

Mycobacterium tuberculosis Detection in Diverse Clinical Specimens by GeneXpert MTB/RIF: A Large-Scale Retrospective Study

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Purpose: *Mycobacterium tuberculosis* (MTB) infection poses a significant global health challenge, with conventional diagnostic methods like acid-fast smear and culture techniques exhibiting limitations in sensitivity and efficiency. This retrospective study examined 4098 clinical samples from China-Japan Friendship Hospital between March 2018 and March 2019, focusing on the diagnostic performance of GeneXpert MTB/RIF across different specimen types.

Methods: The study encompassed various sample types, including bronchoalveolar lavage fluid (BALF), sputum, lung tissues, hydrothorax, and others. All samples were performed via acid-fast-staining, GeneXpert MTB/RIF and culture.

Results: GeneXpert MTB/RIF demonstrated superior sensitivity (81.46%) and specificity (98.98%) in respiratory specimens compared to tissues (62.50%) and hydrothorax (46.15%). Notably, in acid-fast-staining-negative samples, GeneXpert MTB/RIF showed sensitivity and specificity of 73.43% and 98.8%, respectively, and reduced false negatives of acid-fast staining. Furthermore, the study explored Cyclical Threshold (CT) values, revealing associations with bacterial load and sample types.

Conclusion: The findings highlight the importance of considering sample types in MTB diagnosis and underscore the potential of GeneXpert MTB/RIF as a valuable diagnostic tool, especially in respiratory specimens, contributing to improved tuberculosis management strategies. Additionally, the study recommended direct GeneXpert MTB/RIF testing of hydrothorax samples instead of acid-fast staining, further enhancing diagnostic accuracy and efficiency.

Keywords: acid-fast staining, threshold cycle, diagnosis, GeneXpert MTB/RIF, *Mycobacterium tuberculosis*, tuberculosis

Introduction

Mycobacterium tuberculosis (MTB) infection remains a significant global challenge.¹ Approximately 1.2 million people succumb to MTB each year, with nearly 10 million new cases reported annually.² China is also considered a high-burden region for MTB.³ TB is a major public health concern worldwide, ranking above HIV/AIDS and PTB/EPTB diagnosis exhibits serious challenges owing to paucibacillary nature of specimens and localization of disease at sites that are

difficult to access.^{4,5} Until the COVID-19 pandemic, TB was the foremost cause of death from a single infectious agent and is now the second leading infectious killer after COVID-19.^{6,7}

For decades, acid-fast staining and traditional culture techniques have been considered the mainstays of MTB diagnostics. While the sensitivity of acid-fast staining and its consistency was compromised by various factors,⁸ culture, the gold standard for detecting tuberculosis, was time-consuming.⁹ Both methods could effectively meet the clinical needs for MTB diagnosis. Since 2010, a rapid and accurate nucleic acid amplification test for diagnosing MTB emerged, marking a significant leap forward in tuberculosis diagnosis. GeneXpert MTB/RIF used for MTB detection, provided results within 2 hours with high sensitivity and specificity, representing a major improvement over traditional detection techniques.¹⁰ GeneXpert MTB/RIF is a cartridge-based NAAT (CBNAAT), using *rpoB* (Rv0664) that concomitantly identifies TB and rifamycin resistance.¹¹ In 2010, GeneXpert MTB/RIF was co-developed by Cepheid and FIND, later the WHO endorsed the use of GeneXpert MTB/RIF (is automated, hemi-nested real-time PCR) for pulmonary tuberculosis diagnosis that has played a major role for high TB burden countries including India towards achieving the “End-TB Goal”.¹² Afterward, the WHO endorsed GeneXpert MTB/RIF for rapid detection of pediatric-TB including other extrapulmonary tuberculosis types and could be used as an alternative of traditional acid-fast staining for initial detection of adult TB patients.¹³

Due to its high sensitivity, GeneXpert MTB/RIF has been recommended by guidelines for the early and precise diagnosis and management of tuberculosis patients.¹⁴ It has been proven to effectively reduce clinical costs.¹⁵ Although GeneXpert MTB/RIF exhibits excellent diagnostic performance for pulmonary tuberculosis using sputum samples, its diagnostic efficacy in extrapulmonary tuberculosis remains suboptimal, and studies evaluating its performance in non-sputum specimens are limited.^{7,16–19} Several factors, including low bacterial load and sample processing methods, may contribute to diagnostic failures of GeneXpert MTB/RIF in certain clinical settings.²⁰ Therefore, the influence of different specimen types on GeneXpert MTB/RIF results should not be overlooked and merits further investigation. The GeneXpert MTB/RIF Ultra, the advanced iteration of the GeneXpert MTB/RIF, exhibits enhanced sensitivity and demonstrates improved diagnostic performance across various specimen types.^{11,21} But WHO has not yet recommended it as the gold standard diagnostic method.²¹ Additionally, due to the low sensitivity of acid-fast staining and variations in disease progression, 40–50% of MTB patients might have negative acid-fast staining results, leading to potential missed diagnoses.²² Therefore, the evaluation of MTB diagnosis using GeneXpert MTB/RIF in conjunction with acid-fast staining as a preliminary screening test is worthy of exploration.

Hence, this study aims to conduct a retrospective investigation of 4098 clinical samples collected from China-Japan Friendship Hospital (CJFH) between March 2018 and March 2019. The research intended to explore the influence of different sample types on the results of GeneXpert MTB/RIF in clinical MTB diagnosis.

Materials and Methods

Ethics Statement

Permission to use the information in the medical records of the patients for research purposes was granted by the Ethics Committee of the China-Japan Friendship Hospital (CJFH) (2022-KY-133). Our research is in line with the exemption type of informed consent and ethics approval that

Using identifiable human body materials or data for research, it is no longer possible to locate the subject, and the research project does not involve personal privacy disclosure or commercial interests.

As this is a retrospective cohort study based on previous clinical diagnosis and treatment results, the Ethics Committee of the China-Japan Friendship Hospital granted the study exemption status. In addition, we declare that this study is in line with the ethical guidelines of the Declaration of Helsinki, and the patient-related data is strictly confidential.

