

Letter to the Editor

**Multicenter Evaluation of the Molecular Line Probe Assay for Multidrug Resistant *Mycobacterium Tuberculosis* Detection in China***

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In order to evaluate the performance of a molecular Hain line probe assay (Hain LPA) for rapid detection of rifampicin and isoniazid resistance of *Mycobacterium tuberculosis* in China, 1612 smear positive patients were consecutively enrolled in this study. Smear positive sputum specimens were collected for Hain LPA and conventional drug susceptibility testing (DST). The sensitivity and specificity of Hain LPA were analyzed by using conventional DST as golden reference. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for rifampicin resistance detection were 88.33%, 97.66%, 81.54%, and 98.62%, respectively. The sensitivity, specificity, PPV and NPV for isoniazid resistance detection were 80.25%, 98.07%, 87.25%, and 96.78%, respectively. These findings suggested that Hain LPA can be an effective method worthy of broader use in China.

Tuberculosis (TB) remains a serious health problem worldwide and it ranks as the second leading cause of death from a single infectious agent. Multidrug resistant TB (MDR TB), defined as resistance to at least rifampicin and isoniazid, threatens TB control programs, especially in developing countries and more than one half of these cases occurred in China, India, and the Russian Federation^[1]. It is estimated that about 120,000 MDR TB cases occur every year in China based on data from national drug resistant survey in 2007^[2].

In China, conventional drug susceptibility testing (DST) was widely used as the MDR TB detection method. However, this method usually takes about 2 to 3 months to yield the result and usually delay the diagnosis and appropriate treatment of MDR TB. Therefore, the rapid detection of MDR TB plays a very important role for MDR TB treatment, thereby

reducing morbidity, mortality, economic costs, the further transmission of infection, and the emergence of extensive drug-resistant (XDR) strains. Hain Line probe assay (Hain LPA) is a fast and reliable method to determine resistance to rifampicin and isoniazid by detecting *rpoB*, *katG*, and *inhA* genes mutation and was evaluated in a variety of settings^[3-4]. In 2008, Hain LPA was endorsed by the WHO for MDR TB screening^[5]. Yet, there is no any relative multicenter evaluation data for clinical diagnosis reference in China.

The main purpose of this study was to determine the performance characteristics of Hain LPA for detecting rifampicin and isoniazid resistance using smear positive sputum samples in routine settings. From April to October 2010, the study was conducted in Hohhot city of Inner Mongolia Autonomous region, Kaifeng city of Henan province, Lianyungang city of Jiangsu province, and Yongchuan District of Chongqing. Sputum specimens (three specimens per patient) were collected from smear-positive TB patients at county-level TB clinics and submitted to city TB hospitals twice a week.

The specimen with the highest smear grade from each patient was selected for Hain LPA and solid culture and DST. Conventional DST for detecting rifampicin and isoniazid resistance were performed using proportion method on L-J media^[6]. In order to prevent DNA contamination, strict separation of reagent preparation, specimen preparation, amplification, and hybridization were assumed. A negative control was included in each run of conventional DST. DNA was extracted from processed sputum specimens by heating suspensions in a heating block followed by incubation on ultrasonic bath and centrifugation. PCR was performed using

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HotStar Taq DNA Polymerase, and the number of PCR cycles was 40. 20 µL PCR product was used for hybridization. After hybridization, membrane strips were attached to the evaluation sheet and were read and interpreted by a trained laboratory technician.

For the specimens with inconsistent results between DST and LPA testing, we further amplified and sequenced the corresponding drug-resistance related gene fragments by the methods reported previously^[7]. Results from sequencing were entered into the Basic Local Alignment Search Tool (BLAST), an international data bank (www.ncbi.nlm.nih.gov/BLAST), and were compared with the corresponding genes of *M. tuberculosis* strain H37Rv. Sensitivity,

specificity, PPV, and NPV of the LPA assay for detecting rifampicin and isoniazid resistance were calculated in comparison to conventional DST by using SPSS 15.0 software.

