Mol Biol Rep (2011) 38:2185–2192
DOI 10.1007/s11033-010-0347-0

Application of Genotype MTBDRplus in rapid detection of the Mycobacterium tuberculosis complex as well as its resistance to isoniazid and rifampin in a high volum laboratory in Southern China

Lei Zhang · Yuanxing Ye · Lina Duo · Tingting Wang · Xingbo Song · Xiaojun Lu · Binwu Ying · Lanlan Wang

Received: 16 March 2010 / Accepted: 6 September 2010 / Published online: 18 September 2010

Abstract The alarmingly worsening epidemics of drug-resistant tuberculosis (TB) call urgent need for a simple method for the rapid detection of drug-resistant TB in clinical settings. In an attempt to establish a rapid procedure for laboratory diagnosis of TB and investigate the local TB epidemiology, molecular line probe assay of the Genotype MTBDRplus was used to identify Mycobacterium tuberculosis complex (MTBC) and detect mutations conferring resistance to two most active first-line drugs against TB: Rifampin and Isoniazid. 96 acid-fast bacillus (AFB) smear- positive sputums and 18 PCR-positive non-sputum specimens have been determined for the MTBC and resistance to Rifampin and Isoniazid. The MTBC detection rates in two sources of specimens were 93.8% (90/96) and 77.8% (14/18) respectively. The overall drug resistance (Rifampin or Isoniazid) occurred in 34.6% (36/104). Resistance to rifampin (RMP) was 28.8% (30/104) and 25% (26/104) was to Isoniazid (INH), in which high level drug resistance accounted for 88.5% (23/26) and low level drug resistance accounted for 7.7% (2/26). Multidrug resistance (MDR), defined as resistant to both RMP and INH, was found in 19.2% (20/104) of clinical samples, which was double that of official statistics. In addition, 63.3% (19/30) RMP-resistant mutations were identified in the region of RopB 530–533 and 57.9% (11/19) were the S531L mutation. 84.6% (22/26) of resistance to INH was mediated by Kat S315T1 mutations which conferred the high-level resistance to INH. The Genotype MTBDRplus line probe assay is a suitable and applicable method for establishing the rapidness in detection of drug-resistant TB in clinical laboratory. It will be a valuable addition to the conventional TB diagnostic approaches.

Keywords Mycobacterium tuberculosis · Drug resistance · Genotype MTBDRplus · Epidemiology

L. Zhang · Y. Ye · L. Duo · T. Wang · X. Song · X. Lu · B. Ying (✉) · L. Wang (✉)
Department of Laboratory Medicine, West China Hospital of Sichuan University, 37 Guoxue Alley, Chengdu 610041, Sichuan Province, People's Republic of China
e-mail: docybw@gmail.com

L. Wang
e-mail: huaxiwangll@gmail.com

Introduction

Tuberculosis is a severe infectious disease with high morbidity and mortality. Since the 1990s, incidence of tuberculosis has been on the rise. According to the WHO, there were 9.4 million incident cases of TB, 11.1 million prevalent cases of TB and 1.82 million deaths from TB in 2008 globally. TB ranks second only to human immunodeficiency virus as a cause of death from an infectious agent [1–3]. The situation is further complicated by increasing emergence of drug resistant TB. RMP and INH are most important drugs to treat TB clinically. Resistance to the two drugs at the same time is defined as multidrug resistance (MDR). MDR-TB can prolong the treatment period and lower the cure rate. And thus, increased toxic and side effects of these drugs and treatment cost create a huge burden to the patient [4–7]. In 2006 the United States Center for Disease Control and Prevention and the WHO issued warning reports on the prevalence of extensive drug-resistant tuberculosis (XDR-TB) which exhibits additional resistance to any one of the fluoroquinolones and to at least one of the injectable second-line drugs. Under these circumstances clinical treatment of TB will be seriously limited and many helpless cases will be witnessed. Dual

infections of TB and HIV, especial drug-resistant TB and HIV, will accelerate TB disease progression and has a high risk of mortality, posing new challenge to TB control [4, 6, 7]. China ranks the second on the list of high TB burden countries issued in 2009 by WHO with 2.5 million cases and 1.3 million new cases in 2008. The global anti-Tuberculosis Drug Resistance Surveillance report was updated in 2009 in which 4 big cities and 4187 patients in China was included. According to the report, the incidence of resistance to any drug ranges from 15.4 to 36.1% and to at least RMP and INH from 2.3 to 7.3% in China [3, 5, 7, 8].The situation in China should be more serious than has been estimated since the relatively backward development in TB laboratory diagnosis.

