



Evaluation of GeneXpert MTB/RIF for detecting *Mycobacterium tuberculosis* in a hospital in China

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Abstract

Objective: To evaluate the performance of GeneXpert MTB/RIF in diagnosing pulmonary tuberculosis (TB) in China.

Methods: This cross-sectional study included sputum specimens of 240 suspected TB cases. Specimens were examined by light microscopy for the presence of acid-fast bacilli, which were cultured by the BACTEC MGIT 960 (M960) system and detected by the GeneXpert MTB/RIF assay. The positive rate, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and average turnaround time of methods were evaluated.

Results: The positive rate was 36.6% (87/238) for the GeneXpert MTB/RIF assay and 34.0% (81/238) by M960 culture, with no significant difference between methods ($\chi^2 = 0.33$, $p > 0.05$). According to culture results, sensitivity of the GeneXpert MTB/RIF assay was 84.0% (68/81), specificity was 87.8% (129/147), the PPV was 78.2% (68/87), and the NPV was 87.2% (129/148). The agreement for results between Gene Xpert MTB/RIF and the M960 system was 82.8% and the Kappa value was 0.73.

Conclusion: The GeneXpert MTB/RIF assay is a simple, rapid, and accurate test for detecting *Mycobacterium tuberculosis* in sputum specimens.

Keywords

Tuberculosis, GeneXpert MTB/RIF, China

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Introduction

Tuberculosis (TB) is a deadly infectious disease that is caused by *Mycobacterium tuberculosis* (M.tb) worldwide. In 2014, 9.6 million people were estimated to have fallen ill with TB and 1.5 million people died because of TB worldwide.¹ China has

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the third highest number of incidents and deaths from TB (after India and Indonesia). China accounts for 9.7% of cases of TB globally. The estimated number of incident and fatal cases of TB was 930,000 and 38,000, respectively, in 2014 (1). Therefore, TB remains one of the world's largest threats.

At present, the major problem in managing TB is lack of an accurate and rapid diagnostic test for *M.tb*. Rapid detection of *M.tb* in infected patients is essential for diagnosis and treatment of TB because of the high risk of transmission from person to person.² In China, peripheral antituberculosis clinics usually rely on acid-fast staining and the conventional Löwenstein–Jensen culture method in conjunction with assessment of clinical symptoms and radiographic evidence to diagnose TB.³

Culture is considered the gold standard technique for diagnosing TB, but it is slow and may take 2 to 8 weeks. Although sputum smear microscopy for acid-fast bacilli (AFB) is rapid and inexpensive, it has poor sensitivity.² Therefore, rapid detection of *M.tb*, which is essential for early diagnosis and treatment, improving patients' outcomes, and taking effective public health measures, relies on nucleic acid amplification techniques.⁴ Several molecular methods have been developed in recent years for rapid detection of *M.tb* and drug resistance in clinical samples, including line probe assays and real-time polymerase chain reaction (PCR).

GeneXpert mycobacterium tuberculosis (MTB)/rifampicin (RIF) is a new cartridge-based, automated and rapid molecular diagnostic device that performs sample processing and hemi-nested real-time PCR analysis in a single, hands-free step for identifying *M.tb* and rapid detection of rifampicin (RIF) resistance in sputum samples.^{5,6} This study aimed to evaluate the performance of the GeneXpert MTB/RIF system in diagnosing TB in a hospital in China.

Materials and methods

This cross-sectional study included data that were collected between July 2014 and October 2014 at Zhejiang Hospital where there are 600 estimated cases of TB annually. One sputum specimen from each patient with the clinical suspicion of TB (cough of ≥ 2 weeks and fever, weight loss of greater than 3 kg or dyspnoea, and having radiographic imaging features of TB) was collected before treatment. Medical information of the patients, such as age, sex, occupation, address, and clinical signs and symptoms, were recorded by doctors.

Quality controls

Quality control of each batch was conducted with the positive control strain H37Rv (ATCC 27294) and the negative control strain *Escherichia coli* (ATCC 25922). If a positive control stain was identified as negative or a negative control strain was identified as positive by smear, the M960 system, or GeneXpert MTB/RIF, all tests of that batch were then repeated.

Reproducibility of testing

Reproducibility of the GeneXpert MTB/RIF assay was evaluated using a blinded panel of five AFB smear-positive (1+) and five AFB smear-negative sputum specimens. Each specimen was tested in triplicate on three different occasions.

AFB smears

Before processing of specimens, smears were performed and stained by the Ziehl–Neelsen (Z-N) method and examined with a light microscope for the presence of AFB.