Sample Processing and Definitions

The clinical and laboratory data were collected from the medical record system and laboratory information system of China-Japan Friendship Hospital (CJFH) between March 2018 and March 2019, retrospectively. Patients who underwent

acid-fast staining, GeneXpert MTB/RIF (Cepheid, Inc., Sunnyvale, CA, USA), and culture (Bactec Myco/F lytic culture and Bactec mycobacterial growth indicator tube 960 system) were included in the study. The sample data included the sample collection site, the results of acid-fast staining, culture system and GeneXpert MTB/RIF results including the Cyclar Threshold (CT) values.

Fluid samples such as bronchoalveolar lavage fluid (BALF), hydrothorax, etc. were centrifuged at 3000 rpm for 3 min, and then subjected to acid-fast staining and culture. Long or large tissues were minced with sterile scissors or scalpel blades, and then the tissue samples were placed in a 10 mL glass container and grinder (Naitong Industrial Products Co., Ltd., Guangzhou, China) to grind and homogenize, then the acid-fast staining and GeneXpert MTB/RIF were employed considered as MTB positivity.

Statistical Analysis

To better describe diagnostic performance in detecting MTB, relevant indicators for methodological evaluation including sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Likelihood Ratio (LR), and κ value, were obtained via Microsoft Excel 16. The chi-square test and Fisher's exact test were performed to compare the impact of different samples on GeneXpert MTB/RIF results using the GraphPad Prism 8, the two-side P value of <0.05 was considered statistically significant.

Data Availability

The original data presented in the current study are all included in the article. Further inquiries can be directed to the corresponding author.

Results

Clinical Characteristics

In the present study, a total of 4098 specimens were collected from CJFH between March 2018 and March 2019. After excluding individuals who met the exclusion criteria, there were 4098 samples included in the final analysis. Among these, 2405 (58.7%) were male. The age of the cohorts ranged from 14 to 97 years with the median age being 61 years. The types of clinical specimens from which used for MTB detection included BALF (1809, 44.14%), sputum (1633, 39.85%), lung tissues (350, 8.54%), hydrothorax (283, 6.91%), and others (23, 0.56%), as shown in Figure 1 and Table 1.

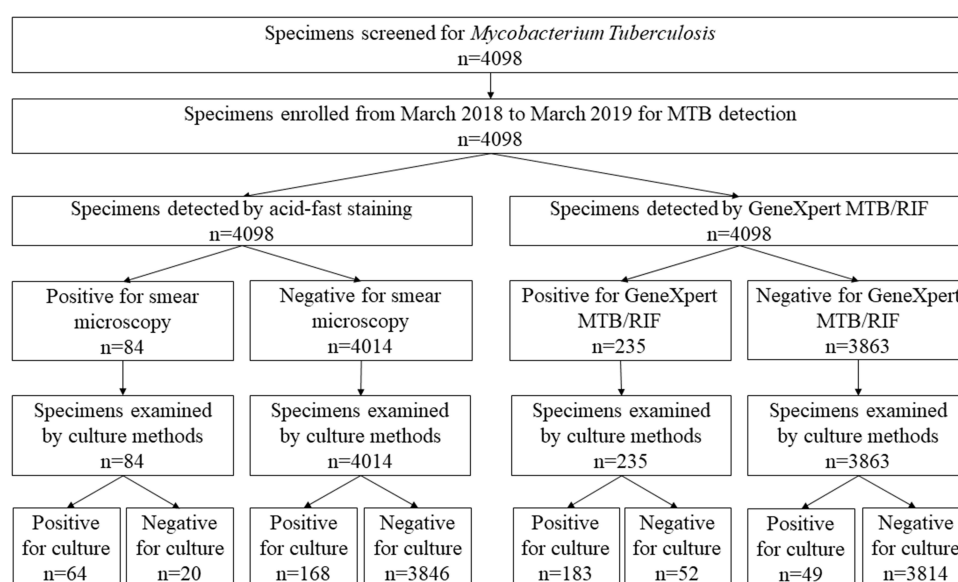


Figure 1 Diagnostic accuracy of GeneXpert MTB/RIF and acid-fast staining for detection of MTB from suspected tuberculosis patients based on retrospective data.

Table 1 Characteristics of All Patients and Samples Investigated in This Study

Characteristic	Value
Age, median(range)(yr)	61 (14–97)
Gender	
Male	2405(58.69%)
Female	1693(41.31%)
Specimen type	
BALF	1809(44.14%)
Sputum	1633(39.85%)
Tissue	350(8.54%)
Hydrothorax	283(6.91%)
Others*	23(0.56%)
Total	4098(100%)

Note: *Others include urine, cerebrospinal fluid, pus, ascites and other samples.

Performance of the Acid-Fast Staining and the GeneXpert MTB/RIF in MTB Detection

In the study involving 4098 samples, sequential application of acid-fast staining and GeneXpert MTB/RIF revealed higher specificity for GeneXpert MTB/RIF (98.65%, 3814/3866) compared to acid-fast staining (99.48%, 3846/3866) as summarized in Table 2, Figures 1 and 2A. The sensitivity of GeneXpert MTB/RIF (78.88%, 183/232) surpassed that of acid-fast staining (27.59%, 64/232). GeneXpert MTB/RIF demonstrated slightly greater accuracy in MTB-positive

Table 2 Detection Performance of GeneXpert MTB/RIF and Acid-Fast Staining in All Kinds of Samples, and the Diagnostic Efficiency of GeneXpert MTB/RIF Among Acid-Fast Staining Grouped Samples

