Xpert MTB/RIF test for rapid diagnosis of *Mycobacterium tuberculosis* and simultaneous detection of multidrug-resistant tuberculous bacillus

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[Abstract] Tuberculosis (TB) is endemic in China with high prevalence of multiple-drug resistant tuberculous bacilli (MDR-TB). The incidence of new cases of TB reaches 1,300 thousand annually. Among them, 5.7 percent are MDR-TB. Staining for acid-fast bacilli in sputum and clinicoradiological examination have been the main diagnostic tools for TB, particularly pulmonary TB. However, the positive rate of sputum Ziehl-Neelsen stain for sputum is disappointedly low, merely 28% in newly-diagnosed TB. Moreover, the radiological manifestations of the patients suspected of TB are often non-specific. All these facts call for a simple, accurate and rapid diagnostic method to overcome this bottleneck, which hinders the success of satisfactory TB control in China.

Employing both hemi-nested RT-PCR and beacon technology with fluorescent probes, the MTB/RIF diagnostic assay specifically amplifies, thus helps detect the rpoB gene, which is unique to *Mycobacterium tuberculosis* and also a biomolecular-marker of rifampin resistance. As a semi-quantitative analysis, the quantity of *Mycobacterium tuberculosis* in samples is reflected by the threshold of PCR cycles during MTB/RIF assay. With *Mycobacterium tuberculosis* culture as the standard reference, for sputum samples from patients suspected of suffering from pulmonary TB, overall diagnostic sensitivity of MTB/RIF assay is 73.1%–90.0% with a specificity of 99.0%–99.5%. For detection of rpoB gene mutations responsible for rifampin-resistance, the sensitivity is 97.2% and specificity is 98.3%. Following sample loading, the system can automatically complete the diagnostic process and report the results within 2 hours. Targeting the rpoB gene specifically, there is no cross-reaction with non-tuberculosis mycobacteria or other common respiratory pathogens. In addition to sputum samples, the system can be used to detect *Mycobacterium tuberculosis* in various body fluids (including pleural effusion, urine, cerebrospinal fluid and even bronchoalveolar lavage fluid) and lung tissue biopsy samples. As for sensitivity, the assay is comparable to *Mycobacterium tuberculosis* culture (for the latter, mean interval between loading and result-reporting is 16 or 30 days (depending upon the culture medium used), and it is 100 times longer than Ziehl-Neelsen stains. Compared with conventional laboratory diagnostic approaches, this assay is much simpler and biohazardous aerosol-free.

[Key words] tuberculosis, multidrug-resistant; diagnostic tests, routine

[CNC Number] R52; R446-33

[Document Code] A

RMP-resistance is particularly amenable to rapid molecular detection since more than 95% of all RMP-resistant strains contain mutations localized within the 81bp core region of the bacterial RNA polymerase rpoB gene, encoding the active site of the enzyme (Figure 2).

Furthermore, mutations occurring in this region are highly predictive of RMP-resistance, whereas susceptible strains invariably have the same wild-type nucleotide sequence. In addition, the rpoB core region is flanked by *M. tuberculosis*-specific DNA sequences. Thus, it is possible to test *M. tuberculosis* and RMP-resistance strain simultaneously by targeting a single amplicon generated using PCR technology. Moreover, RMP-resistance is strongly, although not invariably, indicative of MDR-TB (defined as concomitant resistance to isoniazid, another key antituberculosis agent)[3,7].

The Xpert MTB/RIF test utilizes molecular beacon technology to detect the amplified *M. tuberculosis* DNA sequences and its mutations responsible for Rifampin-resistance in a semi-nested RT-PCR assay (Figure 2). Five different nucleic acid hybridization probes are used in the same multiplex reaction. Each probe is complementary to a different target sequence within the rpoB gene of RIF-susceptible MTB and is labeled with a differently colored fluorophore. Taken together, these overlapping probes span the entire 81bp core region of the rpoB gene. The molecular beacons were designed to specifically hybridize with amplified wild-type rpoB sequences. A mutation within these sequences interferes with hybridization so that the conformational integrity of the probe may be retained in a non-fluorescent state. Thus, a mutation anywhere in the core region of the rpoB gene results in either delayed onset or complete suppression of fluorescence of the corresponding molecular beacon (Figure 2)[3,7].

2 Operation

As illustrated in Figure 1, the Xpert MTB/RIF test is a disposable cartridge-based assay that operates in a self-contained, fully-integrated and automated platform (The GeneXpert system, Cepheid, Sunnyvale, CA, USA). It incorporates microfluidics technology and fully-automated nucleic acid analysis capable of extracting (ultrasonic lysis),
concentrating, detecting and semi-quantifying targeted nucleic acid sequences from clinical samples including sputum, decontaminated sputum pellets, various body fluids such as pleural fluid, cerebral spinal fluid, urine, pus, bronchoalveolar lavage fluids and even homogenized lung tissues.  

*M. tuberculosis* is detected by the five overlapping molecular probes that are collectively complementary to the entire 81bp rpoB core region. *M. tuberculosis* is identified when at least two of the five probes give positive signals with a cycle threshold (CT) less than 38 and that differ by no more than a prespecified number of cycles.