Culture and drug susceptibility test

One sputum specimen was divided into two portions. One portion was decontaminated

by the N-acetyl-L-cysteine-NaOH method.⁷ The decontaminated specimen was inoculated to the liquid medium of the BACTEC MGIT 960 (M960) system (Becton Dickinson, USA) for detection of growth of mycobacteria. The culture method was performed by following the standard procedure of the manufacturer. Positive isolates were tested for susceptibility to first-line antituberculosis drugs (isoniazide, RIF, streptomycin, and ethambutol) by the M960 system. This method was performed by following the standard procedure of the M960 SIRE kit. These isolates were tested for the presence of M.tb or nontuberculous mycobacteria (NTM) by PNB and TCH medium growth tests.

GeneXpert MTB/RIF assay

Another portion of the specimen was tested by the GeneXpert MTB/RIF assay. This assay was performed as described previously.^{5,8} Briefly, 2.0 ml of GeneXpert MTB/RIF sample reagent was added to 1.0 ml of sputum specimen using a sterile pipette. The closed specimen container was manually agitated twice during 15 min at room temperature and then 2 ml of the inactivated material was transferred to the test cartridge.

Statistical analysis

Data are presented as mean \pm SD (range) or n (%). The recovery and (or) contamination rates of the three systems were compared using the χ^2 test. *P* values < 0.05 were considered to be statistically significant and statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) for Windows. The sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and Kappa value were calculated using Excel 2013 software.

Ethics approval

The study was approved by the Ethics Committee of Zhejiang Hospital. All patients in the study provided written informed consent and were told that this study would not increase their costs and pain.

Results

Sputum specimens of 240 patients (112 females and 128 males) were collected. All of the patients had a cough for ≥ 2 weeks and fever, weight loss of greater than 3 kg or dyspnoea, and radiographic imaging features of TB. The median age of patients was 36.5 years (range, 16–78 years).

The consistency of the reproducibility test of GeneXpert MTB/RIF was 100% in 90 tests of 10 sputum specimens.

As shown in Table 1, among the 240 sputum specimens, 45 were smear-positive for AFB and 83 were culture-positive for mycobacteria. Two of the 83 isolates were confirmed as NTM. Ten culture tubes were considered contaminated. A total of 87 were positive and three tests of GeneXpert MTB/RIF failed.

The positive rates of Z-N smear, M960 culture, and GeneXpert MTB/RIF for M.tb were 18.9% (45/238; 95% confidence interval [CI], 13.9 to 23.9), 34.0% (81/238; 95% CI, 28.0 to 40.1), and 36.6% (87/238; 95% CI, 30.4 to 42.7), respectively. The differences between GeneXpert MTB/RIF and Z-N smear and between M960 culture and Z-N smear were significant (χ^2 was 18.49 and 13.99, respectively, both $p < 0.01$). However, there was no significant difference between GeneXpert MTB/RIF and M960 culture ($\chi^2 = 0.33$, $p > 0.05$).

According to culture results, sensitivity analysis of the GeneXpert MTB/RIF assay and Z-N smear examination were carried out. Sensitivity of the GeneXpert MTB/RIF test was 84.0% (68/81; 95% CI, 76.0 to 91.9), specificity was 87.8% (129/147; 95% CI, 82.5 to 93.1), the PPV was 78.2% (68/87; 95% CI,

Table 1. Results, and sensitivity, specificity, and predictive values of the GeneXpert MTB/RIF test and Z-N smear method according to culture results.

	M960 culture										Total	Sensitivity (%; 95% CI)	Specificity (%; 95% CI)	PPV (%; 95% CI)	NPV (%; 95% CI)	
	M.tb					NTM										
	P	N	C	P	N	C	P	N	C	P						N
GeneXpert	P	68	15	4	0	0	0	0	0	0	0	87	84.0	87.8	78.2	87.2
MTB/RIF	N	13	129	6	2	0	0	0	0	0	0	150	(76.0–91.9)	(82.5–93.1)	(69.5–86.8)	(81.8–92.6)
	F	0	3	0	0	0	0	0	0	0	0	3				
Total		81	147	10	2	0	0	0	0	0	0	240				
Z-N smear	P	42	2	1	0	0	0	0	0	0	0	45	51.9	98.6	93.3	75.1
	N	39	145	9	2	0	0	0	0	0	0	195	(41.0–62.7)	(96.8–100.0)	(86.0–100.0)	(69.0–81.2)
Total		81	147	10	2	0	0	0	0	0	0	240				

M960, BACTEC MGIT 960 system; PPV, positive predictive value; NPV, negative predictive value; Z-N, Ziehl-Neelsen; M.tb, *Mycobacterium tuberculosis*; NTM, nontuberculosis mycobacteria; P, positive; N, negative; F, failure; C, contaminated; CI, confidence interval.