Currently no identification and drug sensitivity program for Mycobacterium tuberculosis is carried out in most of clinical microbiology labs in large general hospitals in China. Laboratory diagnosis of TB remains in a stage of acid-fast staining and primary culture. Therefore, rapid and accurate identification of TB and detection of drug resistance will not only help with the choice of optimal clinical treatment, it will also assist in the prevention of spread of drug-resistant strains and in the reasonable utilization of available anti-TB drug resources. It is highly significant to TB control in China. Collective studies show that the resistance to RMP mainly involves the mutation of the rpoB gene coding RNA polymerase subunit β and that mutations in the katG, inhA and ahpC gene were strongly associated with INH resistance. Mutations in katG and inhA were closely related to the high-level and low-level INH resistant respectively [6, 9, 10]. In this paper the Genotype MTBDRplus line probe hybridization system is used to identify the MTBC in 114 clinical samples and to detect mutations in rpoB, katG, inhA in an attempt to collect regional epidemiological information of drug-resistant TB and to explore a new mode incorporating the Genotype MTBDRplus for rapid laboratory diagnosis of TB.

Materials and methods

Clinical specimens

Ninety-six acid-fast bacillus smear-positive sputum specimens were analyzed. These specimens included clinical specimens that had been received by the Microbiology Laboratory in West China Hospital of Sichuan University from July 2009 to October 2009. Also included are eighteen non-sputum, smear-negative samples (10 csfs, 8 urines) tested positive for TB DNA via real-time fluorescence PCR (TB PCR Fluorescence Diagnostic Kit, Qiagen) in the Molecular Diagnostic Lab, West China Hospital of Sichuan University. Standard strain H37Rv (ATCC27294) was used.

Decontamination of samples

Sample was processed using the NALC/NAOH method according to the CDC publication "Public health mycobacteriology: a guide for the level III laboratory".

DNA extraction

For specimens tested positive for TB DNA, the extracted DNA by NucliSens EasyMag (BioMerieux) was directly input into The Genotype MTBDRplus assay. For sputum specimens, The Genotype MTBDRplus instructions were followed and GenoLyse DNA Extraction Kit was used. Briefly, 500 µl of decontaminated sputum was taken, centrifuged at 10,000×g for 15 min and the supernatant was discarded. The pellet was lysed in 100 µl of lysis buffer (Lys-A) at 95°C for 5 min, then mixed with 100 µl of neutralization buffer (A-NB). The supernatant was collected as template after centrifugation at full speed for 5 min.

Multiplex amplification

Amplification was done following the manufacture's instructions. The reaction system consists of 35 µl primer nucleotide mix, 5 µl 10× reaction buffer for HotStar Taq, 2 µl 25 mmM $MgCl_2$ buffer, 1U HotStar Taq polymerase and 5 µl DNA template in a total reaction volume of 50 µl. The multiplex PCR was performed according to the protocol: denaturation at 95°C for 15 min; 10 cycles of denaturation at 95°C for 30 s and elongation at 58°C for 120 s; an additional 30 cycles of denaturation at 95°C for 25 s, annealing at 53°C for 40 s, and elongation at 70°C for 40 s; and a final extension at 70°C for 8 min. The amplification products were verified by electrophoresis on 3% agarose gel.

Hybridization and coloration

According to The Genotype MTBDRplus manufacture's instructions, the biotin-labeled PCR product was chemically denatured and hybridized to a strip with specific oligonucleotide probes and then color was developed by alkaline phosphatase mediated reaction. Briefly, denaturation of PCR product was conducted by mixing 20 µl denaturation solution (DEN) with 20 µl PCR product in hybridization well and incubating the mixture at room temperature for 5 min. Then DNA-STRIPs with prior markers were introduced into the wells. The hybridization tray was placed smoothly into the pre-heated automatic

hybridization machine GT-Blot 20 (Hain Lifescience GmbH, Nehren, Germany). The hybridization and coloration were finished in 2 h.

Probe arrangement and detection strategies

Three controls were set on the DNA-STRIP. One probe is complementary with the MTBC specific region of 23S rRNA gene (tub) and should be always positive for all MTBC strains, one probe for internal control (AC) should be positive when the amplification performed correctly and one line (CC) must develop when conjugate binding and substrate coloration occur efficiently. Three probes specific for rpoB, katG and inhA gene are locus controls and should be positive when TUB documents the presence of MTBC. 8 rpoB wild-type probes (WT1 to WT8) encompass the region of the rpoB gene coding amino acids 505 to 534. 4 other probes are specific for the most common mutations: D516V, H526Y, H526D, and S531L (rpoMUT1, MUT2, MUT3 and MUT4, respectively). 3 probes are specific for S315 region of katG. One is the wild-type probe (katG WT), while two others (katG MUT1 and MUT2) are designed for the AGC-to-ACC (S315T1) and the AGC-to-ACA (S315T2) mutation. 6 probes are designed for promoter region (−8, −15 and −16) of inhA gene. Two wild-type probes, inhA WT1 and WT2, cover −15, −16 and −8 nucleic acid positions respectively, four others (inhA MUT1, MUT2, MUT3A and MUT3B) detect mutations of C15T, A16G, T8C and T8A severally. Probe arrangements on nitrocellulose strip see Fig. 1. The Genotype MTBDRplus results were interpreted as described in manufacture's instruction.