Sample Types		Sensitivity	Specificity	PPV	NPV	LR+	LR-	Kappa
All sample	Smear microscopy	64/232 27.59%	3846/3866 99.48%	64/84 76.19%	3846/4014 95.81%	53.06	0.73	0.387
	GeneXpert MTB/RIF	183/232 78.88%	3814/3866 98.65%	183/235 77.87%	3814/3863 98.73%	58.43	0.21	0.771
	GeneXpert MTB/RIF (smear positive)	63/64 98.44%	14/20 70.00%	63/69 91.30%	14/15 93.33%	3.28	0.02	0.749
	GeneXpert MTB/RIF (smear negative)	120/168 71.43%	3800/3846 98.80%	120/166 72.29%	3800/3848 98.75%	59.52	0.29	0.706
All respiratory sample	Smear microscopy	63/205 30.73%	3219/3237 99.44%	63/81 77.78%	3219/3361 95.78%	55.27	0.7	0.421
	GeneXpert MTB/RIF	167/205 81.46%	3204/3237 98.98%	167/200 83.5%	3204/3242 98.83%	79.91	0.19	0.814
	GeneXpert MTB/RIF (smear positive)	62/63 98.41%	14/18 77.78%	62/66 93.94%	14/15 93.33%	4.43	0.02	0.81
	GeneXpert MTB/RIF (smear negative)	105/142 73.94%	3190/3219 99.1%	105/134 78.36%	3190/3227 98.85%	82.08	0.26	0.751
BALF	Smear microscopy	24/91 26.4%	1710/1718 99.5%	24/32 75.0%	1710/1777 96.2%	52.8	0.74	0.374
	GeneXpert MTB/RIF	75/91 82.4%	1698/1718 98.8%	75/95 78.9%	1698/1714 99.1%	68.67	0.18	0.796
	GeneXpert MTB/RIF (smear positive)	23/24 95.8%	5/8 62.5%	23/26 88.5%	5/6 83.3%	2.55	0.07	0.636
	GeneXpert MTB/RIF (smear negative)	52/67 77.6%	1693/1710 99%	52/69 75.4%	1693/1708 0.991%	77.6	0.23	0.755

(Continued)

Table 2 (Continued).

Sample Types		Sensitivity	Specificity	PPV	NPV	LR+	LR-	Kappa
Sputum	Smear microscopy	39/114 34.2%	1509/1519 99.3%	39/49 79.6%	1509/1584 95.3%	48.86	0.66	0.456
	GeneXpert MTB/RIF	92/114 80.7%	1506/1519 99.1%	92/105 87.6%	1506/1528 98.6%	89.67	0.19	0.829
	GeneXpert MTB/RIF (smear positive)	39/39 100%	9/10 90%	39/40 97.5%	9/9 100%	10	0	0.935
	GeneXpert MTB/RIF (smear negative)	53/75 70.7%	1497/1509 99.2%	53/65 81.5%	1497/1519 98.6%	88.38	0.30	0.746
Lung tissue	Smear microscopy	1/16 6.25%	333/334 99.70%	1/2 50.00%	333/348 95.69%	20.83	0.94	0.102
	GeneXpert MTB/RIF	10/16 62.50%	323/334 96.71%	10/21 47.62%	323/329 98.18%	19.00	0.39	0.515
	GeneXpert MTB/RIF (smear positive) **	1/2 50.00%	ND*	1/1 100.00%	ND*	ND*	ND*	0
	GeneXpert MTB/RIF (smear negative)	9/19 47.40%	323/329 98.20%	9/15 60.0%	323/333 97.0%	26.33	0.536	0.506
Hydrothorax	Smear microscopy	ND*	283/283 100.00%	ND*	272/283 96.11%	ND*	ND*	0
	GeneXpert MTB/RIF	6/11 54.55%	265/272 97.43%	6/13 46.15%	265/270 98.15%	21.23	0.47	0.478
	GeneXpert MTB/RIF (smear positive)	ND*	ND*	ND*	ND*	ND*	ND*	ND*
	GeneXpert MTB/RIF (smear negative)	6/11 54.5%	265/272 97.43%	6/13 46.15%	265/270 98.15%	21.23	0.47	0.478

Notes: *ND, not detected. Since the denominator is 0, it cannot be calculated. **The number of samples is too small and the results are for reference only.

diagnosis (PPV=77.87%, LR+=58.43) compared to acid-fast staining (PPV=76.19%, LR+=53.06). Notably, GeneXpert MTB/RIF exhibited superior negative predictive value (NPV=98.73%, LR-=0.21) compared to acid-fast staining (NPV=95.81%, LR-=0.73). The impact of different operators or experimental locations was more pronounced for acid-fast staining ($kappa=0.387$) than for GeneXpert MTB/RIF ($kappa=0.771$).

The MTB Diagnostic Efficacy on Different Specimen Types

The impact of specimen type on diagnostic efficacy was taken into account, as shown in Table 2 and Figure 2. The testing effectiveness of the GeneXpert MTB/RIF alone was more outstanding than the acid-fast staining only used among different specimen types as that on all samples.

Differences in diagnostic efficacy were observed among different specimen types when using GeneXpert MTB/RIF alone. The GeneXpert MTB/RIF performed best among the respiratory specimens, as shown in Table 2 and Figure 2B. The sensitivity was above 80% ($Sen=81.46\%$), and the specificity was nearly 100% ($Spe=98.98\%$). It also exhibited excellent performance in terms of accuracy and consistency (PPV=83.50%, NPV=98.83%, LR+=79.91, LR-=0.19, $kappa=0.814$). Among the respiratory specimens, the GeneXpert MTB/RIF was particularly effective in the sensitivity and excluding negative samples from BALF ($Sen=82.4\%$, NPV=99.1%, LR-=0.18); meanwhile, the specificity and confirmation of positive samples were higher in sputum ($Spe=99.1\%$, PPV=87.6%, LR+=89.67%) (shown in Table 2, Figure 2C and D). For the tissue and hydrothorax sample, the specificity and NPV were similar to those observed in respiratory specimens ($Spe_{tissue}=96.71\%$, $NPV_{tissue}=98.18\%$; $Spe_{hydrothorax}=97.43\%$, $NPV_{hydrothorax}=98.15\%$). However, the sensitivity, PPV, LR, and consistency of GeneXpert MTB/RIF in tissue and hydrothorax samples significantly differed from those in respiratory samples ($Sen_{tissue}=62.50\%$, $PPV_{tissue}=47.62\%$, $LR+_{tissue}=19.00$, $LR-_{tissue}=0.39$, $kappa_{tissue}=0.515$; $Sen_{hydrothorax}=54.55\%$, $PPV_{hydrothorax}=46.15\%$, $LR+_{hydrothorax}=21.23\%$, $LR-_{hydrothorax}=0.47$, $kappa_{hydrothorax}=0.478$).