69.5 to 86.8), and the NPV was 87.2% (129/148; 95% CI, 81.8 to 92.6). Sensitivity of the Z-N smear test was 51.9% (42/81; 95% CI, 41.0 to 62.7), specificity was 98.6% (145/147; 95% CI, 96.8 to 100.0), the PPV was 93.3% (42/45; 95% CI, 86.0 to 100.0), and the NPV was 75.1% (145/193; 95% CI, 69.0 to 81.2). The differences in sensitivity, specificity, PPV, and NPV between the GeneXpert MTB/RIF test and the Z-N smear test were significant (χ^2 values were 19.15, 13.73, 4.92, and 7.68 respectively, all $P < 0.05$).

The agreement for results between the Gene Xpert MTB/RIF assay and the M960 culture system was 82.8% and the Kappa value was 0.73 (95% CI, 0.61 to 0.86) using Kappa statistics. Nineteen specimens were positive by GeneXpert MTB/RIF, but negative by the M960 system. In the 19 cases, 15 were diagnosed as TB by doctors and the patients received antituberculous treatment. However, the other cases were diagnosed as old pulmonary tuberculosis (two cases) and tuberculosis stable stage (two cases) without treatment.

The mean turnaround time of one GeneXpert MTB/RIF assay result was 2.5 ± 0.5 hours, that of Z-N smear microscopy was 3.5 ± 0.8 hours, and that for a positive result of M960 culture was 12 ± 5 days (range, 5–33 days), with 42 days for a negative result of culture.

Seven cases were detected as RIF-resistant by the GeneXpert MTB/RIF assay. Four of seven cases were confirmed to be MDR-TB by a drug sensitive test using the MGIT SIRE kit. Two of seven cases did not have positive M960 culture results. One of seven cases was detected as rifampin-resistant by the GeneXpert MTB/RIF assay, but rifampin susceptibility was detected using the MGIT SIRE kit.

Discussion

In this study, performance of the GeneXpert MTB/RIF assay with sputum specimens of

patients who were suspected as having pulmonary TB was evaluated. The positive rate was 36.6% by GeneXpert MTB/RIF, and this rate was higher than that (18.9%) by a direct AFB smear examination and by M960 culture (34.0%). However, the difference in positive rate between GeneXpert MTB/RIF and M960 culture was not significant. The sensitivity and specificity of the GeneXpert MTB/RIF assay was 84.0% and 87.8%, respectively. Our finding of sensitivity is similar to that from previous studies (82.1%–90.3%) in several different countries,^{2,9–11} but it is lower than that found in some other studies (99.1% and 97.1%).^{12,13} Our finding of specificity is lower than that (93.8%–100.0%) in some previous studies.^{2,9–13} These wide variations may reflect differences in quality of specimens, collection, transport, and testing times.

Our finding that the Z-N smear is less sensitive than the GeneXpert MTB/RIF test and M960 culture is reasonable because the Z-N smear method requires 5×10^3 to 1×10^4 bacilli/ml of specimen to generate a positive result.¹⁴ However, the GeneXpert assay only requires 131 bacilli/ml of specimen and M960 culture requires as low as 10 to 100 bacilli/ml.^{5,14}

In our study, although the difference in positive rate between the GeneXpert MTB/RIF assay and the M960 culture method was not significant, the positive rate of the former (36.6%) was higher than that of the latter (34.0%). Discovering TB in patients early and treating them in time may be important.

Agreement of results of the GeneXpert MTB/RIF assay and the M960 system was medium (82.8%; Kappa value, 0.73). Nineteen sputum specimens were positive with GeneXpert MTB/RIF, but were negative with the M960 system, and thirteen were negative with GeneXpert MTB/RIF, but were positive with the M960 system. When detecting at the lower limits of any assay, variability is to be expected because of

various factors, such as sampling and processing. Additionally, the GeneXpert MTB/RIF assay detects DNA of M.tb, including live and dead bacilli, but the M960 system only detects living M.tb. Therefore, some bacilli may be killed by sodium hydroxide in processing and cannot be detected by the M960 culture method. The reason why M.tb in some specimens is not detected by GeneXpert TB/RIF, but is detected by the M960 system, could be that there are low numbers of bacilli that are under the lower limit of detection of the GeneXpert TB/RIF assay.

