

Molecular Diagnosis of Skin Mycobacterial Infections

Orion Jorvik*

Department of Dermatology, St Vincent's Hospital, Sydney, Australia

DESCRIPTION

Mycobacteria are a diverse group of bacteria that include *Mycobacterium tuberculosis* (*M. tb*) and a wide range of Non-Tuberculous Mycobacteria (NTM), such as *Mycobacterium leprae*, *Mycobacterium marinum*, and *Mycobacterium ulcerans*. These organisms are often responsible for cutaneous infections, which can range from localized skin lesions to disseminated disease, depending on the species involved and the immune status of the host. Accurate and timely identification of mycobacterial species in skin tissue is important for effective treatment, as different species may require distinct therapeutic approaches. Traditional methods for identifying mycobacteria, such as culture and histopathology, have limitations. Mycobacteria are notoriously slow-growing organisms, with cultures often taking weeks to yield results. Furthermore, histopathological examination may suggest the presence of mycobacteria through the identification of granulomatous inflammation, but it cannot definitively identify the species involved.

PCR Amplification and its steps

Polymerase Chain Reaction (PCR) amplification has revolutionized the diagnosis of mycobacterial infections. PCR allows for the rapid detection and identification of mycobacterial DNA directly from tissue samples, bypassing the need for time-consuming culture techniques. By targeting specific genetic sequences unique to mycobacteria, PCR can provide results within hours to days, significantly speeding up the diagnostic process.

Sample collection and DNA extraction: The process begins with a skin biopsy or other tissue samples from the affected area. Once the tissue is collected, DNA is extracted from the sample. This involves breaking down the cell membranes of the mycobacteria to release their genetic material.

Amplification of mycobacterial DNA: Using PCR, the extracted DNA is amplified to generate multiple copies of a specific mycobacterial gene. This is typically done using primers

that are complementary to conserved regions of the mycobacterial genome, such as the *16S rRNA* gene, *hsp65* gene, or regions within the *rpoB* gene. These genes are commonly targeted because they contain sequences that vary between different species of mycobacteria, allowing for species-level identification.

Detection and identification: Once amplification is complete, the resulting PCR products are analyzed using techniques such as gel electrophoresis, sequencing, or hybridization to species-specific probes. Sequencing is a particularly powerful tool as it provides precise information about the genetic sequence, allowing for accurate species identification by comparing the results to known mycobacterial sequences in genetic databases.

Quantitative PCR (qPCR): In addition to standard PCR, quantitative PCR (qPCR) is often used for more accurate quantification of bacterial load. This can be particularly useful in monitoring the response to treatment in cases of chronic or persistent mycobacterial infections.

Advantages and limitations of PCR

PCR can provide results much faster than traditional culture-based methods, enabling more timely initiation of appropriate therapy. PCR is highly sensitive, allowing for the detection of low levels of bacterial DNA that might be missed by culture or histology. By targeting specific genetic sequences, PCR can accurately identify the species of mycobacteria involved, which is critical for guiding treatment. For instance, infections caused by NTM such as *Mycobacterium avium* or *Mycobacterium abscessus* may require different antibiotics than those used to treat tuberculosis or leprosy. Despite its advantages, PCR is not without its limitations. False-positive results can occur due to contamination of samples with environmental mycobacteria or due to residual DNA from dead bacteria, which may not reflect active infection. Therefore, PCR results must be interpreted in conjunction with clinical findings and other diagnostic tests. Additionally, PCR may not always distinguish between closely related mycobacterial species without further sequencing or specialized assays.

Correspondence to: Orion Jorvik, Department of Dermatology, St Vincent's Hospital, Sydney, Australia, Email: orinjvik@outlook.com

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CONCLUSION

The identification of mycobacterial species in skin tissue using PCR-based amplification techniques represents a significant advancement in the diagnosis of cutaneous mycobacterial infections. By providing rapid, sensitive, and specific identification, PCR allows clinicians to tailor treatment more effectively and improve patient outcomes. However, PCR should be used as part of a comprehensive diagnostic strategy that

includes clinical, histological, and microbiological findings to ensure accurate diagnosis and appropriate management of mycobacterial infections. The slow-growing nature of these organisms, combined with their often nonspecific clinical and histological presentation, makes rapid identification challenging. This delay can have significant consequences for patient management and outcomes, particularly in cases involving drug-resistant strains or unusual NTM species.