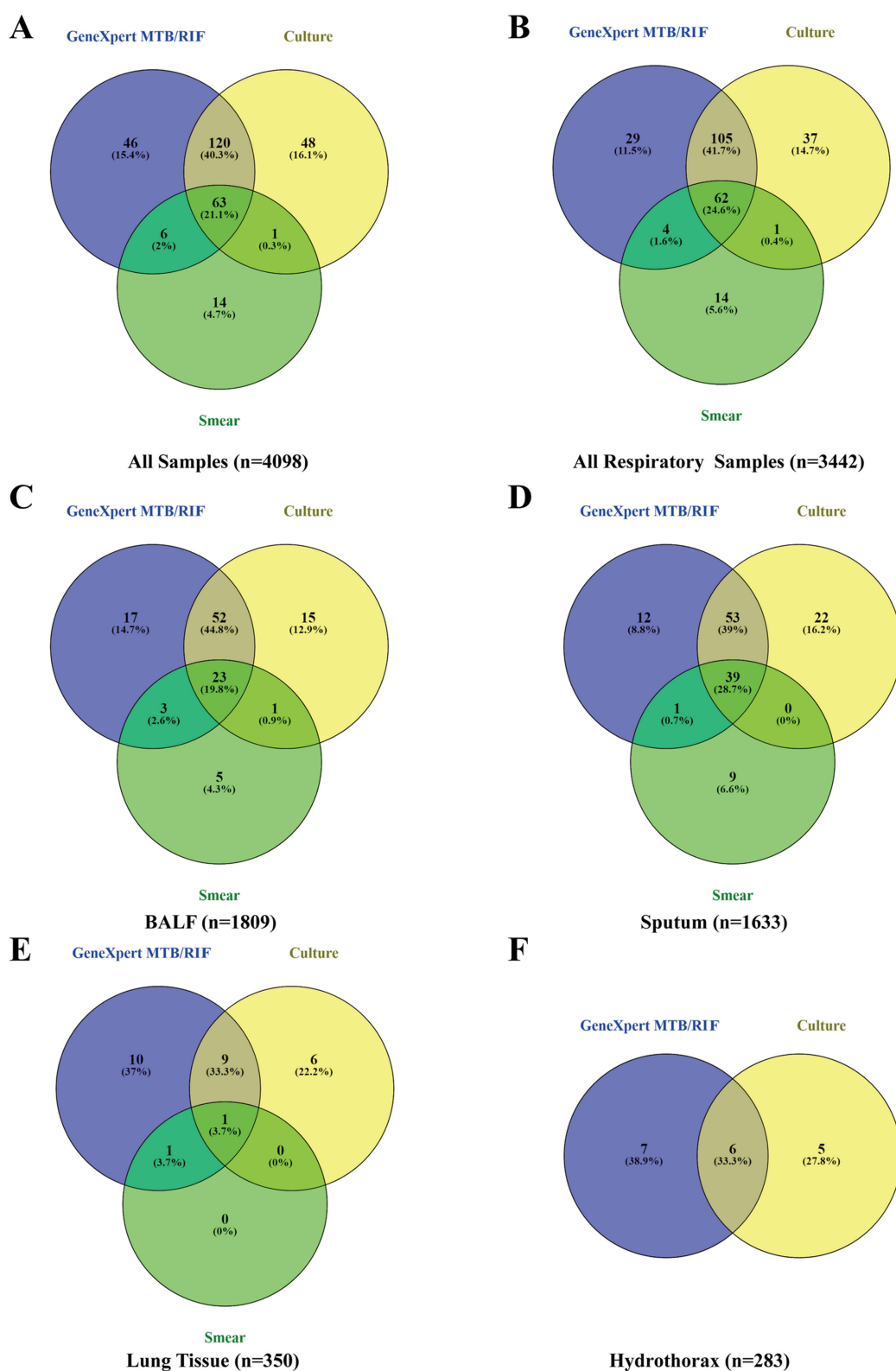


Figure 2 The Venn diagram of the performances of GeneXpert MTB/RIF, culture, and acid-fast staining in detecting *Mycobacterium tuberculosis* on all samples, respiratory samples, BALF samples, sputum samples, lung tissue samples and hydrothorax samples, shown on (A–F) respectively.

(displayed in Table 2, Figure 2E and F). Only a single GeneXpert MTB/RIF-positive sample, considered negative by culture and acid-fast staining, was detected in pus.

Acid-fast staining also performed the best in respiratory specimens similarly to GeneXpert MTB/RIF. The most outstanding performance of the acid-fast staining was its nearly 100% specificity ($\text{Spe}=99.44\%$), but the accuracy and the consistency were extremely poor ($\text{PPV}=77.78\%$, $\text{NPV}=95.78\%$, $\text{LR}+=55.27$, $\text{LR}-=0.70$, $\text{kappa}=0.421$). The diagnostic efficacy between sputum and BALF was similar, except for sensitivity which sputum had a higher sensitivity than BALF ($\text{Sen}_{\text{sputum}}=34.2\%$; $\text{Sen}_{\text{BALF}}=26.4\%$).

The Diagnostic Efficacy of GeneXpert MTB/RIF in Different Acid-Fast Staining Grouped Samples

The samples were then divided into two groups based on acid-fast staining. Both sensitivity and PPV were higher than those observed when using the GeneXpert MTB/RIF alone in smear-positive samples ($\text{Sen}=98.44\%$, $\text{PPV}=91.30\%$), yet the performance of GeneXpert MTB/RIF in smear-negative specimens was similar as the GeneXpert MTB/RIF employed individually. The consistency was also found to be relatively satisfactory ($\text{kappa}_{\text{smear-positive}}=0.749$, $\text{kappa}_{\text{smear-negative}}=0.706$).

