Rapid Detection of HIV-1 subtype C Integrase resistance mutations by the Use of High-Resolution Melting Analysis

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**HIV-1 drug resistance**

1. With increasing numbers of people initiating ART, there have been increasing concerns over the development and transmission of HIV drug resistance (HIVDR).

2. This has significant implications to the efforts to eliminate HIV.
Current status of HIVDR testing

1. Diagnostic laboratory tests in RLS have mainly relied on immunological and clinical evaluations for HIVDR which are not as accurate and efficient.

2. Resistance testing using the Sanger method has been reserved for individuals with virologic failure on second-line ART.

3. HIV treatment guideline panels in the United States and Europe to recommend that HIV-infected individuals be tested for antiretroviral drug-resistant virus prior to initiation or modification of ART.

4. Other genotyping technologies such as point mutations assays (PMAs) are more feasible for such settings, as they are less costly and require less expertise and laboratory infrastructure.
Background
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Rapid (<1 hr), low-cost ($10's), and simple (sample-to-answer) MDx to detect up to 1000 unique DNA sequences/targets.
1. HRMA is a rapid and relatively simple DNA-typing and mutation scanning methodology that genotypes by melting curve analysis.

2. HRMA genotyping is suited to relatively invariant genetic material that presents a challenge to the genotyping of RNA viruses, such as HIV.

3. Sacks *et al* (2017) have however shown that it is possible to genotype 80-90% of HIV samples with high accuracy and sensitivity.
Objectives

1. To optimize an High resolution Melting Analysis for the detection of HIV integrase resistance mutation, N155H.

Specific Objectives

a) To determine genetic relatedness of the Integrase gene by Multiple Sequence Analysis.
b) To design primers and standards for HRMA and nested PCR.
c) To validate the HRMA method.
d) To determine HIV-1 Subtype C integrase mutation (N155H) in plasma samples using the optimized HRMA method.
e) To analyze the melting temperatures for detection of the N155H mutation.
Multiple Sequence alignment on existing Integrase sequence data

Design a consensus subtype C Integrase sequence

Validate the High resolution Melting Analysis using Real Time PCR

Design Primers and Standards
HRMA can be used to distinguish between the wild type and mutant standards.

Figure 1: Melt curve for the WT (red) and MT (blue) standards for set 1.
METHOD

1. RNA extraction
2. cDNA synthesis
3. First round of Nested PCR
4. 2nd round of Nested PCR
5. Sanger sequencing
6. Analysis of results

HRMA
HRMA was used to detect a wild type genotype in a patient sample.

Figure 1: Melt curve showing the WT (red), MT (blue) and one WT sample (purple).
Limitations

1. Synthetic standards were used for optimization of method.
2. The assay is prone to interferences from the naturally occurring polymorphisms associated with HIV
Conclusion

1. HRMA can be used an alternative method for the detection of HIV-drug resistance.

2. HRMA has a high turnaround time, is considerably cheaper than sequencing and has potential for use in near point of care testing therefore is suitable for use in Resource limited settings.
Recommendations

1. Test more clinical samples as an expansion of the validation of the method.
2. Designing a multiplex HRMA PCR that would allow for detection of more than one mutation.
3. Designing longer primers that will reduce the variability in the regions flanking the codon of interest.
4. Decreasing the size of the amplicon to reduce variability in the regions flanking the mutation of interest.
5. Explore combining HRMA and labeled probes to increase specificity and the level of multiplexing.
References


2. Guidelines consolidated guidelines on hiv prevention, diagnosis, treatment and care for key populations. 

3. Treat all: policy adoption and implementation status in countries hiv treatment and care. 2017
Small DNA Differences Matter

Albert Einstein (1879-1955) - Bobo the Chimp (1995-Now) = 1.5% DNA Difference

Thank you