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# Efficacy Study of Molecular Diagnostic Techniques for Monitoring Tuberculosis Relapse

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**Abstract:** The purpose of this article is to analyze the efficacy of molecular diagnostic techniques for monitoring tuberculosis relapse. After analyzing the connotation of molecular diagnostic techniques and their specific application process in tuberculosis relapse monitoring, a total of 200 cured tuberculosis patients were selected (100 in the experimental group and 100 in the control group). During the 12-month follow-up period, the experimental group was monitored by molecular diagnostic techniques, while the control group was monitored by traditional techniques. Finally, by comparing the performance indicators of the two monitoring methods, as well as the relapse situations and outcomes of patients, it was demonstrated that molecular diagnostic techniques have higher efficacy in tuberculosis relapse monitoring.

**Keywords:** Molecular diagnosis; Tuberculosis; Relapse monitoring; Target amplification

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## 1. Introduction

Tuberculosis is a prevalent disease, and monitoring patients for relapse after treatment is crucial for safeguarding their health and quality of life. On the one hand, monitoring for tuberculosis relapse can identify patients as early as possible, block potential sources of infection, and reduce the risk of infection. On the other hand, by monitoring the evolution of the patient's condition, it can provide data support for optimizing the patient's subsequent treatment and healthcare strategies, and generate personalized treatment plans. Mastering scientific, effective, and accurate monitoring methods is a key factor in improving the efficiency of relapse monitoring, controlling monitoring costs, and maximizing public health benefits.

## 2. Introduction to molecular diagnostic techniques and their application in tuberculosis relapse monitoring

### 2.1. Overview of molecular diagnostic techniques

Molecular diagnostic techniques belong to a type of precision medicine technology that can achieve disease typing, diagnosis, and prognosis assessment by detecting nucleic acids (DNA/RNA), proteins, or other biological molecular markers in biological samples. Its principle is to reveal the essence of diseases at the molecular level, and it has the characteristics of high specificity, high sensitivity, and early diagnosis.

Specifically, the core technical processes of molecular diagnostic techniques include target identification, signal amplification and conversion, and signal detection and analysis. Among them, target identification is to amplify DNA/RNA fragments through technologies such as polymerase chain reaction (PCR) to improve detection sensitivity, and then use labeled probes for specific binding to target sequences for the detection of protein markers. Signal amplification and conversion use PCR amplification technology to amplify target DNA exponentially and detect it using fluorescence signals or electrophoresis. Signal detection and analysis use chemiluminescence or fluorescence technology to monitor the amplification products in real-time, such as quantitative PCR (qPCR).

By utilizing the characteristics of DNA polymerase synthesizing new DNA strands during the PCR process, combined with a fluorescently labeled probe or dye, the progress of the PCR reaction can be measured by monitoring the increase of fluorescence signals in real-time. The entire molecular diagnosis process usually involves technologies such as gene chips, PCR technology, next-generation sequencing (NGS) high-throughput testing technology, microfluidics, and lab-on-a-chip technology.

### 2.2. Application of molecular diagnostic techniques in tuberculosis relapse monitoring

In the monitoring of tuberculosis relapse, the application process of molecular diagnostic techniques includes sample collection and pretreatment, nucleic acid extraction and purification, target amplification and detection, rapid drug-resistance detection, data analysis, and result interpretation.

#### 2.2.1. Sample collection and pretreatment

For the dynamic monitoring of tuberculosis patients, samples are collected after treatment and then compared and analyzed. In this process, sample processing is a key step to improve the quality of analysis. Medical institutions use N-acetylcysteine-sodium hydroxide for liquefaction and decontamination to remove interfering bacteria. Subsequently, a high-speed centrifuge is used to enrich Mycobacterium tuberculosis, ensuring higher accuracy of the sample in subsequent detections. In terms of quality control, internal reference DNA, such as the human β-actin gene, is introduced to monitor the efficiency of nucleic acid extraction.

#### 2.2.2. Nucleic acid extraction and purification

Nucleic acid extraction and purification are basic steps of molecular diagnostic techniques. Based on the pretreated samples, efficient extraction is achieved through magnetic bead methods or silica membrane adsorption methods. At the same time, specific capture probes are designed for MTB DNA drug-resistance genes, such as rpoB and katG, to further improve the detection accuracy.