In the respiratory specimens, GeneXpert MTB/RIF showed better efficiency after the samples were grouped by acid-fast staining. GeneXpert MTB/RIF did better in the sensitivity, PPV and LR- of the smear-positive sample ($\text{Sen}=98.41\%$, $\text{PPV}=93.94\%$, $\text{LR}-=0.02$). Furthermore, samples negative by both acid-fast staining and GeneXpert MTB/RIF can be confidently identified as negative with a high degree of certainty ($\text{Spe}=99.1\%$, $\text{NPV}=98.85\%$). The acid-fast-staining-negative patients with a positive GeneXpert MTB/RIF had a higher risk of suffering from MTB ($\text{LR}+=82.08$). However, compared to BALF samples, GeneXpert MTB/RIF exhibited superior diagnostic efficacy in detecting MTB in acid-fast-staining-positive sputum samples ($\text{Sen}_{\text{BALF}}=95.8\%$, $\text{Spe}_{\text{BALF}}=62.5\%$, $\text{PPV}_{\text{BALF}}=88.5\%$, $\text{NPV}_{\text{BALF}}=83.3\%$, $\text{LR}+_{\text{BALF}}=2.55$, $\text{LR}-_{\text{BALF}}=0.07$, $\text{kappa}_{\text{BALF}}=0.636$; $\text{Sen}_{\text{sputum}}=100.0\%$, $\text{Spe}_{\text{sputum}}=90.0\%$, $\text{PPV}_{\text{sputum}}=97.5\%$, $\text{NPV}_{\text{sputum}}=100.0\%$, $\text{LR}+_{\text{sputum}}=10$, $\text{LR}-_{\text{sputum}}=0$, $\text{kappa}_{\text{BALF}}=0.935$). In acid-fast-staining-negative samples, the detection capabilities between the two types of specimens showed little difference ($\text{Spe}_{\text{BALF}}=99.0\%$, $\text{PPV}_{\text{BALF}}=75.4\%$, $\text{NPV}_{\text{BALF}}=99.1\%$, $\text{LR}+_{\text{BALF}}=77.60$, $\text{LR}-_{\text{BALF}}=0.23$, $\text{kappa}_{\text{BALF}}=0.755$; $\text{Spe}_{\text{sputum}}=99.2\%$, $\text{PPV}_{\text{sputum}}=81.5\%$, $\text{NPV}_{\text{sputum}}=98.6\%$, $\text{LR}+_{\text{sputum}}=88.38$, $\text{LR}-_{\text{sputum}}=0.3$, $\text{kappa}_{\text{BALF}}=0.746$), except for in smear-negative patients, where GeneXpert MTB/RIF exhibited a stronger ability to detect MTB positivity in BALF ($\text{Sen}_{\text{BALF}}=77.6\%$; $\text{Sen}_{\text{sputum}}=70.7\%$). Therefore, in acid-fast-staining-positive respiratory specimens, when GeneXpert MTB/RIF testing was negative, there was a high likelihood of excluding MTB infection. However, in acid-fast-staining-negative cases, when GeneXpert MTB/RIF testing was positive, the possibility of infection cannot be ruled out. In contrast, when both acid-fast staining and GeneXpert MTB/RIF are negative, there was a strong basis for excluding MTB infection.

However, GeneXpert MTB/RIF in samples from other types yielded far fewer effective results compared to its usage in respiratory samples. As shown in Table 2. The sensitivity and the PPV in acid-fast-staining-negative tissue and acid-fast-staining-negative hydrothorax sample was below 60% ($\text{Sen}_{\text{tissue}}=47.4\%$, $\text{PPV}_{\text{tissue}}=60.0\%$; $\text{Sen}_{\text{hydrothorax}}=54.5\%$, $\text{PPV}_{\text{hydrothorax}}=46.15\%$), and the reliability and consistency are also not high ($\text{LR}+_{\text{tissue}}=26.33$, $\text{LR}-_{\text{tissue}}=0.536$, $\text{kappa}_{\text{tissue}}=0.506$; $\text{LR}+_{\text{hydrothorax}}=21.23$, $\text{LR}-_{\text{hydrothorax}}=0.47$, $\text{kappa}_{\text{hydrothorax}}=0.478$). Specificity and NPV were at an acceptable level ($\text{Spe}_{\text{tissue}}=98.20\%$, $\text{NPV}_{\text{tissue}}=97.0\%$; $\text{Spe}_{\text{hydrothorax}}=97.43\%$, $\text{NPV}_{\text{hydrothorax}}=98.15\%$). The diagnostic performance of the combined application in samples with acid-fast staining positivity had not been evaluated due to the limited number of samples with acid-fast staining positivity. Hence, in tissue and hydrothorax samples, when both acid-fast staining and GeneXpert MTB/RIF testing were negative, there was greater confidence in excluding MTB infection.

Impact of Specimen Types and Acid-Fast Staining on the CT of GeneXpert MTB/RIF

Different specimen types, owing to their varied processing methods, may have an impact on the results of GeneXpert MTB/RIF. The GeneXpert MTB/RIF cycle threshold value (CT) of all 235 GeneXpert MTB/RIF-positive samples was collected, and the values across different specimen types were compared. The median CT value of all culture-positive and all culture-negative samples was 23 and 26, respectively, showing a statistical difference between the two groups ($P=0.0001$) (Figure 3A). In the BALF samples, the median CT values for culture-negative and culture-positive samples were 23 and 27.5, respectively, with a statistically significant difference ($P=0.0009$) (Figure 3A). In sputum specimens,