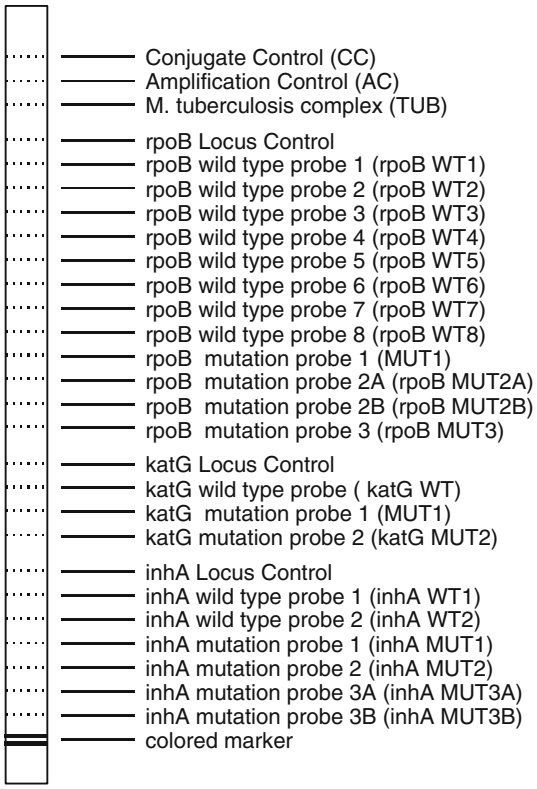

Fig. 1 Genotype MTBDRplus probe arrangement on nitrocellulose strip

Results

Amplification and electrophoresis

Among 114 clinical specimens, 91.2% (104/114) correctly showed two groups of amplification fragments with different sizes: around 100 and 160 bp, with identical pattern to the H37RV. These fragments were suggested to have the approximate length of 110, 115, 120, and 166 bp, corresponding to the amplicon of inhA, MTBC 23S rRNA, katG, and ropB gene, separately. However, the 63 bp product of amplification control failed to show up in most lanes where banding profiles of the other 4 amplicons could be visible. A competition disadvantage may exist for the amplification control in the multiplex PCR system, which was reflected by a relatively weak banding in the AC area. 8.8% (10/114) showed absence of the expected bands, among which 22.2% (4/18) were from the non-sputum samples detected positive by PCR and 6.3% (6/96) were from the smear-positive specimens. The failure of amplification of related genes may in part account for the presence of Mycobacterium other than tuberculosis (MOTT) (parts of results see Fig. 2).

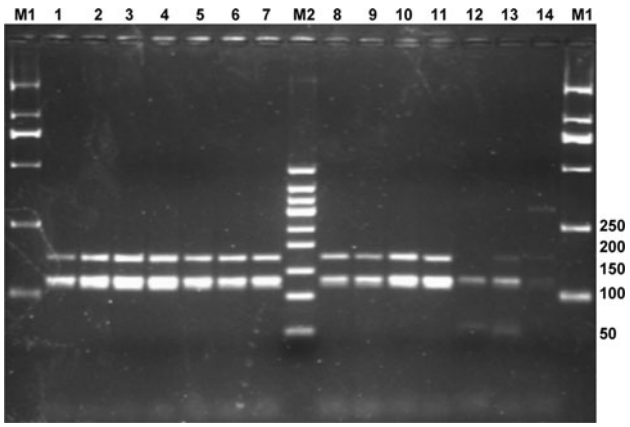

Fig. 2 Multiplex amplification of targeted genes in MTBC. PCR products were electrophoresised on 3% agarose gel. M1 and M2, molecular weight marker indexing of fragment sizes: 50, 100, 150, 200, and 250 bp. 1 represents standard strain of H37Rv and 2–14 clinical specimen. 12, 13 and 14 failed to produce corresponding amplicons of targeted genes. The 60 bp band of amplification control can only be viewed in the lanes of 12 and 13 due to competitive inhibition

Line probe hybridization assay

Through the assay, 91.2% (104/114) were confirmed to be the MTBC with the co-presence of control band of CC, AC and TUB. 6.3% (6/96) were identified as nontuberculosis mycobacteria combining the microscopy of positive smear and hybridization result with CC and AC positive but TUB negative, consistent with the electrophoresis profiles showing the lack of related bands. The MTBC detection rate was 93.8% (90/96) for the smear-positive sputum and 77.8% (14/18) for the non-sputum specimens screened positive by PCR. Figure 3 shows parts of the hybridization results.

Epidemiology of drug-resistant TB

The overall drug resistance (resistant to RMP or INH) was 34.6% (36/104). Resistance to RMP was 28.8% (30/104) and 25% (26/104) was resistant to INH, among which 88.5% (23/26) were due to the high-level INH resistance and 7.7% (2/26) to the low level INH resistance. In addition, 3.8% (1/26) harbored both the high-level and the low-level INH resistance mutation. 19.2% (20/104) were resistant to both RMP and INH (MRD). The inclusion of the retreated patients at 35% (40/114) of the investigated population may justify the relatively higher level of drug resistance observed in this study, compared with the WHO reports statistically based on incident cases. In the RMP-resistant TB, 33.3% (10/30) were sensitive to INH and 66.7% (20/30) were resistant to INH, among which 83.3% (5/6) were high-level INH resistance and 16.7% (1/6) were low-level INH resistance. In the INH-resistant TB, 23.1% (6/26) were sensitive and 76.9% (20/26) were resistant to RMP. It indicates that resistance to RMP or INH tends to occur concomitantly. In other words, multidrug resistance is more common than monoresistance to either RMP or INH.