The internal control, *B. globigii*, is positive when the single *B. globigii*-specific probe produces a CT less than 38. The standard user interface indicates the presence or absence of *M. tuberculosis* and the presence or absence of RFP-resistance, and a semi-quantitative estimate of *M. tuberculosis* load are all defined by the CT range ([high, <=16; medium, 16–22; low, 22–28; very low, >28]). Assays that are negative for both *M. tuberculosis* and the *B. globigii* internal control are reported as invalid assays. When performed on unprocessed sputum samples, results are reported within 2 hours with less than 15 minutes of hands-on-time.

### 3 Sensitivity, specificity and clinical impact

Prospective multi-center studies performed in either reference laboratories or laboratories at district and sub-district level demonstrated that the specificity of the MTB/RIF assay for the diagnosis of TB to be very high (97%–100%). The limit of detection of the assay is 131 cfu/ml of *M. tuberculosis* in sputum (95% CI 106–176). This compares with the limits of detection of automated Mycobacterial liquid culture ranging between 10 and 1000 cfu/ml, and compares with an estimated 10,000 cfu/ml for smear microscopy. Henceforth, the MTB/RIF assay has a sensitivity approximately two orders of magnitude greater than smear microscopy, and approaches that of *M. tuberculosis* culture.
Testing a single sputum sample with the MTB/RIF assay detects 98%–100% of sputum smear-positive pulmonary TB, and between 57% and 83% of sputum smear-negative disease with very high specificity in adults suspected of pulmonary TB. Among patients with smear-negative, culture-positive TB, the addition of a second MTB/RIF test increases the sensitivity by 12.6% and a third by 5.1%, to a total sensitivity of 90.2% [9].

In addition to its high specificity and sensitivity, MTB/RIF assay could facilitate the diagnosis of pulmonary TB and RFP-resistance as well as prompt initiation of anti-tuberculosis treatment. Median time for detection of pulmonary TB for the MTB/RIF test was 0 day [interquartile range (IQR) 0–1], compared with 1 day (IQR 0–1) for smear microscopy, 30 days (IQR 23–43) for solid culture, and 16 days (IQR 13–21) for liquid culture. Median time for detection of resistance was 20 days (IQR 10–26) for line-probe assay and 106 days (IQR 30–124) for conventional drug-susceptibility test. Taking sequencing results into account, the MTB/RIF test correctly detected rpoB mutations (RFP-resistance) with 99.1% sensitivity and 100% specificity. Use of the MTB/RIF test reduced median time from diagnosis to initiation of anti-tuberculosis chemotherapy for smear-negative tuberculosis from 56 days (IQR 39–81) to 5 days (IQR 2–8) [10]. Furthermore, there was no cross-reaction with non-tuberculosis Mycobacterium or a range of common respiratory pathogens [7–10].

In children suspected of pulmonary TB, MTB/RIF assay is a reliable test for rapid diagnosis of TB when used on induced sputum specimens: with mycobacterial culture as the reference standard, MTB/RIF assay when done on two induced sputum samples detected twice as many cases (75.9%, 95%CI 64.5–87.2) as did smear microscopy (37.9%, 95%CI 25.1–50.8), detecting all of 22 smear-positive cases and 61.1% of smear negative cases [11].

4 Bio-safety, cost and other issues

MTB/RIF test operates at temperature ranging from 15 to 30°C, even in high-humidity environment. It is easy to train health workers in its use. Treatment with a sodium hydroxide and isopropanol-containing sample reagent can reduce the viability of M. tuberculosis in sputum at least 10^6-fold to reduce biohazard risk, so there is virtually no risk of bio-hazard and no need for a specific biological safety environment [3,7].

However, there are some unresolved issues with this test: cost-benefit in resource-limited settings and false positive result though very rare concerning RFP-resistance. In addition, a few operational headaches such as annual recalibration of the instrument, 18-month shelf-life of the cartridges and safe disposal of large volumes of plastic cartridges must be taken into consideration [3,7].
5 Conclusion

The Xpert MTB/RIF assay, brings great expectations for rapid diagnosis of pulmonary, even extra-pulmonary TB with simultaneous identification of MDR-TB. With heavy burden of TB and high-prevalence of MDR-TB in China, as recommended by WHO and STAG-TB, rapid and extensive implementation of this automated system in major referral hospitals might enormously improve TB control, facilitating the diagnosis or rendering important diagnostic clues for exclusion of TB, providing important and timely laboratory information of MDR-TB and guiding the prompt initiation of appropriate anti-tuberculosis chemotherapy, and significantly reducing transmission of TB and MDR-TB, thereby potentially decreasing morbidity associated with diagnostic delay, dropout and mistreatment.

However, at the present stage, we suggest that the Xpert MTB/RIF assay should be considered as a follow-up test after smear microscopy, though as advocated by STAG-TB and WHO 'the MTB/RIF test should be used as the initial diagnostic test in individuals suspected of having MDR-TB', whereas positive MTB/RIF test for RFP-resistance in patients from low MDR-TB prevalent regions should be confirmed by M. tuberculosis culture.

No potential conflict of interest relevant to this article was reported.

[Reference]


(Received: 2012-08-01; Revised: 2012-09-05)
(Edited by SHEN Ning, ZHANG Jin-tong)