
Total	+	114	32	91.9%	75.9%	83.7%
	-	10	101			
Smear-positive	+	96	7	92.3%	30.0%	86.8%
	-	8	3			
Smear-negative	+	18	25	90.0%	79.7%	81.1%
	-	2	98			

MTB, Mycobacterium tuberculosis; SAT, simultaneous amplification and testing.

**Table 4** Identification of MTB clinical isolates by DST, RDB and DNA sequencing

Strain no.	DST	RDB	DNA sequencing
1	None	N1 missing	WT
1	None	306N, 306M missing	WT
1	R, I, S, E	S531L, 43M, M306L	S531L(TCG/TTG) K43R(AAG/AGG) M306L(ATG/GTG)
1	I	WT	P388P(CCG/CCA)
1	R	D516V, 15N, 15M missing	D516V(GAC/CCA)
1	R, I, S	H526D, 315M, 88M	H526D(CAC/GAC) S315T(AGC/ACC) K88R(AAG/AGG)
1	R	H526D	H526D(CAC/AAC)
2	I	315M	S315T(AGC/ACC)
1	R, I, S	S531L, 315M, 43M	S531L(TCG/TTG) S315T(AGC/ACC) K43R(AAG/AGG)
1	E	WT	WT
42	None	WT	WT

DST, drug susceptibility testing; E, EMB-resistant; I, INH-resistant; MTB, *Mycobacterium tuberculosis*; R, RFP-resistant; RDB, reverse dot blot; S, SM-resistant; WT, wild-type sequencing.

960, can hasten the detection process, results can still take an average of 7–9 days for smear-positive samples.<sup>18</sup> This relatively long waiting period might contribute to less than optimal TB treatment. In developing countries, a rapid, simple, accurate and sensitive laboratory method that can be used to detect TB is urgently required.

In our study, we evaluated the clinical utility of SAT for suspected TB patients and demonstrated high sensitivity and specificity for all sputum samples. The sensitivity and specificity of SAT were significantly higher than those of culture, with a moderate agreement rate of 83.7% ( $\kappa=0.75$ ). There were 32 clinically diagnosed PTB patients who were SAT-positive but culture-negative, which we attribute to the high sensitivity of SAT in clinical samples. The results of our study were consistent with those of Cui *et al.*,<sup>12</sup> which showed that SAT had 67.6% sensitivity and 100% specificity, with excellent agreement with culture ( $\kappa=0.91$ ).

SAT enables concurrent nucleic acid amplification and real-time fluorescence detection with the use of a special MTB isothermal RNA amplification primer and an optimised probe technology, with M-MLV polymerase and enhanced transcriptional activity of T7 RNA polymerase. SAT took only 4 hours to perform and specifically detected MTB, which addressed the limitation of traditional culture methods in differentiating between MTB and NTM. In our study, the sputum samples of six confirmed NTB patients were SAT-negative while all were positive in culture and identified as NTM by the CDC in Hangzhou. These findings suggested that SAT was both a sensitive test for diagnosing TB from sputum samples and also a tool that can raise suspicion of NTM lung disease in patients who are smear-positive and SAT-negative.<sup>13</sup>

Xpert MTB/RIF resistance PCR is a rapid and sensitive method that can detect DNA and has been extensively studied in resource-limited settings. SAT detects RNA, which is relatively more labile than DNA and quickly degrades after the pathogens die, and therefore is suitable for monitoring active TB and drug efficacy. In contrast, tests that previously targeted DNA detection were not fully utilised because of problems such as easy cross-contamination in the laboratory due to improper

operation and inability to distinguish live from dead bacteria.<sup>19–21</sup> SAT might reduce the risk of laboratory contamination and lower false-positive rates.<sup>13</sup>

However, in the present study, SAT yielded false-negative results in six patients, probably due to the presence of inhibitors of enzymatic amplification, suboptimal target extraction, low starting concentration, or uneven distribution of mycobacteria in the samples.<sup>22,23</sup> However, the false-negative rate of SAT was lower than that of culture.

