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ORIGINAL RESEARCH ARTICLE

Analysis of the detection efficacy of Xpert MTB/RIF, Lowenstein–Jensen medium culture, and fluorescence smear microscopy methods for *Mycobacterium tuberculosis* diagnosis

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Abstract

Objectives: Tuberculosis (TB) remains a major global health threat, necessitating accurate, rapid, and cost-effective diagnostic methods to improve early detection and control of the disease. The traditional methods for detecting *Mycobacterium tuberculosis* (MTB) in clinical testing are fluorescence smear microscopy (FSM) and Lowenstein–Jensen medium culture (LJMC) methods. With the development of molecular diagnostics, the Xpert MTB/rifampicin assay (Xpert) has become increasingly used. This study compared Xpert with traditional FSM and LJMC using sputum specimens to study the application value in the diagnosis of TB.

Methods: A total of 342 examination reports were included. Sputum samples from all study subjects were tested using FSM, LJMC, and Xpert methods.

Results: The Xpert method showed a significantly higher positive rate than FSM, and a slightly higher rate than LJMC.

Conclusion: Among all sputum samples from suspected or confirmed TB patients, Xpert detected all cases that were positive by FSM, while both Xpert and LJMC missed positives found by each other. FSM also detected a small number of positives not detected by LJMC. Overall, Xpert had the highest positive detection rate, and FSM the lowest. Although FSM is the fastest and least expensive (costing only one-seventh of Xpert), it demonstrated the lowest sensitivity, with its positive rate being roughly half that of Xpert or LJMC.

Keywords: Acid-fast bacilli; Fluorescence smear microscopy; Lowenstein–Jensen medium culture; *Mycobacterium tuberculosis*; Rifampicin resistance; Xpert *Mycobacterium tuberculosis*/Rifampicin assay

1. Introduction

Tuberculosis (TB), mainly transmitted through airborne particles, remains widespread around the world and poses a serious threat to people's health.[1-3] It urgently needs to be effectively curbed. According to the Global Tuberculosis Report released by the World Health Organization (WHO) on October 29, 2024, there were 10.8 million new TB patients globally in 2023, with the disease causing 1.25 million deaths.[4] For the treatment of TB, rifampicin (RIF) has been a cornerstone;[5] however, some *Mycobacterium* species are resistant to the drug, significantly undermining the treatment efficacy.[6] Continuous exploration and improvement of rapid, accurate detection methods for *Mycobacterium tuberculosis* (MTB) and its resistance to RIF are the key to preventing and controlling TB.[7,8] For a long time, the most widely used methods for laboratory diagnosis of TB have been fluorescent smear microscopy (FSM) and the Lowenstein–Jensen medium culture (LJMC) for detecting acid-fast bacilli (AFB) in sputum smears.[9,10]

RIF is a broad-spectrum antibiotic drug with strong antimicrobial activity against MTB. When MTB is affected by factors such as inappropriate drug combination, insufficient dosage, intermittent use of drugs, and the spread of AIDS, mutations in the *rpoB* gene occur, resulting in incorrect nucleotide coding and misalignment of the amino acid sequence of crucial enzymes. This affects the binding of drugs to the target enzymes, leading to their resistance.[11,12] RIF resistance is usually detected using LJMC and Xpert methods.

However, the sensitivity of FSM is low[13] and no longer meets the requirements of current clinical standards. Although LJMC has been considered the gold standard method for TB detection, its turnaround time often exceeds 1 month,[14,15] which is inadequate for timely clinical decision-making.

Fortunately, according to the WHO Meeting Report of a Technical Expert Consultation on the non-inferiority analysis of Xpert MTB/RIF Ultra compared to MTB/RIF (2017),[16] and China's WS 288-2017: Diagnosis for Pulmonary TB issued by the National Health Commission,[17] the Xpert method has, since 2017, been included alongside LJMC in TB diagnostic protocols in Chinese hospitals. Moreover, Xpert offers fully automated operation, with a detection time of no longer than 2 h,[18] making it more compatible with the needs of clinical testing.

This study uses clinical data from the Infectious Diseases Hospital of the First Affiliated Hospital of the University of Science and Technology of China (USTC) to evaluate the clinical application value of Xpert detection technology by processing and comparing selected cases tested using the FSM, LJMC, and Xpert methods. With particular attention to Xpert, this study aims to verify whether Xpert can completely replace LJMC as the gold standard, or whether each method has distinct advantages and should be used complementarily. The findings will provide data-driven insights to inform TB diagnostic strategies in clinical settings, facilitating hospitals or other institutes in establishing appropriate testing standards.

