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Title: Dual TaqMan probes for the detection of rifampin resistance in Mycobacterium tuberculosis

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Body: Mutations (SNP) at codons 526, 531 of rpoB gene of MTB are considered to be biomarkers of resistance to rifampin (RMP). The aim of the study was to develop 96-well plate qPCR platform with dual TaqMan probes for the prediction of RMP resistance in MTB. Methods. A total number of 122 DNA extracts from MTB cultures were studied. SNPs in 516, 526, 531 codons of rpoB gene were detected by autosequencing and qPCR with dual TaqMan probes (labeled with FAM and JOE for identification of wild and mutated codons correspondingly). Results. We present a 96-well PCR plate method for identification of SNPs in rpoB gene of MTB. Each 96-well PCR plate is organized as follows: 1) 12 DNA extracts can be investigated collectively; 2) four wells are used for each DNA extract to identify MTB and mutations in 516, 526, 531 codons; 3) one set of controls per 6 DNA extracts is provided including positive, and negative (with and without mutations), and no-template controls for each codon tested. The entire assay includes the following steps:1) filling wells with DNA-extracts and PCR reaction mixtures using repeater pipette; 2) amplification and data bioanalysis including: a) detection of fluorescence threshold using method of negatives with 20% tolerance; b) allele discrimination analysis based on detection of differences in JOE and FAM fluorescence. The results obtained with this real time PCR design agreed well with DNA sequencing data. Out of 122 DNA extracts (488 reactions) 8 samples were false negative (7%) versus sequencing data. This 96-well PCR plates diagnostic platform resembles ELISA and represents an adequate method for the specific and rapid detection of RMP resistance in MTB cultures.