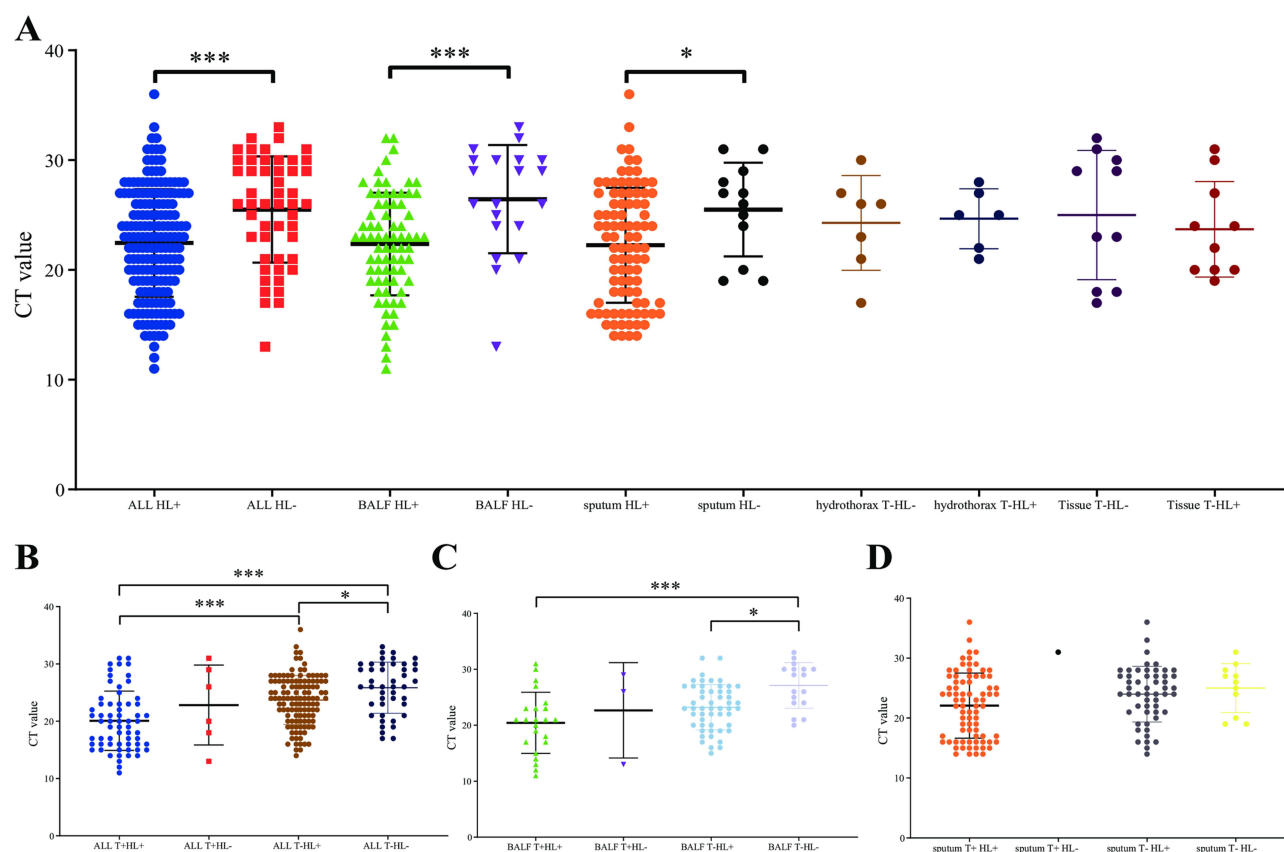


Figure 3 The cyclic threshold (CT) value of GeneXpert MTB/RIF on all samples, BALF samples and sputum samples grouped by acid-fast staining and culture results, showing on (A–D) respectively.

Notes: HL+ and HL- denote culture-positive and culture-negative results, respectively; T+ and T- indicate acid-fast staining-positive and acid-fast staining-negative results, respectively. Asterisks (***) represent statistical significance levels of $P < 0.01$ and $P < 0.05$, respectively.

the median CT values for culture-negative and culture-positive samples were 22.5 and 26.5, and a statistically significant difference was also observed ($P=0.423$) (Figure 3A). However, there was no difference between the culture-negative and culture-positive tissue samples ($P=0.5813$), in which the median CT values were 23 and 26, respectively (Figure 3A). The median CT values for culture-negative and culture-positive samples from hydrothorax were 25 and 26, separately, with no statistical difference between the two groups ($P>0.9999$) (Figure 3A). There was no statistical difference between the GeneXpert MTB/RIF-negative and GeneXpert MTB/RIF-positive groups of different specimen types ($P_{\text{positive}}=0.7267$, $P_{\text{negative}}=0.7405$).

The acid-fast staining and culture results served as grouping criteria and divided samples of each specimen type into four groups: acid-fast-staining-positive and culture-positive (T+HL+), acid-fast-staining-positive and culture-negative (T+HL-), acid-fast-staining-negative and culture-positive (T-HL+), and acid-fast-staining-negative and culture-negative (T-HL-). The impact of different specimen types and acid-fast staining on GeneXpert MTB/RIF results was analyzed, with detailed results presented in Figure 3. Among all samples, the median CT values for T+HL+, T+HL-, T-HL+, and T-HL- groups, as displayed in Figure 3B, were 20, 23, 24, and 26, respectively, with statistically significant differences ($P<0.0001$). Statistical differences within groups were observed between T+HL+ vs T-HL-, T+HL+ vs T-HL+, as well as T-HL+ vs T-HL- ($P_{\text{ALL T+HL+ vs ALL T-HL-}}<0.0001$, $P_{\text{ALL T+HL+ vs ALL T-HL+}}<0.0001$, $P_{\text{ALL T-HL+ vs ALL T-HL-}}=0.043$), indicating that acid-fast smear results had an impact on the CT values. In BALF samples, as shown in Figure 3C, the median CT values for T+HL+, T+HL-, T-HL+, and T-HL- groups were 21, 26, 23, and 29, respectively, with significant intergroup differences ($P=0.0003$). Pairwise comparisons revealed statistical differences between BALF T+HL+ and BALF T-HL-, as well as BALF T-HL+ and BALF T-HL- within the groups ($P_{\text{BALF T+HL+ vs BALF T-HL-}}<0.0001$, $P_{\text{BALF T-HL+ vs BALF T-HL-}}=0.0153$). However, in sputum specimens, the median CT values for T+HL+, T+HL-, T-HL+, and T-HL- groups were 22.5, 31, 24, and 26, respectively, exhibiting significant

intergroup differences ($P=0.0348$) (Figure 3D), though pairwise comparisons revealed no statistical differences ($P>0.1$). Due to the lack or minimal presence of acid-fast-staining-positive samples in hydrothorax and tissue samples, no comparisons were made between these types. The results indicate that specimen types had an impact on GeneXpert CT values after initial grouping by acid-fast staining. GeneXpert MTB/RIF had higher accuracy in detecting MTB in BALF.