2. Materials and methods

2.1. Patient data collection

This study collected examination reports from 362 patients who received treatment at the TB Clinic of the Infection Hospital Area of the First Affiliated Hospital of the USTC, from July 2021 to June 2022. Among them, 146 were suspected TB patients with lung shadows (age range: 9 – 90 years), 176 were confirmed TB patients (age range: 9 – 81 years), and 10 were patients with old TB (Table 1). Patient classification was based on the diagnosis recorded by the clinician in the diagnosis section of their medical report.

Sputum samples from all patients were collected and tested using three distinct diagnostic methods to determine the positive detection rate. Patients diagnosed with old TB

Table 1. Characteristics of patients

Group	Age (years)	Gender		Total (*n*)	Percentage (%)
		Male (*n*)	Female (*n*)		
Suspected TB	<30	33	23	56	38.36
	30–40	11	2	13	8.90
	40–50	10	5	15	10.27
	50–60	17	12	29	19.86
	≥60	25	8	33	22.60
	Subtotal	96	50	146	100
Diagnosed with TB	<30	32	26	58	35.62
	30–40	17	15	32	20.55
	40–50	10	8	18	10.96
	50–60	23	13	36	17.81
	≥60	21	11	32	15.07
	Subtotal	103	73	176	100
Total	<30	65	49	114	39.84
	30–40	28	17	45	13.82
	40–50	20	13	33	10.57
	50–60	40	25	65	20.33
	≥60	46	19	65	15.45
	Total	199	123	322	100

Abbreviation: TB: Tuberculosis.

were excluded from the analysis, as all 10 such cases tested negative using the three diagnostic tests.

2.2. Reagents and instruments

For the Xpert method, the testing instrument (Figure 1A) and the supporting reagents were provided by Cepheid (USA). For the FSM method and LJMC methods, the Auramine O staining kit and LJMC medium were provided by Zhuhai Beso Biotechnology (China). The fluorescence microscope (Figure 1B) and incubator used for these methods were provided by Olympus (Japan).

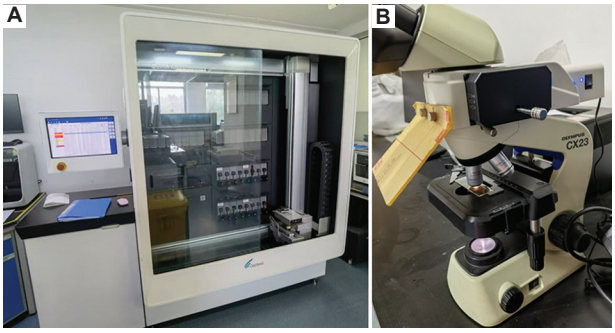

Figure 1. Equipment used in the test. (A) Equipment used for Xpert *Mycobacterium tuberculosis*/rifampicin assay. (B) Fluorescence microscope.

2.3. FSM methodology for detecting AFB in sputum smears

The FSM method was conducted according to the instructions provided by the manufacturer, following three standardized steps: smearing, staining, and microscopic examination (Figure 2).

2.3.1. Smearing

After numbering the slides (Cat. No. 7105 Microscope Slides, Yancheng Feizhou Glass Co., LTD., China), a disposable wooden stick was used to pick the thicker part of the sputum and evenly apply it on the slide until the text underneath became legible. After the slides naturally dried, they were heat-fixed.

2.3.2. Staining

The slides were covered with Auramine O Staining Solution A (0.01 g auramine + 10 mL 95% alcohol + 100 mL 5% carbolic acid) for 5 min, before slowly rinsing them with clean water along one end of the slide. The slides were then covered with Auramine O Staining Solution B (3% hydrochloric acid alcohol) for 1 – 2 min and rinsed similarly. Decolorization was repeated until the smear appeared colorless to the naked eye, then rinsed again.

