Identification of a Mycobacterium tuberculosis-specific gene marker for diagnosis of tuberculosis using semi-nested melt-MAMA qPCR (lprM-MAMA)

ABSTRACT

Tuberculosis (TB) is the deadliest of infectious diseases. TB diagnosis, based on sputum microscopy, culture, and nucleic acid amplification tests (NAATs) to identify its main causative agent, Mycobacterium tuberculosis (MTB), remains challenging. The current available NAATs, endorsed by World Health Organization (WHO), can differentiate MTB from some MTB complex (MTBC) members. Using bioinformatics, we identified a single nucleotide polymorphism (SNP) in lprM (Rv1970) gene that differentiate MTB from other MTBC members. A forward mismatch amplification mutation assay (MAMA) primer was designed for the targeted mutation and was used in a semi-nested melt-MAMA qPCR (lprM-MAMA). Using the optimized protocol, lprM-MAMA was positive with all MTB reference and clinical strains, and negative with other MTBC members, non-tuberculous mycobacteria (NTM) and other non-mycobacterial (NM) reference strains. The limit of detection (LOD) of lprM-MAMA was 76.29 fg. Xpert® MTB/RIF (Xpert)-positive sputum samples were also positive by lprM-MAMA, except for samples classified as having "very low" bacterial load by Xpert. Xpert-negative sputum samples were also negative by lprM-MAMA. In conclusion, lprM-MAMA demonstrated to be a useful tool for specific MTB diagnosis. Further evaluation with higher number of reference strains, including NTM and NM; and sputum samples are required to determine its potential for clinical application.