According to the worldwide estimates of the WHO, the median values of initial multidrug resistance and acquired multidrug resistance were 1.2% and 7.7%, respectively, and there were an estimated 480 000 new MDR-TB patients in 2016<sup>24</sup>. The rapid identification of drug-resistant MTB plays an important role in the diagnosis and treatment of TB patients. Previous studies have clearly shown the resistance mechanisms of first-line anti-TB drugs. Most resistance to RFP resulted from mutations in codons 516, 526 and 531 of the *rpoB* gene encoding its RNA polymerase  $\beta$  subunit.<sup>25</sup> Among INH-resistant strains, 50%–70% had mutations in the *katG* gene, which reduced or inactivated the catalase-peroxidase activity of the drug, and 5%–10% had mutations in the *inhA* gene encoding enoyl-acyl carrier protein reductase.<sup>26</sup> In SM-resistant strains, 64%–68% had a mutation in the *rpsL* gene encoding the ribosomal 30S subunit of the S12 protein.<sup>27</sup> In EMB-resistant strains, 70% had a mutation in the *embB* gene encoding Arab transferase.<sup>28</sup>

In this study, DST was performed using the BACTEC<sup>TM</sup> MGIT<sup>TM</sup> 960 system, but this gold standard needs at least 2 weeks for the results to become available. Sequence analysis is the gold standard for bacterial molecular identification<sup>29</sup> but requires a relatively expensive automated sequencer and well-trained technicians, making its widespread application in clinical laboratories difficult.

RDB was developed for the rapid identification of drug-resistant genotypes and it took only 6–8 hours for the results to become available. It is a useful and inexpensive test for detecting most drug-resistant tuberculosis (DR-TB) isolates and can be routinely used in TB reference laboratories.<sup>30</sup> RDB uses specific biotin-modified primers to amplify related resistance genes. PCR products are then degenerated and modified with biotin, hybridised with a specific linear probe tagged on the membrane, and coloured by the biotin/peroxidase system, with final results based on the signal strength of spot hybridization.

Mokrousov *et al.*<sup>11</sup> reported the first attempt to combine different targets in a single assay for predicting anti-TB drug resistance. The reproducibility, sensitivity and specificity of RDB were superior to those of the routine phenotypic method reported by van Rie *et al.*<sup>31</sup> Similarly, our study demonstrated good diagnostic performance for RDB, with coincidence rates of 92.4% with DST and 98.1% with DNA sequencing.

According to the RDB results, two drug-sensitive strains failed to show the correct colour dots, and the mutation probe dots were negative. These incorrect results might have been caused by less probe combination ability, inhibitor interference, or shorter chromogenic and hybridization time. Neither RDB nor DNA sequencing showed any mutations in the *katG* or *inhA* gene in one INH-resistant strain. This implied the possibility of other resistance mechanisms. Approximately 6%–13% of mutations in the *ahpC* gene encoding alkyl peroxide enzyme have been described.<sup>32</sup> Therefore, the negative detection rate resulting from a lack of specific probes in frequent mutants can be easily corrected by adding appropriate probes on the membrane.<sup>33</sup> One INH-resistant strain showed negative RDB results but had a silent mutation in *katG* gene codon 388 (CCG/

CCA). It is unclear whether a similar mutation with a single base change can cause drug resistance. RDB and DNA sequencing did not show any changes in the embB gene in one EMB-resistant strain, but this was not unexpected as current literature showed that 30% of EMB-resistant strains do not have mutations in the embB gene.<sup>34</sup> The results implied the existence of other resistance mechanisms or gene mutations in this group. Clinicians should assess these results in conjunction with the clinical situation. As the number of drug-resistant strains was limited in the present study, the frequencies of gene mutations cannot be further discussed. Nevertheless, our study demonstrated nine drug-resistant strains among 53 confirmed PTB patients (17.0%), including three MDR-TB (5.7%) patients, which accounted for 33.3% of the DR-TB patients. The resistant gene mutated types in our study covered the most commonly encountered examples. Future studies with more samples and gene mutations are required.

A high throughput test for MTB drug resistance testing is required in China and similar endemic areas. RDB can simultaneously detect several drug-resistant genes and significantly increases the detection rate of MDR strains, since it is a simple, rapid and reliable method for screening for TB, as DST is time consuming and DNA sequencing needs special and expensive equipment.

In summary, SAT is a sensitive, accurate and fast method for the direct detection of MTB in clinical settings, while RDB can rapidly screen DR-TB patients and detect gene mutations without the aid of expensive equipment. A combination of SAT and RDB is a promising tool for rapidly identifying PTB patients and monitoring drug resistance in clinical laboratories.

### Take home messages

- ▶ Simultaneous amplification testing (SAT) can rapidly detect *Mycobacterium tuberculosis*, and as the positive rate, sensitivity and specificity are higher than in traditional methods, it can reduce false-negative diagnoses.
- ▶ Reverse dot blot (RDB) is a useful and inexpensive test for the rapid identification of drug-resistant genotypes as it takes only 6–8 hours, and has high coincidence rates with drug susceptibility testing and DNA.
- ▶ A combination of SAT and RDB is promising for rapidly diagnosing pulmonary tuberculosis and monitoring drug resistance in clinical laboratories.

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