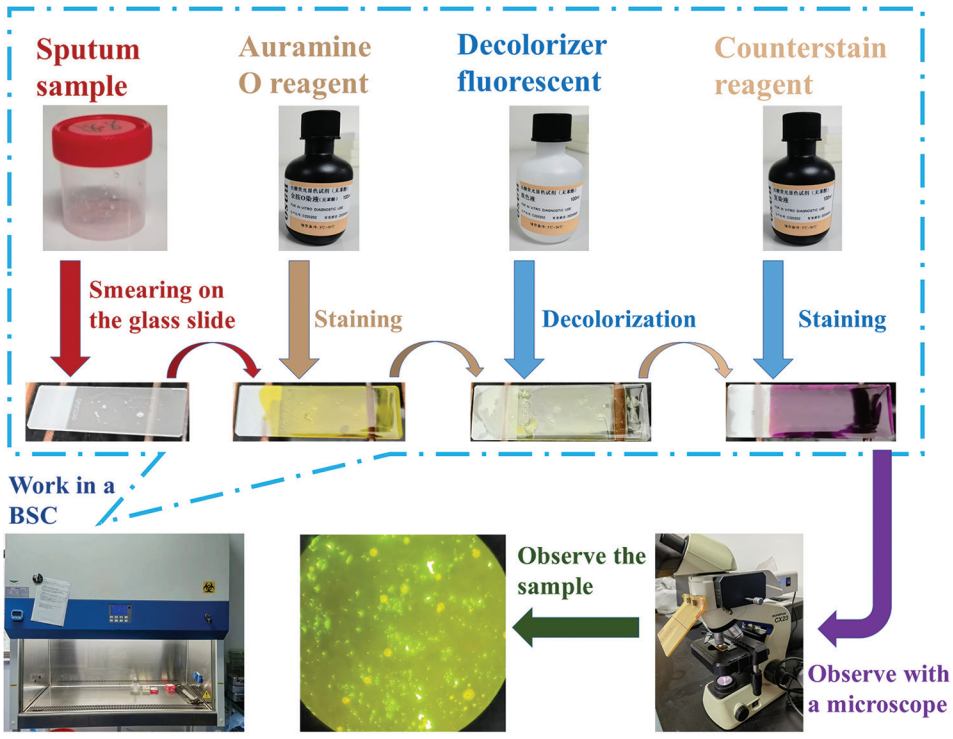

Figure 2. Operation process of FSM method
Abbreviations: BSC: Biosafety cabinet; FSM: Fluorescence smear microscopy.

Finally, the slides were stained with Auramine O Staining Solution C (0.5% potassium permanganate solution) for 1 – 2 min, rinsed similarly, and then drained.

2.3.3. Microscopic examination

Under a dark background using a 40× fluorescence microscope, AFB appeared as bright yellow–green rods that were slightly curved. AFB counts were reported as follows: (1) Negative (–) if no AFB in 30 fields; (2) Reported as the exact number if 1 – 9 AFB in 30 fields; (3) Positive (1+) if 10 – 100 AFB in 30 fields; (3) Positive (2+) if average 1 – 10 AFB per field; (4) Positive (3+) if average 11 – 200 AFB per field; and (5) Positive (4+) if average more than 200 AFB per field. In actual clinical testing, all cases reported as (1+), (2+), (3+), or (4+) were eventually diagnosed as positive (+).

2.3. LJMC methodology for detecting AFB in sputum samples

A 1 mL sputum sample of patients was treated with 1 – 2 volumes of 4% sodium hydroxide (NaOH) (Roche Diagnostics Suzhou Co., LTD, China) and allowed to liquefy for 20 min. A few drops of the liquefied sputum were then inoculated into LJMC medium and incubated at 37°C for several weeks.

Typical MTB colonies appear on the medium surface as opaque, dry, rough, light-yellow, cauliflower-like protrusions. Once such colonies are observed, they are picked and subjected to standardized smear preparation, staining, and microscopic observation under a 100× oil immersion objective for confirmation. A specimen was reported as MTB-positive only after these confirmatory steps. If a culture was positive, the colonies were further inoculated into drug susceptibility testing media and incubated at 37°C for several weeks. The presence or absence of colony growth in the testing medium determined the drug resistance profile: no growth was reported as RIF-sensitive, while the presence of growth indicated RIF resistance.

2.4. Xpert methodology for detecting AFB in sputum samples

The Xpert MTB/RIF assay for detecting AFB in sputum involved four steps.

2.4.1. Sputum precipitation treatment

Sputum samples were added to a 50 mL pre-treatment tube with a screw cap, mixed with 1 – 2 times volumes of 4% NaOH, and shaken well for several minutes until fully liquefied. Samples were then left at room temperature for 15 min. Subsequently, 45 mL of 0.067M phosphate-buffered saline (PBS) was added to the liquefied sputum and centrifuged at 3000 g for 20 min. After discarding the supernatant, 2 mL of PBS was added to the pellet.