#### 2.2.3. Target amplification and detection

In the target amplification and detection stage, real-time fluorescence quantitative PCR (qPCR) technology is used

to distinguish *Mycobacterium tuberculosis* from non-*Mycobacterium tuberculosis* using labeled probes. At the same time, based on the loop-mediated isothermal amplification (LAMP) technology targeting the MPB64 gene, amplification can usually be completed within 1 hour, and the result can be judged by color development.

#### 2.2.4. Rapid drug-resistance detection

For drug-resistance detection, the Xpert MTB/RIF system is mainly used in combination with real-time fluorescence PCR and melting curve analysis to detect rpoB gene mutations within 2 hours and report MTB positivity and rifampicin resistance. On this basis, based on current probe technology, after PCR amplification, hybridization probes are used, and reverse dot-blot hybridization is used to read the drug-resistance genotypes of rifampicin and isoniazid.

#### 2.2.5. Data analysis and result interpretation

Data analysis and result interpretation aim to comprehensively understand threshold determination and the analysis of drug-resistance gene mutations. In the threshold determination link, the Ct value is used to determine the number of cycles required for the fluorescence signal in qPCR to reach the threshold. A lower Ct value corresponds to a higher bacterial load. Subsequently, based on the melting curve peak pattern, the purity of the bacterial species in the sample is revealed. For the analysis of drug-resistance mutations, the sequencing results are compared with the data in the existing Mycobacterium tuberculosis drug-resistance gene database (such as the TBDrea database). Finally, the types of drug-resistance mutations can be clarified, supporting the assessment of clinical relapse risks and the fine-tuning of subsequent treatment plans. Under the monitoring results, if MTB DNA remains positive or drug-resistance genes appear, it indicates an increased probability of tuberculosis relapse in patients, and consideration should be given to changing to second-line drugs, such as delamanid and bedaquiline [1].

## 3. Influence mechanism of molecular diagnostic techniques on the efficacy of tuberculosis relapse monitoring

### 3.1. Precise identification and dynamic tracking of molecular markers

In the monitoring of tuberculosis relapse, the identification and dynamic tracking of molecular markers are the keys to assessing the relapse risk of tuberculosis patients. Based on high-throughput sequencing and real-time PCR fluorescence technology, medical institutions can accurately identify specific gene fragments of *Mycobacterium tuberculosis* (such as IS6110 and RD9). The presence and dynamic changes in the expression levels of these markers are important indicators for monitoring tuberculosis relapse. At the same time, combined with target amplification and specific probe hybridization, sequence comparison before and after patient treatment can be achieved, and then the tracking of drug-resistance mutations, such as rpoB and katG, can be realized. Compared with traditional culture methods, molecular diagnostic techniques can provide rapid and sensitive means for disease course and dynamic monitoring, effectively identifying signs of relapse and guiding medical institutions to implement targeted and precise intervention management for patients.

### 3.2. Multidimensional analysis of molecular heterogeneity

The molecular heterogeneity of *Mycobacterium tuberculosis* has a direct impact on the relapse risk and drug-resistance characteristics of tuberculosis. The application of molecular diagnostic techniques can reveal the

mutation spectra inside and outside the strain and the heterogeneity of gene expression in multiple dimensions through whole-genome sequencing and single-cell RNA sequencing. Multidimensional analysis methods can provide medical institutions with a comprehensive and in-depth understanding of the strain lineage and drug-resistance mechanisms, especially for different drug-resistance gene mutations, such as the distribution of katG S315T and inhA. Precise analysis of heterogeneity can not only promote the optimization of standard treatment plans but also provide personalized targeted treatment for different patients. At the same time, by using the analysis of molecular heterogeneity, it is possible to quickly determine whether the relapse strain and the primary strain are homologous, and then accurately judge whether the patient's condition is a primary infection or a relapse infection.