Distribution of mutation in the region of rpoB gene in RMP-resistant TB

63.3% (19/30) of RMP-resistant mutations were concentrated in the region of RopB 530–533, covered by WT8, in which 57.9% (11/19) were the S531L mutation, 42.1% (8/19) were the non-S531L mutation in the RopB 530–533 region. 20% (6/30) of the RMP -resistant mutations were located in the RopB 525–529, covered by WT6 and WT7, in which H526D (MUT2b) accounted for 16.7% (1/6) and 83.3% (5/6) belonged to the non-H526D and non-H526Y (MUT2a) mutations in the RpoB 525–529. 20% (6/30) of the RMP-resistant mutations were found in the RopB510–519, included by w2, w3, w4, belonging to the

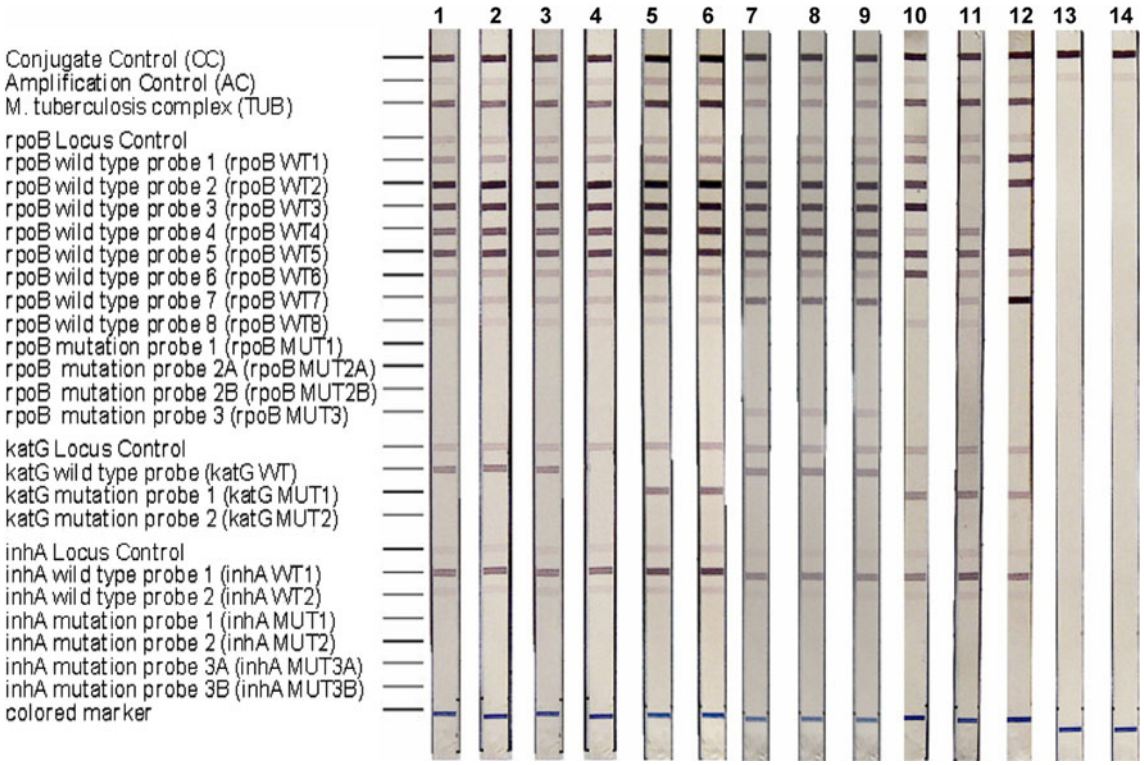

Fig. 3 Line probe hybridization assay results. 1 represents standard strain of H37Rv. 2–14 are clinical specimens. 13 and 14 give uninterpretable results due to lack of control bands