2.4.2. Sputum precipitation pretreatment

A 0.5 mL of sputum precipitate was transferred into a tube with a screw cap. Then, 1.5 mL of sample reagent (SR) was added. The tube was shaken vigorously 10 – 20 times and allowed to stand for 15 min at room temperature.

2.4.3. Preparation of the test cartridge

The processed sample was slowly added to the test cartridge using a special pipette. The lid of the cartridge was then closed, and the assay was initiated within 30 min.

2.4.4. Interpretation of results

According to the manufacturer's instructions, if MTB DNA was detected, the result was categorized based on cycle threshold (Ct) values into four abundance levels: (1) High abundance (HA): Ct <16; (2) Medium abundance (MA): Ct = 16 – 22; (3) Low abundance (LA): Ct = 22 – 28; and (4) Extremely low abundance (ELA): Ct >28.

Cases in which no MTB DNA was detected were considered negative. The presence of RIF resistance was determined only if MTB DNA was detected and a mutation in the *rpoB* gene was identified within a valid Ct value range.

2.4. Statistical analysis

Clinical test data were analyzed using Microsoft Excel 2021 (Microsoft, USA) and IBM Statistical Package for the Social Sciences software. Confusion matrices were constructed to compare the diagnostic methods (Table 2). Key performance indicators were calculated for each diagnostic method–FSM, LJMC, and Xpert–including: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy. These calculations follow standard diagnostic performance evaluation protocols as described in the literature.[19-22]

Table 2. Confusion matrix for diagnostic method comparison

Findings	Positive (standard)	Negative (standard)	Total
Positive (comparison method)	TP	FP	TP+FP
Negative (comparison method)	FN	TN	FN+TN
Total	TP+FN	FP+TN	N

Notes: N: Total quantity of samples tested. TP (true positive): both standard and comparison method are positive; FP (false positive): standard is negative, but comparison method is positive; FN (false negative): standard is positive, but comparison method is negative; TN (true negative): both standard and comparison method are negative.

3. Results

3.1. Analysis of medical records

Although the LJMC method has traditionally been considered the gold standard for detecting AFB in sputum smears, the advent of more advanced technologies such as the Xpert MTB/RIF assay necessitates a reevaluation of diagnostic performance. In our retrospective analysis of 322 medical records, the LJMC method identified 69 positive cases. Among these, 37 cases were negative using the FSM method, and 15 were negative using the Xpert method. The FSM method identified 35 positive cases. Of these, 3 cases were negative by LJMC, and none were negative by Xpert. The Xpert method identified 80 positive cases. Among these, 45 cases were negative using FSM, and 26 were negative using LJMC.

Among the 322 tested samples, the FSM method detected 35 positive cases, yielding a positivity rate of 10.87%. The LJMC method detected 69 positive cases, with a positivity rate of 21.43%. The Xpert method detected 80 positive cases, corresponding to a positivity rate of 24.84%.

Notably, all three FSM-positive cases that were not identified by LJMC were detected by Xpert. Of the LJMC-positive cases, 37 were not detected by FSM, and 15 were not detected by Xpert. Among the Xpert-positive cases, 45 were not detected by FSM, and 26 were not detected by LJMC. These comparisons are summarized in Table 3 and visually represented in Figure 3.

From Table 3, it is evident that the Xpert method identified the highest number of positive cases, while the FSM method identified the fewest. All samples detected as positive by FSM were also identified by Xpert, suggesting that Xpert has broader detection coverage. However, both Xpert and LJMC identified cases that were not detected by the other two methods. This finding implies that, based on current data, neither Xpert nor LJMC alone can be considered a definitive gold standard.

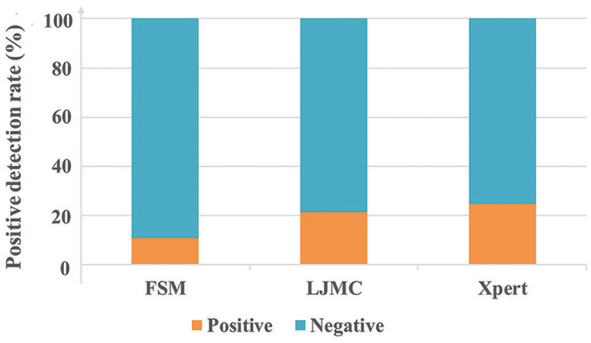

Figure 3. Positive detection rates of FSM, LJMC, and Xpert detection methods
Abbreviations: BSC: Biosafety cabinet; LJMC: Lowenstein-Jensen medium culture.