A total of 1612 smear-positive TB patients were recruited in the present study. Among 1612 specimens, 237 specimens were not used for DST due to negative specimen culture result, culture contamination and Non-Tuberculous Mycobacteria (NTM). However, valid Hain LPA results were obtained from 1471 specimens. Hain LPA results from rest of 141 were either negative for TB or invalid. 1307 specimens in total were therefore valid for evaluation of the performance of Hain LPA test (Figure 1).

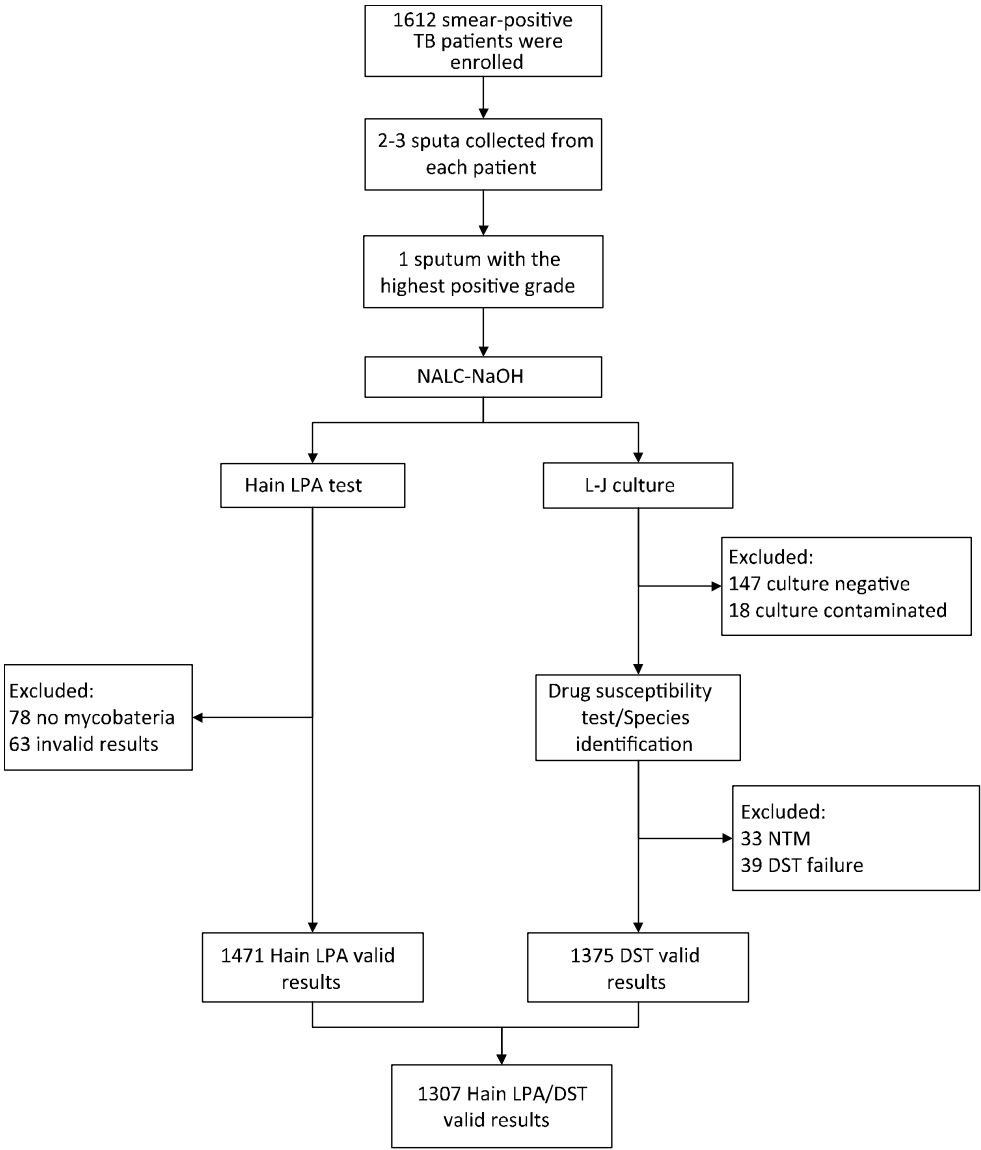


Figure 1. Enrollment and outcomes.