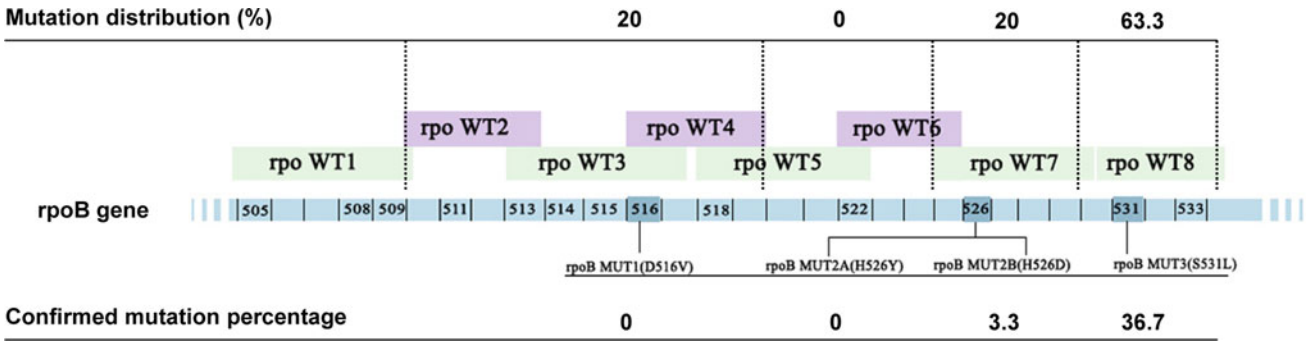

Fig. 4 Mutation distributions in the 81 bp region of rpoB gene. RpoBWT1-8, rpoB wild type probes; rpoB MUT1-3, rpoB mutation probes. The numbers specify the positions of involved amino acids for all mutations reported. The codons at which mutation probes targeted are highlighted. 63.3% mutations fall in the region of 530–533. 1 strain harbors mutations in both 510–519 and 530–533 regions

non-D516V mutations. One RMP-resistant TB simultaneously harbored mutations in both the RopB 530–533 and RopB 510–519 region (covered by WT3, 4 and 8) (Fig. 4).

Distribution of mutation in katG and inhA in INH-resistant TB

88.5% (23/26) of INH-resistant mutations were assigned to KatG mutations conferring the high-level INH-resistance, in which 95.7% (22/23) were identified as Kat S315T1 and 4.4% (1/23) as non-S315T1 and non-S315T2 mutations. 7.7% (2/26) were defined as the C15T mutation of the InhA promoter region mediating low-level INH-resistance. One strain (3.8%) simultaneously harbored high-level S315T1 and low-level T8C mutation.

Discussion

Since tubercle bacillus usually grows slowly, the identification and drug-resistance inspection usually require several weeks. The golden standard of TB diagnosis by culture takes weeks to become positive, and even with the up-to-date automatic culture apparatus it costs an average of 14 days. Another 14 days for additional tests is required to get the information of identification and drug susceptibility and a total of 42 days is needed for the negative report [11–13]. In addition, the culture-based identification and susceptibility tests involve increased consumption of the exclusive culture media and hence place more economic burden on patients, especially in low resource and high burden region [13]. Finally, in order to control the occupational exposure of TB, Chinese government has raised demands on the biosafety settings in laboratories which practice culturing technology of TB. Therefore, the cost and complexity of establishing culture capacity to meet the anticipated need, especially in low income countries where these services are not generally available, present overwhelming challenges [13–15]. For the above reasons, most TB laboratories in the large-sized general hospital in China Mainland have only been equipped with the acid-fast staining and primary culturing and lack resources to provide the information about identification and drug sensitiveness of TB, which present a huge hindrance for domestic tuberculosis control. Therefore, there is an urgent need for laboratory to find a rapid and efficient method for TB testing as a complement to the conventional mycobacterial culture and smear microscopy, and meanwhile to establish a new of MDR-TB diagnostic route for rapid detection of drug-resistant TB.

The multiplex PCR-based solid phase reverse hybridization Genotype MTBDRplus line probe assay (Hain Lifescience GmbH, Nehren, Germany) has been shown to be a rapid and accurate method to detect drug-resistant TB in clinical specimen, especially in growth-positive culture and smear-positive sputum, and it has the potential to shorten the turnaround time to result [16–21]. It is also technically simple to perform and do not require sophisticated equipment when compared with the conventional culture techniques. Briefly, the membrane-bound (DNA-STRIP) probes complementary to the targets of interest captured the corresponding amplicons by hybridization after multiplex amplification and chemical denaturation and then color was developed through an enzyme-mediated staining reaction to indicate the presence of related targets. The overall turnaround time from specimen receipt to reporting susceptibility is dramatically shortened to roughly 4–5 h compared to weeks to months with traditional culture-based assays. In addition, this system reduces the opportunity to operate the biohazard intermediates containing culture-enriched TB, which inevitably lowers the biosafe risks in the laboratory and requires less demanding biosafety facilities.

Multiple reports have shown that the performance of the Genotype MTBDRplus assay in sensitivity and specificity almost comes up to that of conventional culture-based identification and susceptibility testing and its potential applications in laboratory diagnosis of drug-resistant TB