To further evaluate the diagnostic performance, two scenarios were analyzed: (1) assuming Xpert as the reference standard and (2) assuming LJMC as the reference standard. Based on these assumptions, confusion matrices were constructed for each comparison.

3.2. Result with LJMC as the reference standard

When the LJMC method was regarded as the reference standard, and both Xpert and FSM were evaluated as index tests for detecting AFB in sputum smears, confusion matrices were constructed as shown in Tables 4 and 5.

3.3. Result with Xpert as the reference standard

Similarly, when the Xpert was regarded as the reference standard, and both LJMC and FSM were evaluated as the test methods, the corresponding confusion matrices are presented in Tables 6 and 7.

Table 3. Comparison of positive detection rates using the three methods

Method	FSM=0	LJMC=0	Xpert=0	Total positive (*n*, %)
FSM=1	-	3	0	35 (10.87)
LJMC=1	37	-	15	69 (21.43)
Xpert=1	45	26	-	80 (24.84)

Notes: "0" indicates a negative result; "1" indicates a positive result. % is reported as a proportion of total cases (*n*=322).
Abbreviations: FSM: Fluorescence smear microscopy; LJMC: Lowenstein-Jensen medium culture.

Table 4. Confusion matrix for Xpert when LJMC is considered the reference

Findings (*n*)	Positive (LJMC)	Negative (LJMC)	Total
Positive (Xpert)	54	26	80
Negative (Xpert)	15	227	242
Total	69	253	322

Notes: Refer to Table 3 for more details on confusion matrix.
Abbreviation: LJMC: Lowenstein-Jensen medium culture.

Table 5. Confusion matrix for FSM when LJMC is considered the reference

Findings (*n*)	Positive (LJMC)	Negative (LJMC)	Total
Positive (FSM)	32	3	35
Negative (FSM)	37	250	287
Total	69	253	322

Notes: Refer to Table 3 for more details on the confusion matrix.
Abbreviations: FSM: Fluorescence smear microscopy; LJMC: Lowenstein–Jensen medium culture.

3.4. Summary of diagnostic performance

A comparative summary of diagnostic performance metrics–including sensitivity, specificity, PPV, NPV, and Cohen's kappa (κ)—for the various method comparisons is provided in Table 8, derived from the results of Tables 2-7.

When LJMC was used as the reference standard, Xpert showed high consistency with LJMC, demonstrating high sensitivity and strong agreement (high κ). FSM showed only moderate consistency with LJMC, with lower sensitivity and moderate κ values. When Xpert was used as the reference standard, LJMC also demonstrated high agreement with Xpert, indicating strong mutual reliability. FSM again showed moderate consistency, with the lowest sensitivity among the three methods but relatively high specificity.

4. Discussion

The global TB epidemic is becoming an increasingly serious concern, with roughly 9 million new TB cases and about 1.6 – 2.2 million deaths caused by the disease each year.[23] China is one of the eight countries in the world with the highest TB burden[24] and also experiences a high incidence of drug resistance to the disease.[25] Therefore, a sense of urgency has arisen in the prevention and control of the TB epidemic in the country. The emergence of drug-resistant TB is a critical factor slowing the decline in both TB incidence and deaths in China.[26] Strengthening of TB patient monitoring throughout the entire spectrum of prevention, diagnosis, and treatment is thus critically important. In recent years, molecular diagnostic techniques have been adopted for the definitive diagnosis of TB, which has led to the gradual adoption of the Xpert MTB/RIF assay in the molecular diagnosis of TB. Xpert is designed for rapid detection of MTB and mutations in the *rpoB* gene associated with RIF resistance, completing the entire process in approximately 2.5 h.[16] In comparison, the traditional gold standard, LJMC, takes a month to complete.

Table 6. Confusion matrix for LJMC when Xpert is considered the reference.

Findings (*n*)	Positive (Xpert)	Negative (Xpert)	Total
Positive (LJMC)	54	15	69
Negative (LJMC)	26	227	253
Total	80	242	322

Note: Refer to Table 3 for more details on the confusion matrix.
Abbreviation: LJMC: Lowenstein–Jensen medium culture.

Table 7. Confusion matrix for FSM when Xpert is considered the reference

Findings (*n*)	Positive (Xpert)	Negative (Xpert)	Total
Positive (FSM)	35	45	80
Negative (FSM)	0	242	242
Total	35	287	322

Note: Refer to Table 3 for more details on the confusion matrix.
Abbreviation: FSM: Fluorescence smear microscopy.