Among the 1307 specimens, the performance characteristics of Hain LPA were calculated using conventional DST as the standard reference (Table 1). The consistent rate of results for rifampicin between Hain LPA and DST was 96.3% (1258/1307). Among 139 phenotypic rifampicin-resistant specimens, 123 were identified as resistant by Hain LPA test, indicating a sensitivity of 88.5%. Meanwhile, among the 1168 phenotypic rifampicin-susceptible specimens, 1135 were identified as susceptible by Hain LPA, resulting in a specificity of 97.2%. Among the four study sites in our present study, the sensitivity of Hain LPA test for detecting rifampicin resistance in Yongchuan (96.6%) was the highest most likely due to the performance was conducted by experienced laboratory staffs. The test specificity was more than 90.0% in all four study sites The PPV and NPV of Hain LPA test for detecting rifampicin resistance were 78.9% and 98.6%, respectively. The sensitivity for detecting rifampicin was lower when those from other studies^[8] and this might be due to the low sensitivity of the solid culture, which was used as the reference standard in this study while liquid culture has commonly been used elsewhere as the reference standard. Another reason for the lower PPV might be related to the delayed testing due to the delayed submission of sputum specimens to the city level laboratories from the study counties which located far away from the cities.

The consistent rate of the results on detecting isoniazid resistance between DST and Hain LPA was

95.0% (1241/1307) (Table 1). Among the 180 phenotypic isoniazid-resistant specimens, 140 were identified as resistant by Hain LPA, indicating a sensitivity of 77.8%. Meanwhile, among the 1127 phenotypic isoniazid-susceptible specimens, 1101 were identified as susceptible by Hain LPA, indicating a specificity of 97.7%. The PPV and NPV of Hain LPA test for detecting isoniazid resistance were 84.3% and 96.5%, respectively.

We also analyzed the performance of Hain LPA for MDR TB detection. The consistent rate of the results on detecting MDR TB between DST and Hain LPA was 96.6% (1263/1307) (Table 2). Among the 102 MDR TB patients identified by DST, 76 patients were identified as MDR TB by Hain LPA, resulting in a sensitivity of 74.5%. Also, among the 1205 non MDR TB patients identified by DST, 1187 patients were identified as non MDR TB by Hain LPA, indicating a specificity of 98.5%. The PPV and NPV of Hain LPA test for detecting MDR TB were 80.9% and 97.9%, respectively.

Some previous studies showed that approximately 90% of patients with rifampicin-resistant TB might be also resistance to isoniazid^[9]. However, in our present study, we found that 102 (73.38%) MDR TB cases were detected among the 139 rifampicin-resistant cases diagnosed by conventional DST. This might be, most likely, due to high differentiation of *M. tuberculosis* in China. Therefore, we suggest that the Rifampicin resistance could not be used as a indicator of MDR TB in China.

