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DEVELOPMENT OF A REAL-TIME PCR INCORPORATING HIGH RESOLUTION MELTING ANALYSIS TO DETECT RESISTANCE-RELATED SINGLE NUCLEOTIDE POLYMORPHISMS IN THE HIV-1 REVERSE TRANSCRIPTASE GENE

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Introduction: High resolution melting analysis (HRMA) has been shown to successfully detect single nucleotide polymorphisms (SNPs) in human, bacterial and viral genetic material. HRMA is based upon differences in melting curves produced when heating DNA in the presence of a saturating double-stranded DNA-binding fluorescent dye. While HRMA indicates base changes within the entire amplicon, unlabelled, 3' blocked oligonucleotide probes can be incorporated in the assay to signal the presence or absence of a particular genotype.

Materials and Methods: Four PCR assays were designed encompassing codons 100-108, 146-154, 181-191 and 212-229 of the HIV-1 reverse transcriptase gene. Unlabelled probes were then designed to specifically anneal to either the wild-type or mutant sequences, at codon positions 103, 151, 181, 184, 190, 215 and 225. The assays were performed on the LightCycler 480 Real-Time PCR system using LightCycler High Resolution Melting Master (Roche Diagnostics, GmbH). We analyzed 120 patient samples with previously determined genotypes (Sanger population sequencing). Clonal analysis was then performed on three samples to verify the accuracy of the results.

Results: HRMA including 3' blocked oligonucleotide probes correctly genotyped the amino acid sequence in 92% of patient samples at position 103 (K103N), 86% at 151 (Q151M), 92% at 181 (Y181C), 85% at 184 (M184V) and 84% at 190 (G190A). At each position, the samples that could not be genotyped were possibly due to the extent of mismatches between the probe and target. The T215Y/F and P225H mutations produced multiple melting peaks which could not be genotyped. The incorporation of mismatched and deleted bases within the probe did improve the accuracy of detection; whereas inosine, locked nucleic acids, ambiguous bases as well as shorter probes did not improve accuracy. The limit of detection was ~10% of mixed species. Clonal analysis confirmed that HRMA is more sensitive than traditional population sequencing.

Conclusion: HRMA presents a quick, inexpensive, sensitive and accurate method of HIV-1 resistance codon genotyping. Future work involves designing assays for M41L, K65R, D67N, K70R and V106M.