

# Developing Tuberculosis Diagnosis with Polymerase Chain Reaction Techniques

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## DESCRIPTION

The traditional methods of Tuberculosis (TB) diagnosis, such as sputum smear microscopy and culture, have limitations in sensitivity and specificity, especially in paucibacillary and extrapulmonary TB cases. Polymerase Chain Reaction (PCR) has emerged as a powerful molecular diagnostic tool that offers rapid, sensitive, and specific detection of TB. This article delves into the principles, applications, and advantages of PCR in the context of TB diagnosis. PCR works by amplifying specific DNA sequences of *Mycobacterium tuberculosis*, enabling detection even when bacterial levels are low. This technique involves repeated cycles of DNA denaturation, primer annealing, and strand extension to exponentially increase the target DNA. PCR can identify TB in various clinical samples, such as sputum, blood, and tissue biopsies, making it particularly valuable for diagnosing both pulmonary and extrapulmonary TB. Additionally, PCR can detect genetic mutations linked to drug resistance, facilitating the fast initiation of effective treatment regimens.

## Principles and advantages of PCR in TB diagnosis

PCR is a molecular technique that amplifies specific DNA sequences, allowing for the detection of even minute quantities of genetic material. The process involves three steps, such as, denaturation the double-stranded DNA is heated to around 94°C-98°C, causing it to denature into two single strands. Annealing the temperature is lowered to 50-65°C to enable short DNA primers to bind to the complementary sequences on the single-stranded DNA. Extension The temperature is raised to 72°C, allowing the DNA polymerase enzyme to extend the primers and synthesize new DNA strands. These steps are repeated for 25-40 cycles, leading to exponential amplification of the target DNA sequence, making it detectable by various methods. The advantages of PCR were, traditional culture methods can take weeks to yield results, while PCR can provide results within hours to a few days. This rapid turnaround is critical for early diagnosis and treatment initiation. PCR is highly sensitive and can detect low levels of bacterial DNA, making it especially useful in cases with low bacterial load. Its specificity reduces the likelihood of

false-positive results, which can occur with non-specific staining methods. PCR can be applied to a wide range of clinical specimens, making it a versatile tool for diagnosing both pulmonary and extrapulmonary TB. PCR can identify genetic mutations associated with drug resistance, enabling the fast initiation of appropriate treatment and reducing the spread of resistant strains.

## Applications in TB diagnosis

PCR can target specific sequences in the *Mycobacterium tuberculosis* genome, such as the IS6110 insertion element, the 16S rRNA gene, and the *rpoB* gene associated with rifampicin resistance. The application of PCR in TB diagnosis includes, PCR can rapidly detect the presence of *M. tuberculosis* DNA in clinical samples, including sputum, blood, cerebrospinal fluid, and tissue biopsies. This is particularly valuable for diagnosing extrapulmonary TB, where traditional methods often fail. PCR can identify mutations in genes associated with resistance to first-line TB drugs, such as rifampicin and isoniazid. This facilitates the rapid determination of multidrug-resistant TB (MDR-TB) and enables timely initiation of appropriate treatment regimens. Real-time PCR (qPCR) allows for the quantification of bacterial DNA, providing an estimate of the bacterial load in a sample. This is useful for monitoring treatment response and disease progression. Despite its advantages, PCR is not without challenges. False-negative results can occur due to the presence of PCR inhibitors in clinical samples or mutations in primer binding sites. The high cost of PCR and the need for specialized equipment and trained personnel may limit its accessibility in resource-limited settings. Additionally, PCR cannot distinguish between live and dead bacteria, which may lead to positive results even after successful treatment.

## CONCLUSION

Polymerase Chain Reaction has revolutionized the diagnosis of tuberculosis, offering rapid, sensitive, and specific detection of *M. tuberculosis* and drug resistance. Its application in TB diagnosis enhances early detection, enables timely treatment, and

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**Received:** 03-Jun-2024, Manuscript No. MDTL-24-33224; **Editor assigned:** 05-Jun-2024, Pre QC No. MDTL-24-33224 (PQ); **Reviewed:** 19-Jun-2024, QC No. MDTL-24-33224; **Revised:** 26-Jun-2024, Manuscript No. MDTL-24-33224 (R); **Published:** 02-Jul-2024, DOI: 10.35248/2161-1068.24.14.476.

**Citation:** Takshi P (2024) Developing Tuberculosis Diagnosis with Polymerase Chain Reaction Techniques. *Mycobact Dis*.14:476.

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contributes to better disease management and control. As technology advances and becomes more accessible, PCR is

poised to play an even more significant role in the global fight against tuberculosis.