In this study, two isolates of NTM were recovered by the M960 system and identified by the TCH and PNB test, but were negative with the GeneXpert TB/RIF assay. This is because GeneXpert TB/RIF is a nucleic acid amplification test for detection of distinctive DNA of the M.tb complex, exclusive of NTM.⁸ However, M960 is a culture system for microorganisms that cannot be killed by the N-acetyl-L-cysteine-NaOH method. Three samples failed to be detected by the GeneXpert TB/RIF assay. The reason for this lack of detection may be because the sample processing control did not meet the acceptance criteria (i.e., the sample was not properly processed) or PCR was inhibited, and one or more of the probe check control results failed.⁸ The rate of contamination of M960 was 4.2% (10/240). Therefore, a small proportion of cultures are likely to be contaminated by other organisms. As a general rule, a contamination rate of 2%–3% is acceptable in laboratories that receive fresh specimens,¹⁵ and the Chinese Antituberculosis Association suggests that the contamination rate should be controlled at 2%–5%.¹⁶ Therefore, the contamination rate was acceptable in our study. With a four-specimen batch, the average turn-around time (including time taken to process specimens and testing time) of the GeneXpert MTB/RIF assay and Z-N smear method was significantly shorter

(2.5 and 3.5 hours) than that of the M960 system (5–42 days). A shorter turnaround time can help TB patients be diagnosed early and treated in time. Therefore, the GeneXpert MTB/RIF assay has an obvious advantage in management of TB.

In a multicentre implementation study, GeneXpert MTB/RIF test sensitivity for RIF resistance was 94.4% and specificity was 98.3%.⁹ In our study, seven cases were resistant to RIF with the GeneXpert MTB/RIF assay and four cases of these were resistant to RIF with the GeneXpert MTB/RIF and the M960 system. However, a robust conclusion cannot be made about the sensitivity of GeneXpert MTB/RIF for diagnosis of MDR-TB because of the low incidence of MDR-TB in this study.

The GeneXpert MTB/RIF assay is a simple, rapid, and accurate test method for detecting M.tb in sputum specimens, is less dependent on the operator's skills, and staff with minimal training can use the equipment. Although the GeneXpert MTB/RIF assay has these advantages, similar to other tests for M.tb, a negative result cannot exclude the diagnosis of TB, and patients with positive results can also be assessed comprehensively with results of the Z-N smear test, culture, clinical symptoms, and radiographic evidence.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. World Health Organization. Global Tuberculosis Report 2015, Geneva: WHO, WHO/HTM/TB/2015.22.
2. Zeka AN, Tasbakan S and Cavusoglu C. Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. *J Clin Microbiol* 2011; 49: 4138–4141.
3. Xia H and Zhao YL. The application prospect of Xpert MTB/RIF in China. *The Journal of Practical Medicine* 2013; 29: 3799–3800.
4. Centers for Disease Control and Prevention (CDC). Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. *MMWR Morb Mortal Wkly. Rep* 2009; 58: 7–10.
5. Blakemore R, Story E, Helb D, et al. Evaluation of the analytical performance of the Xpert MTB/RIF assay. *J Clin Microbiol* 2010; 48: 2495–2501.
6. Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol* 2010; 48: 229–237.
7. Gullans CR Sr. Digestion-decontamination procedures. In: Isenberg HD (ed.) *Clinical microbiology procedure handbook*. Washington, DC: ASM Press, 1992, pp.3.4.1–3.4.14.
8. Bodmer T and Ströhle A. Diagnosing Pulmonary Tuberculosis with the Xpert MTB/RIF Test. *J Vis Exp* 2012; 62: e3547.
9. Boehme CC, Nicol MP, Nabeta P, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *Lancet* 2011; 377: 1495–1505.
10. Bajrami R, Mulliqi G, Kurti A, et al. Comparison of GeneXpert MTB/RIF and conventional methods for the diagnosis of

- tuberculosis in Kosovo. *J Infect Dev Ctries* 2016; 10: 418–422.
11. Heidebrecht CL, Podewils LJ, Pym AS, et al. Assessing the utility of Xpert[®] MTB/RIF as a screening tool for patients admitted to medical wards in South Africa. *Sci Rep* 2016; 6: 19391.
 12. Bojang AL, Mendy FS, Tientcheu LD, et al. Comparison of TB-LAMP, GeneXpert MTB/RIF and culture for diagnosis of pulmonary tuberculosis in The Gambia. *J Infect* 2016; 72: 332–337.
 13. Bunsow E, Ruiz-Serrano MJ, López Roa P, et al. Evaluation of GeneXpert MTB/RIF for the detection of *Mycobacterium tuberculosis* and resistance to rifampin in clinical specimens. *J Infect* 2014; 68: 338–343.
 14. Diagnostic standards and classification of tuberculosis in adults and children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. *Am. J. Respir. Crit. Care Med* 2000; 161(4 Pt 1): 1376–1395.
 15. Zhao P, Yu Q, Chen L, et al. Evaluation of a liquid culture system in the detection of mycobacteria at an antituberculosis institution in China; A retrospective study. *J Int Med Res* 2016; 44: 1055–1060.
 16. Song YY, Zheng HW and Zhao YL. The development of TB laboratory in China. *Chin J Antituberc* 2014; 36: 764–768. [in Chinese].