DOUBLE-STEP MULTIPLEX REAL TIME PCR WITH MELTING CURVE ANALYSIS FOR DETECTION AND DIFFERENTIATION OF MYCOBACTERIA IN SPUTUM

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Abstract. Mycobacterium tuberculosis (M. tb) is a causative agent of tuberculosis, a worldwide public health problem. In recent years, the incidence of human mycobacterial infection due to species other than M. tb has increased. However, the lack of specific, rapid, and inexpensive methods for identification of mycobacterial species remains a pressing problem. A diagnostic test was developed for mycobacterial strain differentiation utilizing a double-step multiplex real time PCR together with melting curve analysis for identifying and distinguishing among M. tb, M. bovis BCG, other members of M. tb complex, M. avium, and non-tuberculosis mycobacteria. The assay was tested using 167 clinical sputum samples in comparison with acid-fast staining and culturing. Using only the first step (step A) the assay achieved sensitivity and specificity of 81% and 95%, respectively. The detection limit was equivalent to 50 genome copies.

Keywords: Mycobacterium tuberculosis complex, DNA melting curve, real time PCR, non-tuberculous mycobacteria

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