Table 1. Performance of Hain LPA for the Rifampicin and Isoniazid Resistance Detection

Drug	Hain LPA	DST		Total	Sensitivity (95% CI)	Specificity (95% CI)	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
		R	S					
Rifampicin	R	123	33	156	88.5% (83.2%-93.8%)	97.2% (96.2%-98.1%)	78.9% (72.4%-85.3%)	98.6% (97.9%-99.3%)
	S	16	1135	1151				
	Total	139	1168	1307				
Isoniazid	R	140	26	166	77.8% (71.7%-83.9%)	97.7% (96.8%-98.6%)	84.3% (78.8%-89.9%)	96.5% (95.4%-97.6%)
	S	40	1101	1141				
	Total	180	1127	1307				

Table 2. Performance of Hain LPA for MDR TB Detection

Hain LPA	DST		Total	Sensitivity (95% CI)	Specificity (95% CI)	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
	MDR	Not MDR					
MDR	76	18	94	74.5% (66.1%-83.0%)	98.5% (97.8%-99.2%)	80.9% (72.9%-88.8%)	97.9% (97.1%-98.7%)
Not MDR	26	1187	1213				
Total	102	1205	1307				

For 49 cases with inconsistent rifampicin resistance results between DST and Hain LPA, the rifampicin resistance-determining region (RRDR) of *rpoB* gene was sequenced for rifampicin resistance confirmation. Among the 33 strains with Hain LPA-rifampicin resistant and DST-rifampicin sensitive results, 19 strains showed mutations in the *rpoB* gene. It means that the conventional DST has lower sensitivity. No mutation was found in the other 14 cases. Among the 16 Hain LPA-rifampicin-sensitive and DST-rifampicin-resistant cases, no mutation was observed in 10 cases. The other 6 cases showed mutations at codon 531 in the *rpoB* gene. This might be due to that the resistant bacteria population only accounted for a minor ratio among the total germ population.

Findings from our present study suggest that LPA assay can be directly used for detecting sputum specimen and could be applied in clinical laboratory when a rapid sensitivity assay is required for MDR TB diagnosis or for screening purpose. In addition, Hain LPA is a easy assay to be performed and this method can shorten the turnaround time of drug resistance report compared with conventional DST. However, this assay can't be considered as a full alternative in the future to conventional DST due to the limitation that only some of the mutations are targeted in this study. Studies are therefore needed for China National Tuberculosis Program to further explore the characteristics of Hain LPA for its application .

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REFERENCES

1. WHO. Global tuberculosis report 2014. Geneva, Switzerland: World Health Organization, 2014.
2. Zhao YL, Xu SF, Wang LX, et al. National survey of drug-resistant tuberculosis in China. *N Engl J Med*, 2012; 366, 2161-70.
3. Bang D, Bengard Andersen A, Thomsen VO. Rapid genotypic detection of rifampicin- and isoniazid-resistant *Mycobacterium tuberculosis* directly in clinical specimens. *J Clin Microbiol*, 2006; 44, 2605-8.
4. Lacombe A, Garcia-Sierra N, Prat C, et al. GenoType MTBDRplus Assay for Molecular Detection of Rifampicin and Isoniazid Resistance in *Mycobacterium tuberculosis* Strains and Clinical Samples. *J Clin Microbiol*, 2008; 46, 3660-7.
5. WHO: Policy Statement. Molecular Line Probe Assays for Rapid Screening of patients at risk of multidrug resistant tuberculosis (MDR-TB). 2008. http://www.who.int/tb/dots/laboratory/lpa_policy.pdf [2014-03-16].
6. Kent PT, Kubica GP. Public health mycobacteriology: a guide for the level III laboratory. Atlanta, GA: Centers for Disease Control, 1985; 55-60.
7. Aslan G, Tezcan S, Serin MS, et al. Genotypic analysis of isoniazid and rifampicin resistance in drug resistant clinical *Mycobacterium tuberculosis* complex isolates in southern Turkey. *Jpn J Infect Dis*, 2008, 61, 255-60.
8. Yadav RN, Singh BK, Sharma SK, et al. Comparative evaluation of GenoType MTBDRplus line probe assay with solid culture method in early diagnosis of multidrug resistant tuberculosis (MDR-TB) at a tertiary care centre in India. *PLoS One*, 2013; 8, 1371-80.
9. Pang Y, Lu J, Wang YF, et al. Study of the rifampicin monoresistance mechanism in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*, 2013; 57, 893-900.