have been widely validated. Neonakis et al., showed that for the direct detection of Mycobacterium tuberculosis complex bacteria the overall sensitivity and specificity obtained with GenoType MTBDRplus were 97.9 and 100% [16]. Causse et al., reported that the Genotype MTBDRplus had a correctness of 100% in RMP susceptibility prediction and 94.5% in INH, compared with routine culture testing [17]. Barnard et al., showed that when applied to smear-positive sputum sample, the Genotype MTBDRplus, compared with traditional culturing methods, has the sensitivity, specificity, negative predict value and positive predict value of 98.8, 100, 100 and 99.7% respectively for detection of rifampicin resistance; 94.2, 99.7, 99.1, and 97.9%, respectively, for detection of isoniazid resistance; and 98.8, 100, 100, and 99.7%, respectively, for detection of multidrug resistance [22]. Nikolayevskyy, et al., also proved that an excellent agreement exists between the Genotype MTBDRplus and culture-based drug sensitivity test when applied to smear-positive sputum sample with the sensitivity of 96.2, 97.4, 97.1% and specificity of 90.7, 83.3, 88.9% for the detection RMP and INH resistance and MDR [21]. Additionally, a systematic evaluation of meta-analysis was recently conducted and suggested that direct testing of RMP and INH resistance with the Genotype MTBDRplus is high sensitive and specific, allowing for prompt detection of MDR-TB [23].

Besides the Genotype MTBDRplus, there is currently another commercially available line probe assay for the rapid detection of drug resistance in M. tuberculosis: INNO-LiPA Rif TB Assay (Innogenetics) for detecting resistance to rifampicin, but the Genotype MTBDRplus could simultaneously inspect both the INH and RMP for the drug-resistance and even fluoroquinolone and amikacin/capreomycin susceptibility could be resolved with the Genotype MTBDRsl under the Genotype series [11, 12, 24, 25]. Furthermore, a specialized automatic hybridization machine was developed to speed and standardize the complex and error-prone process of hybridization and therefore enjoys greater advantage.

The Genotype MTBDRplus has been suggested by the manufacturer to be applied only to smear-positive sample and under this condition stability and performance will be guaranteed. In this study, the non-respiratory, PCR-positive specimen has been for the first time subjected to the Genotype MTBDRplus assay for MTBC confirmation and drug-resistance profiling. The DNA prepared by NucliSens EasyMag (BioMerieux) from 18 PCR-positive specimen, was directly input into the Genotype MTBDRplus assay and 77.8% (14/18, CT < 30) developed the CC, AC and TUB line. It was suggested that the PCR-positive samples with initial input TB load been high enough are suitable for the Genotype MTBDRplus system to promptly obtain the information about identification and drug susceptibility, thus offering the potentials for rapid examination of clinical non-sputum tubercular specimen. A similar exploration of Genotype MTBDplus application in smear-negative and culture-positive sputums was attempted and a high proportion of interpretable result (80%) was obtained [22]. Based on these data, we proposed a new laboratory diagnosis route with Genotype MTBDRplus as a central platform allowing rapid identification and detection of drug resistance for TB (Fig. 5). For samples confirmed as smear-

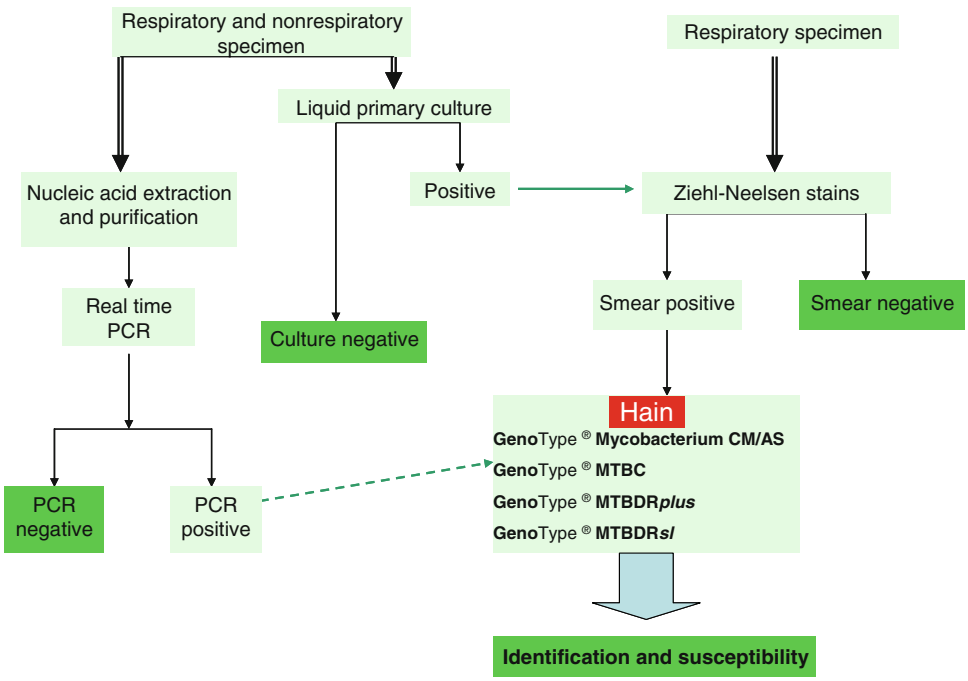

Fig. 5 The proposed new flow chart for rapid TB testing in clinical laboratory. The *parallel arrows* indicate that the specific test application from physicians is involved. The *broken arrow* suggests that the indicated further approach to identification and susceptibility is applicable under certain condition (CT < 30 in this experiment)

positive by microscopic examination (including culture-positive samples with subsequent smear positive) the Genotype MTBDRplus assay can be directly applied. For samples determined by real-time PCR as positive with CT less than 30, the previously extracted DNA in PCR test was directedly put into the Genotype MTBDRplus assay. The new procedures will markedly shorten the time to results of identification and susceptibility of TB from several weeks to 2 days for samples applied for microscopy or PCR examination or roughly 15 days for samples applied for culture examination, and therefore it is of great significance to improve the rapidness of laboratory diagnosis of TB.