The results of this study showed that the positivity rate of the Xpert test was significantly higher than that of the FSM and slightly higher than LJMC in both suspected and confirmed TB patients. The FSM method is, by far, the fastest method for detecting MTB, which is easy and simple to operate and requires no special equipment. Furthermore, the reagents used in FSM are relatively inexpensive. In addition, the operator's picking of the sputum is randomized so as to prevent bias. In this study, FSM exhibited the lowest positive detection rate, and all the positive results detected were confirmed by Xpert, although some were not identified by LJMC.

Although LJMC had been the gold standard for detecting MTB, it takes about eight weeks to complete the culture process for the test due to the long natural reproduction cycle of the bacterium–at least 18 h to reproduce one generation.[27] Xpert effectively addresses the shortcomings of both FSM and LJMC by combining high sensitivity with rapid results, enabling same-day diagnosis and treatment planning. This feature is particularly important for early TB control and initiation of appropriate therapy.

In the study, both Xpert and LJMC detected four RIF-resistant cases, three of which were concordant. Xpert

Table 8. Key diagnostic parameters when LJMC or Xpert is considered the reference

Standard	Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	OC	RC	κ
LJMC	Xpert	78.26	89.72	67.50	93.80	0.873	0.644	0.643
	FSM	46.38	98.81	91.43	87.11	0.876	0.724	0.551
Xpert	LJMC	67.50	93.80	78.26	89.72	0.873	0.644	0.643
	FSM	43.75	100	100	84.32	0.860	0.697	0.539

Notes: $0.4 \leq \kappa < 0.6$: medium consistency; $0.6 \leq \kappa < 0.8$: highly consistent.
Abbreviations: FSM: Fluorescence smear microscopy; LJMC: Lowenstein-Jensen medium culture; NPV: Negative predictive value; OC: Overall consistency: PPV: Positive predictive value; RC: Relative consistency; κ: Cohen's kappa coefficient.

Table 9. Overall comparison of LJMC, FSM, and Xpert methods

Method	Time span	Test complexity	Detection rate	Cost (USD)
Xpert	2.5 h	Simple	Very high	97.50
LJMC	>1 month	Complex	High	4.90
FSM	<1 h	Common	Low	13.90

Note: The price and period data for the three tests in were obtained from the Infectious Diseases Hospital of the First Affiliated Hospital of USTC.
Abbreviations: FSM: Fluorescence smear microscopy; LJMC: Lowenstein–Jensen medium culture.

demonstrated the ability to detect resistance-associated mutations in the *rpoB* gene with high sensitivity. However, due to the limited number of RIF-resistant samples in this study, a comparative analysis of the two methods' diagnostic accuracy for drug resistance could not be performed. Future studies with a larger sample size of resistant cases are warranted.

Although FSM has the lowest positive detection rate, LSM offers the advantage of rapid testing–delivering results within one hour—and lower cost per test, which is only one-seventh that of Xpert. While LJMC is the most affordable option and has a detection rate second only to Xpert, it requires a testing period exceeding 1 month. Xpert, although the most expensive, combines the strengths of both other methods, providing a high detection rate and rapid turnaround. These comparisons are summarized in Table 9.

This study has several limitations. Due to the limited sample size, we were unable to perform statistical analyses on the correlation between the test results of one method and the negative results of others, or between test outcomes and demographic factors such as age and gender. Ongoing data collection and future analyses are planned to address these aspects.

Taken together, Xpert technology offers a fully automated platform for the detection of MTB and RIF resistance with high sensitivity, short processing time, and minimal risk of cross-contamination. These features make Xpert a valuable tool for improving TB diagnosis and monitoring, particularly in primary healthcare settings. While our findings support that Xpert can fully replace FSM for TB detection, Xpert and LJMC each have diagnostic advantages and cannot fully substitute one another. Integration of both methods may provide complementary diagnostic value in clinical practice.

5. Conclusion

The data presented in this study indicate that the Xpert method can fully replace the FSM methods for the detection of TB. However, when compared to the LJMC method, Xpert and LJMC methods are not entirely interchangeable. Currently, at the Infectious Diseases Hospital of the First Affiliated Hospital of USTC, it is mandatory to test samples from all TB patients admitted for treatment using the LJMC, Xpert, and FSM methods.

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Conflict of interest

The authors declare no competing interests.

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of University of Science and Technology of China (USTC) (2024KY-384).

Consent for publication

Not applicable.

Availability of data

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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