Discussion

In this study, the effect of the specimen type on the GeneXpert MTB/RIF performance was investigated. The GeneXpert MTB/RIF demonstrated superior better on respiratory specimens than other samples. The diagnostic performance of GeneXpert MTB/RIF in hydrothorax and tissue samples was similar, yet not as robust as in respiratory samples across all metrics. The predominant representation of respiratory specimens reflects the importance of these samples in pulmonary MTB diagnosis, consistent with the report from WHO.³ Although all specimens analyzed with the GeneXpert MTB/RIF demonstrated high specificity (all Spe were above 98%), the sensitivity of different samples varied significantly. The sensitivity and the specificity of the GeneXpert MTB/RIF on respiratory samples were 81.46% and 98.8%, respectively, which was much higher than that on tissue (62.5%, 96.7%) and hydrothorax (54.6%, 98.2%). This finding aligns with that Akhter et al who reported the sensitivity and specificity were 52.2% and 100% on the biopsy samples, respectively.²³ These results contrast significantly with findings from other researchers. They reported a sensitivity as high as 72.2%, and even up to 85.5%, in tissue samples.^{24–26} This variation may be closely linked to differences in sample collection and processing. The sensitivity of GeneXpert MTB/RIF in hydrothorax samples varied significantly, ranging from 16.6% to 63.6%. It may be attributed to differences in the choice of reference standards and the processing methods, such as centrifugation.^{25–31} However, the specificity across studies (98.6–100%) was similar to our findings. The accuracy and reliability of GeneXpert MTB/RIF in tissue and hydrothorax samples were significantly lower compared to its performance in respiratory samples, similar to other literature.^{25,26,30} In our study, the differences were observed in the specificity and sensitivity of GeneXpert MTB/RIF between the administration on sputum (Sen=80.7%, Spe=99.1%) and BALF (Sen=82.4%, Spe=98.8%) samples, but the variations were not substantial, as similar with others.^{32–34} Detecting BALF in patients with limited sputum is clinically more significant.^{34–36} Thus, GeneXpert MTB/RIF has significant advantages in the detection of respiratory samples.

However, it was recommended that direct GeneXpert MTB/RIF testing of hydrothorax samples rather than acid-fast staining according to our results. No positive result was detected in hydrothorax by acid-fast staining, while 54.6% (6/11) positive samples were detected via GeneXpert MTB/RIF. In previous studies, the sensitivity of the acid-fast staining was far lower than that of the GeneXpert MTB/RIF.^{24,37} So, we recommended direct GeneXpert MTB/RIF testing of hydrothorax samples instead of acid-fast staining based on our experiments and related literature.

However, despite its advantages, GeneXpert MTB/RIF demonstrated limited sensitivity in certain non-pulmonary specimens. In our study, approximately 22% of culture-positive tissue samples and 27.8% of culture-positive hydrothorax specimens were not detected by GeneXpert MTB/RIF. These findings suggest that the assay may not be suitable as a standalone diagnostic tool for extrapulmonary tuberculosis, particularly in resource-limited settings. Notably, a negative GeneXpert MTB/RIF result in non-pulmonary specimens does not definitively rule out TB infection, and supplementary diagnostic methods, such as culture, histopathology, or immunological assays, remain necessary to achieve accurate diagnosis.

In our study, the GeneXpert MTB/RIF in various samples, particularly respiratory samples, effectively excluded true-negative samples among the acid-fast-staining-negative samples. In our research, when GeneXpert MTB/RIF was applied to all acid-fast staining-negative samples, the sensitivity and specificity were 73.4% and 98.8%, respectively. Furthermore, the combination of acid-fast staining and GeneXpert MTB/RIF, with its high specificity, NPV, and low LR-, provides substantial confidence in excluding infection for samples negative in both tests. Among these, the results of respiratory samples were similar to the total samples, with only sputum samples having slightly lower sensitivity than BALF (Sen_{sputum}=70.7%; Sen_{BALF}=77.6%). Our results are consistent with previous studies, which reported sensitivities ranging from 72% to 80.3%.^{38–42} However, Dorman et al reported a sensitivity of only 46% in 137 sputum acid-fast-staining-negative but culture-positive samples when tested with GeneXpert MTB/RIF.⁴³ Kumar et al found sensitivity, specificity, PPV, and NPV of 55.8%, 98.3%, 78.4%, and 95.1%, respectively, in acid-fast-staining-smear-negative cases,⁴⁴ while Ngangue et al reported a sensitivity of approximately 53%.⁴⁵ The reduced bacterial load in sputum

samples may explain the lower sensitivity in acid-fast-staining-negative cases, emphasizing the impact of different sample types on GeneXpert MTB/RIF results. The application of GeneXpert MTB/RIF in non-respiratory samples that are acid-fast-staining negative but culture-positive significantly reduces sensitivity and PPV compared to respiratory samples, emphasizing the influence of sample type on sensitivity again. However, research on non-respiratory samples with acid-fast-staining -negative but culture-positive results was limited. Additionally, across all samples, GeneXpert MTB/RIF demonstrated a sensitivity and PPV of 98.44% and 91.3%, respectively, in acid-fast-staining-positive samples. In sputum and bronchoalveolar lavage fluid, the sensitivity was 100% and 98.41%, respectively. For tissue, hydrothorax, and other samples, there was insufficient data due to a low number of positive acid-fast staining. These results were consistent with previous studies, indicating that GeneXpert MTB/RIF can effectively confirm MTB infection and minimize false positives.⁴² Furthermore, 48 cases with negative acid-fast staining and cultures but positive GeneXpert results were observed. We speculated that it may be due to the presence of MTB DNA or intact MTB (viable or non-viable) in the samples or a combination of both. Some MTB patients may still test positive for MTB nucleic acid in sputum even after receiving tuberculosis treatment.⁴⁶

