

Abstract

Introduction

High resolution melting analysis (HRMA) accurately, rapidly and cost effectively detects single nucleotide polymorphisms by monitoring DNA dissociation kinetics. This technology was applied to HIV samples to assess whether it could be used to detect clinically relevant drug resistance mutations.

Methods

HRMA-PCR assays incorporating unlabeled probes were designed to genotype 12 mutation codons in the HIV-1 *p66/p51* of engineered plasmids and 116 HIV-1 samples.

Results

HRMA correctly genotyped 63%-88% of the K103N, Y181C, M184V, Q151M and G190A mutations. Each assay had a 1.7%-3.4% discordance, most of which was due to the increased analytical sensitivity of HRMA (~5-20%). Only mutant K65R and V106M were correctly identified while the 41, 67, 70, 215 and 225 codons could not be genotyped. Assay modifications had some success in masking the affects of polymorphisms.

Conclusion

These assays can be used for genotyping selected major HIV-1 resistance mutations and should be further developed as a resistance surveillance tool.

Development of a real-time PCR incorporating high resolution melting analysis to screen HIV-1 samples for resistance-related codons. David Sacks August 2010