### 3.3. Visualization of host-microenvironment interactions

From the perspective of host-microenvironment interactions, the use of molecular diagnostic techniques in tuberculosis relapse monitoring enables visual monitoring. Using multiple chromatography-mass spectrometry and cell imaging technology, medical institutions can quickly analyze the interactions between the host immune response and pathogens, thereby assessing the immune homeostasis of the host after treatment. Such interactions can effectively reveal the regulatory mechanisms of cytokines such as interferon and tumor necrosis factor during relapse. At the same time, combined with RNA in situ hybridization and multispectral imaging technology, medical institutions can effectively observe the co-localization of pathogen distribution and host cell responses in tissue sections. Visual analysis can promote a deeper understanding of tuberculosis reactivation in medical institutions and provide strong support for the research and development of new medical treatments and therapies [2].

## 4. Analysis of the application effect of molecular diagnostic techniques in tuberculosis relapse monitoring

To objectively understand the efficacy of molecular diagnostic techniques in tuberculosis relapse monitoring, two hundred cured tuberculosis patients were selected from a public tertiary-grade A hospital in this study. Among them, one hundred patients were monitored by traditional relapse monitoring methods after treatment and set as the control group, and 100 patients were monitored by molecular diagnostic techniques after treatment and set as the experimental group. This article will demonstrate that molecular diagnostic techniques have higher efficacy than traditional monitoring methods by comparing the relapse monitoring results of the two groups of patients.

### 4.1. Research subjects

The research subjects of this study were 200 cured tuberculosis patients from a public tertiary-grade A hospital, aged 18–65 years old, including 100 male patients and 100 female patients. The samples were selected through strict case screening to ensure that all 200 subjects had no serious complications. In terms of monitoring methods, a 100 patients in the control group were monitored by traditional relapse monitoring methods, that is, sputum smear microscopy + Lowenstein-Jensen culture. Another hundred patients in the experimental group were monitored by molecular diagnostic techniques after treatment, that is, Xpert MTB/RIF combined with CRISPR-Cas detection.

The follow-up period of this study was set to 12 months after the patients received treatment and follow-up visits were conducted on average every 3 months. The main follow-up data included the tuberculosis relapse rate,

detection sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). In terms of data collection, the etiological test results, clinical symptoms, imaging features, and treatment history of the patients in the control group and the experimental group were recorded [3–5].

## 4.2. Comparison of the effects of different monitoring methods

### 4.2.1. Comparison of performance indicators

The detection rates, sensitivities, specificities, PPVs, NPVs, detection time, and single-test costs of traditional monitoring methods and molecular diagnostic techniques were compared, as shown in **Table 1**.

**Table 1.** Comparison of performance indicators between traditional monitoring methods and molecular diagnostic techniques for tuberculosis recurrence

| Indicators | Traditional monitoring method (Control group) | Molecular diagnosis (Experimental group) |
|---|---|---|
| Detection rate | 72% (72/100) | 95% (95/100) |
| Sensitivity | 65% | 98% |
| Specificity | 89% | 97% |
| Positive predictive value (PPV) | 75% | 96% |
| Negative predictive value (NPV) | 85% | 98% |
| Detection time cost | Sputum smear: 24 hours<br>Culture: 21 days | 2 hours |
| Single-test cost (Yuan) | 150 (sputum smear)<br>300 (culture), total 450 yuan | 500 yuan |

By comparing the two monitoring methods, the molecular diagnostic technique used in the experimental group has a sensitivity 33 percentage points higher than that of the traditional monitoring method and its specificity is significantly better than that of the traditional method (97% for molecular diagnosis vs. 89% for the traditional method). At the same time, the PPV and NPV of the molecular diagnostic technique reach 96% and 98%, respectively, which are much higher than 75% and 85% of the traditional monitoring method. In addition, in terms of time cost, molecular diagnosis only takes 2 hours, while the entire process of the traditional monitoring method takes 21 days. Although the cost of molecular diagnosis is slightly higher than that of the traditional monitoring method, considering the indicator performance and time cost, molecular diagnosis actually has a lower cost [6–8].

### 4.2.2. Comparison of patient relapse rates and clinical outcomes

**Table 2** shows the comparison of relapse rates and clinical outcome data of 100 patients in the control group and the experimental group, including the relapse rates within 12 months, early relapse situations, and symptom remission times of the two groups of patients.

Based on the data comparison in **Table 2**, among the 100 patients in the experimental group using molecular diagnostic techniques, the relapse rate within 12 months is just 5%, with the early relapse rate controlled at 2%. This is significantly lower compared to the relapse rates observed with traditional monitoring methods. This shows that molecular diagnostic techniques, with an average of one test every 3 months, can quickly detect the signs of tuberculosis relapse. In addition, the imaging abnormality rate and symptom remission time of the experimental group are much lower than those of the control group [9, 10].