The CT values of GeneXpert MTB/RIF appeared to be closely associated with bacterial load and sample types. We observed statistically significant differences in CT values between culture-positive and culture-negative samples ($P < 0.05$). Within the culture-positive group, there were statistically significant differences between acid-fast-staining-positive and smear-negative samples ($P < 0.05$), while within the culture-negative group, there were statistically significant differences between smear-positive and smear-negative samples ($P < 0.05$). This correlation was only observed in the BALF sample group. Although there were differences in median CT values in the sputum sample group, there was no statistical difference between any two groups. It was reported that GeneXpert MTB/RIF could detect MTB with a bacterial load of 10^2 .⁴⁷ In 2019, Irene Najjingo et al confirmed that there was a moderate correlation between GeneXpert MTB/RIF CT values and acid-fast-staining grading. The correlation between GeneXpert MTB/RIF CT values and time to positive culture was relatively weak. It was found that an increase of one unit in GeneXpert MTB/RIF CT values corresponded to a 2.57-day increase in time to positive culture.⁴⁸ Kui Li et al analyzed 980 sputum specimens and found differences when the bacterial load was within 1+ on microscopic examination (all $P < 0.05$). There was a strong negative correlation between CT values and acid-fast staining bacterial load ($P < 0.0001$), and when $CT < 16$, the diagnostic sensitivity and specificity were both 100.00%.⁴⁹ Mary Mansfield also elaborated on the relationship between bacterial load and CT values of GeneXpert MTB/RIF.⁵⁰ Acid-fast staining and culture reflect bacterial load. Our study also confirmed that the bacterial load carried by different sample types had an impact on the CT values of GeneXpert MTB/RIF. Above all, recent studies suggest that lower CT values may be associated with higher transmission risk, greater disease activity, and increased likelihood of smear positivity, although standardized clinical thresholds remain to be defined. We propose that CT values, when interpreted in combination with clinical and radiological findings, may aid clinicians in assessing disease severity and in triaging patients for isolation or treatment prioritization.

Despite the comprehensive evaluation presented in this study, some limitations should be acknowledged. The impact of varying operator skills or experimental locations on acid-fast staining results suggested a potential source of variability that should be addressed in future studies. Additionally, the limited number of acid-fast-staining-positive samples in tissue and hydrothorax samples constrained the evaluation of combined diagnostic performance in these specimen types.

Due to the clinical context, we were unable to apply traditional molecular tools like PCR or loop-mediated isothermal amplification (LAMP) in our study, both of which have certain limitations. PCR, for example, can yield false results and may not detect a broad range of pathogens. PCR can produce false results and may not detect a broad range of pathogens.⁵¹ LAMP, while rapid, can suffer from non-specific primer interactions, false positives, and contamination risks.^{52,53} These challenges underscore the need for alternative methods like the one we developed.

This study provides a comprehensive evaluation of GeneXpert MTB/RIF performance across diverse clinical specimen types, offering practical insights into its application in real-world settings. The inclusion of CT value analysis adds semi-quantitative depth that may assist clinical decision-making. However, the study has limitations, including a limited number of positive cases in certain specimen types and its single-center retrospective design, which may affect the generalizability of the findings.

Conclusion

In summary, a retrospective evaluation of 4098 clinical samples collected from CJFH between March 2018 and March 2019, assessing the application of GeneXpert MTB/RIF in different types of samples was conducted. Our study provides valuable insights into the clinical characteristics and diagnostic performance of GeneXpert MTB/RIF in different specimen types. The research findings support the practicality of GeneXpert MTB/RIF as a highly sensitive and specific diagnostic tool, especially in respiratory specimens. It was recommended that direct GeneXpert MTB/RIF testing of hydrothorax samples rather than acid-fast staining. The GeneXpert MTB/RIF showed promise, particularly in reducing the false-negative rate of acid-fast staining, optimizing the sensitivity of acid-fast-staining-positive samples, and assessing the exclusion accuracy of samples negative for both acid-fast staining and GeneXpert MTB/RIF. The impact of observed sample types on the CT values of GeneXpert MTB/RIF was also observed.

Abbreviation

AFS, Acid-Fast Stain; BALF, Broncho Alveolar Lavage Fluid; CT, Threshold Cycle; *Mtb*, *Mycobacterium tuberculosis*.

Ethics Statement

Permission to use the information in the medical records of the patient for research purposes was granted by the Ethics Committee of the China-Japan Friendship Hospital (2022-KY-133). Our research is in line with the exemption type of informed consent and ethics approval that “Using identifiable human body materials or data for research, it is no longer possible to locate the subject, and the research project does not involve personal privacy disclosure or commercial interests”. As this is a retrospective Cohort study based on previous clinical diagnosis and treatment results, the Ethics Committee of the China-Japan Friendship Hospital granted the study exemption status. In addition, we declare that this study is in line with the ethical guidelines of the Declaration of Helsinki, and the patient-related data is strictly confidential.

Acknowledgments

We wish to thank the support of Changping Laboratory.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by National High Level Hospital Clinical Research Funding (grant no. 2024-NHLHCRF-LX-01-0101, grant no. 022-NHLHCRF-YS-02, and grant no. 2022-NHLHCRF-LX-01-0208). Elite Medical Professionals Project of China-Japan Friendship Hospital (grant no. ZRJY2023-QM08), the Natural Science Foundation of China (grant no. 82470007), Chinese Academy of Medical Sciences (CAMS) Innovation Fund for Medical Sciences (grant no. CIFMS 2021-I2M-1-048 and grant no. 2021-I2M-1-030), Beijing Research Ward Excellence Program BRWEP2024W114060103, Noncommunicable Chronic Diseases-National Science and Technology Major Project (grant no. 2023ZD0506200 and grant no. 2023ZD0506203) and this work has been supported by the New Cornerstone Science Foundation.

Disclosure

All authors report no conflicts of interest in this work.

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