Studies show that about 95% of resistance to RMP are associated with the rpoB mutations which are found to cluster mainly in the region of 507–533 containing 27 amino acids (81 bp nucleic acid equivalents of mutation hotspot) [6, 9, 26]. The Genotype MTBDRplus has eight wild-type probes for the rpoB, covering amino acids in the rpoB from 505 to 533, and four other probes specific for hotspot mutations D516V, H526Y, H526D and S531L (See Fig. 5). In this study, 40% (12/30) of the resistance to RMP belong to the hotspot mutations S531L and H526D, accounting for 36.7% (11/30) and 3.3% (1/30) respectively and 60% (18/60) are non-hotspot resistance related mutations, recorded as simultaneous absence of the corresponding index lines for wild and mutant type of probes. The detailed information about these non-hotspot mutations can be approached by sequencing. The detection rate of the hotspot mutations D516V and H526Y is zero, which is apparently different from the reported mutation distribution of S531L at 41.4% and H526Y at 44.8%, reflecting the difference in the epidemiology. Besides, one MDR stain concurrently harbors two RMP-resistant mutations. One is in the region of RopB 510 to 519 (represented by lack of W3, W4 wild-type probes and MUT1 mutation probe), with estimated mutation of 517 or 516 (but rather than D516V), and the other in the region of 530 to 533 (but rather than the S531L mutation) recorded as simultaneous absence of W8 and MUT3 probes.

Resistance to INH has been proved to be essentially mediated by mutation in katG, inhA and ahpC gene, in which 40–95% of resistance is defined as the high-level drug-resistance (0.4 μg/ml) due to the katG mutations. Among the katG mutations, 75–90% are recognized as mutations in the 315th amino acid, which mainly result in S315T1 (agc-acc) and S315T2 (agc-aca) mutation [6, 9, 10, 27]. The Genotype MTBDRplus has designed the 1 wild-type and 2 mutation-type probes (S315T1, S315T2) for covering these essential changes in katG315. In the experiment, 88.5% of INH resistances were attributed to katG mutations which confer high level resistance to INH, among of which 95.7% were identified as S315T1 mutation. One (4.4%) non-S315T1 and non-S315T2 mutation in katG315 requires confirmation by sequencing. Known studies show that 8% to 20% of INH resistance are defined as the low-level drug resistance (0.1 μg/ml) mainly caused by the mutations in the promoter region of inhA gene, involving −15, −16 and −8 locus. The Genotype MTBDRplus has developed 2 wild-type probes (WT −15/−16 and WT −8) and 4 mutation-type probes, covering mutations of C15T, A16G, T8C and T8A, for detection of INH low-level drug resistance. In the experiment, we have observed that the low-level drug-resistance proportion was 7.7%, close to the low limit of reported range. Besides, we also discovered one MDR stain which bore both the high-level and the low-level drug-resistance related mutations, S315T1 and T8C. We speculate that its resistance to INH has evolved from the acquiring of the low-level drug-resistance by T8C then to obtaining the high-level drug-resistance by S315T1, and finally retaining the two drug-resistance mutations, which may also reflect the transformation of selection pressure exerted by the environment. The mutations in the ahpC promoter region are usually as complementary mutations to the katG mutations, and thus there is no corresponding capture probe set in the Genotype MTBDRplus due to limited space in the supporting strip.

Conclusions

As a simple, quick and efficient method for rapid laboratory diagnosis of TB, the multiplex PCR-based Line Probe Hybridization of Genotype MTBDRplus successfully reveals the high burden of drug resistant TB in local region of a city in Southern China. Its application and popularization will help better solve the long-standing problem of laboratory diagnostic delay by traditional culturing method in China. The establishment of new approaches into which the Genotype MTBDRplus is incorporated will greatly improve the level of diagnosis and treatment of tuberculosis.

Acknowledgments The authors wish to thank Willis Ko for thoughtful editing contributions and Clinical Microbiology Laboratory staffs for kind support in specimen collection.