**Table 2.** Comparison of recurrence rates and clinical outcomes between the control group and the experimental group

| Indicators | Molecular diagnostic technique (Experimental group) | Traditional monitoring method (Control group) | *P*-value |
|---|---|---|---|
| Relapse rate within 12 months | 5% (5/100) | 18% (18/100) | < 0.01 |
| Early relapse (≤ 6 months) | 2% (2/100) | 12% (12/100) | < 0.05 |
| Imaging abnormality rate | 8% (8/100) | 25% (25/100) | < 0.01 |
| Symptom remission time (days) | 14 ± 3 | 21 ± 5 | < 0.001 |

## 5. Conclusion

Based on this study, it can be concluded that compared with traditional tuberculosis relapse monitoring methods, molecular diagnostic techniques can comprehensively improve the early-warning level of tuberculosis relapse based on highly sensitive pathogen detection and rapid drug-resistance gene analysis, reducing the probabilities of early and long-term relapse. In the future, medical and health institutions can promote the large-scale and popularized application of molecular diagnostic techniques in areas with limited medical resources through medical insurance policies and the technical training of medical staff, to achieve high-quality relapse monitoring of tuberculosis patients and improve the patients' living standards.

## Disclosure statement

The authors declare no conflict of interest.

## References


[1] Zhao Y, Tu X, Wang N, et al., 2024, Comparison and Application Analysis of the Positive Detection Contributions of Three Etiological Detection Methods for Pulmonary Tuberculosis Patients. Chinese Journal of Antituberculosis, 2024: 1–12.

[2] Gu J, Zhang P, 2025, Evaluation of the Application Value of Three Mycobacterium Tuberculosis Detection Methods in a General Hospital. Journal of Tuberculosis and Lung Diseases, 6(1): 68–72.

[3] Ke Z, Tao X, Zhang X, et al., 2024, Application Progress of Molecular Biology Techniques in the Diagnosis of Multidrug-Resistant/Rifampicin-Resistant Tuberculosis. Zhejiang Journal of Integrated Traditional Chinese and Western Medicine, 34(11): 1073–1077.

[4] Mairihaba K, Maiwulajiang Y, Alimire A, et al., 2024, Analysis of the Spatial Clustering and Influencing Factors of Tuberculosis Relapse Among Patients in Kashgar Region, Xinjiang From 2013 to 2020. Modern Preventive Medicine, 51(14): 2501–2506.

[5] Sun Q, Wang X, Fu J, et al., 2024, Analysis of the Relapse Situation and Influencing Factors of Newly Treated Tuberculosis Patients After Successful Treatment in Nantong City, Jiangsu Province From 2013 to 2020. Disease Surveillance, 39(10): 1270–1275.

[6] Zhao Y, Tu X, Wang N, et al., 2024, Comparison and Application Analysis of the Positive Detection Contributions of Three Etiological Detection Methods for Pulmonary Tuberculosis Patients. Chinese Journal of Antituberculosis, 2024: 1–12.

[7] Yan G, Wang X, Wang Y, et al., 2025, Diagnostic Value of Metagenomic Next-Generation Sequencing for Suspected Osteoarticular Tuberculosis Patients. Chinese Journal of Antituberculosis, 47(2): 175–180.

[8] Ke Z, Tao X, Zhang X, et al., 2024, Application Progress of Molecular Biology Techniques in the Diagnosis of Multidrug-Resistant/Rifampicin-Resistant Tuberculosis. Zhejiang Journal of Integrated Traditional Chinese and Western Medicine, 34(11): 1073–1077.

[9] Shang Y, Li Q, Feng M, et al., 2023, Analysis of the Results of the Proficiency Testing of Molecular Diagnostic Techniques for Tuberculosis in Shanxi Province. Journal of Shanxi Health Vocational College, 33(5): 64–65.

[10] Li S, Wang Y, Shu W, et al., 2023, New Progress in the Research and Development of Tuberculosis Laboratory Diagnostic Techniques. Chinese Journal of Antituberculosis, 45(5): 446–453.