

# **Aplicación de métodos moleculares para la identificación de las especies del complejo *Mycobacterium tuberculosis***

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**INTRODUCTION:** The *Mycobacterium tuberculosis* complex includes the following species: *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium bovis*-BCG, *Mycobacterium microti*, *Mycobacterium caprae*, *Mycobacterium pinnipedii*, and *Mycobacterium canettii*. These species cause tuberculosis in humans and animals. Identification of mycobacterial strains has classically been performed by phenotype study. Over the last years, laboratories have developed several molecular techniques to differentiate between these species. The aim of this study is to evaluate these methods and develop a simple, fast, identification scheme.

**MATERIAL AND METHODS:** We analyzed 251 strains randomly obtained from the strains studied in 2004, and 797 strains received by the Reference Laboratory between 2005 and 2007. Phenotype characterization of 4183 strains isolated during that period was done by studying the colony morphology, characteristics in culture, nitrate reduction, niacin accumulation, and growth in the presence of thiophen-2-carboxylic acid hydrazide 10µg/mL and pyrazinamide 50µg/mL. The molecular identification scheme designed was as follows: 1) *gyrB* PCR-RFLP with *RsaI*, *TaqI* or *SacII* and *hsp65* RFLP/PCR with *HhaI*, and 2) multiplex-PCR to determine the presence/absence of the RD9 and RD1 regions.

**RESULTS:** The results showed 100% agreement between phenotype study and the molecular scheme.

**DISCUSSION:** This molecular identification scheme is a simple and fast method, with 100% sensitivity and specificity, that can be implemented in most clinical laboratories at a low cost.