References

1. WHO (2009) Global tuberculosis control: epidemiology, strategy, financing: WHO report 2009: World Health Organization
2. WHO (2009) Global tuberculosis control: a short update to the 2009 report: World Health Organization
3. Glaziou P, Floyd K, Raviglione M (2009) Global burden and epidemiology of tuberculosis. Clin Chest Med 30(4):621–636 vii
4. Chapman AL (2008) Antituberculosis drug resistance: new global data on an emerging global emergency. Clin Med 8(5):478–479

5. Lonnroth K, Raviglione M (2008) Global epidemiology of tuberculosis: prospects for control. Semin Respir Crit Care Med 29(5):481–491
6. Riccardi G, Pasca MR, Buroni S (2009) Mycobacterium tuberculosis: drug resistance and future perspectives. Future Microbiol 4:597–614
7. Wright A, Zignol M, Van Deun A, Falzon D, Gerdes SR, Feldman K et al (2009) Epidemiology of antituberculosis drug resistance 2002–07: an updated analysis of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Lancet 373(9678):1861–1873
8. Aziz MA, Wright A, Laszlo A, De Muynck A, Portaels F, Van Deun A et al (2006) Epidemiology of antituberculosis drug resistance (the Global Project on Anti-tuberculosis Drug Resistance Surveillance): an updated analysis. Lancet 368(9553): 2142–2154
9. Cole ST (1996) Rifamycin resistance in mycobacteria. Res Microbiol 147(1–2):48–52
10. Vilcheze C, Jacobs WR Jr (2007) The mechanism of isoniazid killing: clarity through the scope of genetics. Annu Rev Microbiol 61:35–50
11. Ahmad S, Mokaddas E (2009) Recent advances in the diagnosis and treatment of multidrug-resistant tuberculosis. Respir Med 103(12):1777–1790
12. Ani AE (2008) Advances in the laboratory diagnosis of Mycobacterium tuberculosis. Ann Afr Med 7(2):57–61
13. Grandjean L, Moore DA (2008) Tuberculosis in the developing world: recent advances in diagnosis with special consideration of extensively drug-resistant tuberculosis. Curr Opin Infect Dis 21(5):454–461
14. Charles M, Pape JW (2006) Tuberculosis and HIV: implications in the developing world. Curr HIV/AIDS Rep 3(3):139–144
15. Zar HJ (2004) Tuberculosis in the developing world. Pediatr Pulmonol Suppl 26:53–54
16. Neonakis IK, Gitti Z, Baritaki S, Petinaki E, Baritaki M, Spandidos DA (2009) Evaluation of GenoType mycobacteria direct assay in comparison with Gen-Probe Mycobacterium tuberculosis amplified direct test and GenoType MTBDRplus for direct detection of Mycobacterium tuberculosis complex in clinical samples. J Clin Microbiol 47(8):2601–2603
17. Causse M, Ruiz P, Gutierrez JB, Zerolo J, Casal M (2008) Evaluation of new GenoType MTBDRplus for detection of resistance in cultures and direct specimens of Mycobacterium tuberculosis. Int J Tuberc Lung Dis 12(12):1456–1460
18. Hillemann D, Rusch-Gerdes S, Richter E (2007) Evaluation of the GenoType MTBDRplus assay for rifampin and isoniazid susceptibility testing of Mycobacterium tuberculosis strains and clinical specimens. J Clin Microbiol 45(8):2635–2640
19. Lacoma A, Garcia-Sierra N, Prat C, Ruiz-Manzano J, Haba L, Roses S et al (2008) GenoType MTBDRplus assay for molecular detection of rifampin and isoniazid resistance in Mycobacterium tuberculosis strains and clinical samples. J Clin Microbiol 46(11): 3660–3667
20. Miotto P, Piana F, Cirillo DM, Migliori GB (2008) Genotype MTBDRplus: a further step toward rapid identification of drug-resistant Mycobacterium tuberculosis. J Clin Microbiol 46(1): 393–394
21. Nikolayevskyy V, Balabanova Y, Simak T, Malomanova N, Fedorin I, Drobniewski F (2009) Performance of the Genotype MTBDRPlus assay in the diagnosis of tuberculosis and drug resistance in Samara, Russian Federation. BMC Clin Pathol 9:2
22. Barnard M, Albert H, Coetzee G, O'Brien R, Bosman ME (2008) Rapid molecular screening for multidrug-resistant tuberculosis in a high-volume public health laboratory in South Africa. Am J Respir Crit Care Med 177(7):787–792
23. Bwanga F, Hoffner S, Haile M, Joloba ML (2009) Direct susceptibility testing for multi drug resistant tuberculosis: a meta-analysis. BMC Infect Dis 9:67
24. Makinen J, Marttila HJ, Marjamaki M, Viljanen MK, Soini H (2006) Comparison of two commercially available DNA line probe assays for detection of multidrug-resistant Mycobacterium tuberculosis. J Clin Microbiol 44(2):350–352
25. Palomino JC (2009) Molecular detection, identification and drug resistance detection in Mycobacterium tuberculosis. FEMS Immunol Med Microbiol 56(2):103–111
26. Telenti A, Imboden P, Marchesi F, Lowrie D, Cole S, Colston MJ et al (1993) Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. Lancet 341(8846):647–650
27. Hazbon MH, Brimacombe M, Bobadilla del Valle M, Cavatore M, Guerrero MI, Varma-Basil M et al (2006) Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant Mycobacterium tuberculosis. Antimicrob Agents Chemother 50(8):2640–2649