Isothermal Point Mutation Detection: Toward a DNA-based Rapid Diagnostic for Sickle Cell Anemia

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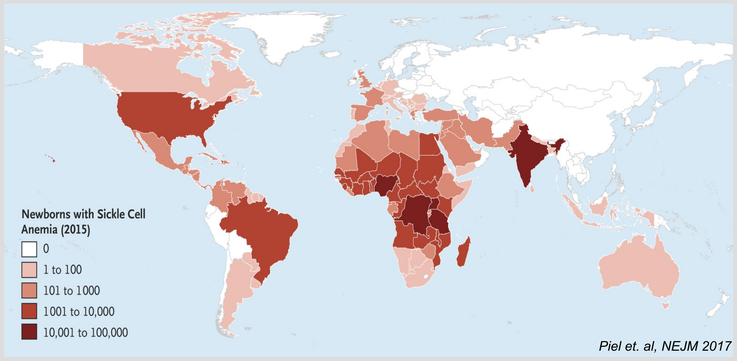
Background

- Sickle cell disease (SCD) is a life-threatening inherited blood disorder that affects over 300,000 newborns globally per year
- Disproportionately impacts low-resource settings, with 90% of SCD occurring in low- and middle-income countries
- Gold-standard diagnostic methods are inaccessible in low-resource settings due to infrastructure requirements and cost
- Current point-of-care tests suffer from poor specificity, non-intuitive readout, and incompatibility with blood transfusions

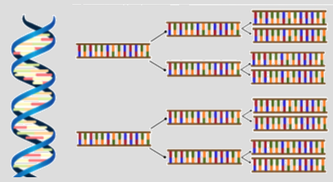


Our Approach

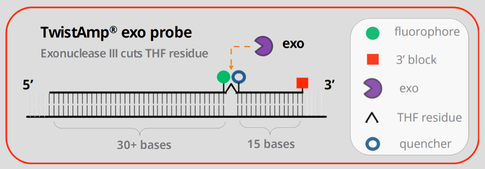
A **DNA-based diagnostic** that detects the point mutation in the beta-globin gene that forms sickled hemoglobin (HbS)

- **Recombinase polymerase amplification (RPA)** isothermally and selectively amplifies genomic DNA coding for HbS or hemoglobin A (HbA)
- A **fluorescence-based readout** and subsequent analysis identifies the genotype of the template nucleic acid

Recombinase Polymerase Amplification

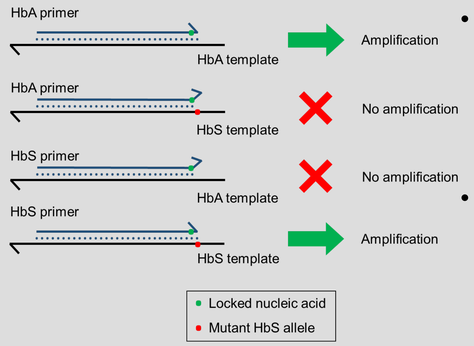


- Isothermal nucleic acid amplification method
- Replaces PCR and the need for thermal cycling
- Operates at 37-42°C and can be incubated against the human body



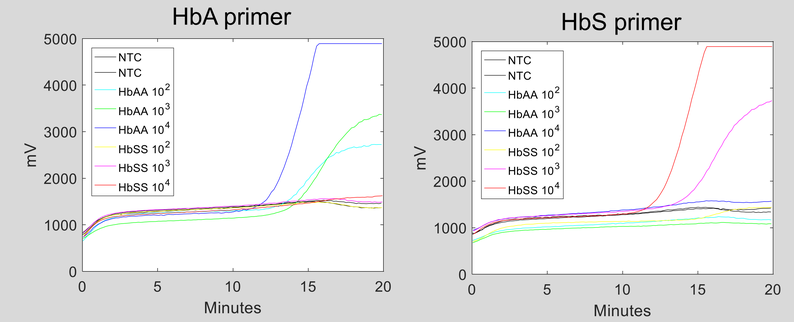
- Reaction products can be detected by a sequence-specific oligonucleotide probe

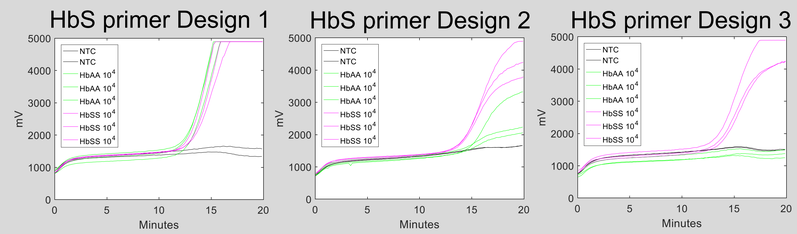
Isothermal Point Mutation Detection



- Strategic primer design enables single-nucleotide polymorphism discrimination in a single round of amplification
- Resulting products can be detected with a sequence-specific probe in a fluorimeter or on a lateral flow strip

Fluorescence-based Readout





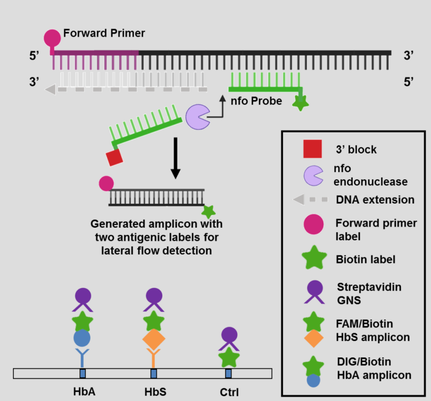
- The two reactions show a concentration-dependent output with amplification of the mismatched template suppressed or delayed
- Primer characteristics are important for discrimination capabilities

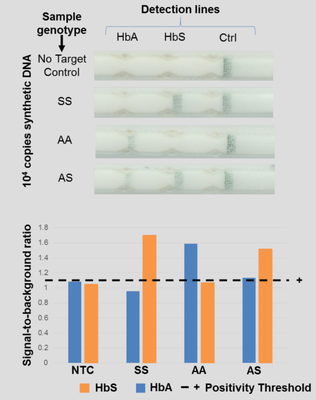
Assay Cost Estimates

Assay Component	Cost (USD)
RPA components	$4.27
Primers and probe	$0.43
Total	**$4.70**

T8-ISO (Axxin)

Multiplexed Paper-based Detection





- Amplification products can also be detected on a lateral flow strip
- Proof of concept data shows that amplification and capture of synthetic templates is maintained in a multiplexed format both visually (top right) and quantitatively with signal-to-background ratio analysis of scanned images (bottom right)

Results with Clinical Samples

Primer performance

Identification of positive samples

Primer	TP	TP + FN	%
HbA	13	13	100.0%
HbS	72	79	91.1%

Identification of negative samples

Primer	TN	TN + FP	%
HbA	63	67	94.0%
HbS	6	6	100.0%

- Blood samples from sickle cell patients at Baylor College of Medicine as well as normal volunteers were analyzed with one or both allele-specific primer(s)
- Amplification of the mismatched template is delayed or suppressed
- Over 90% of results are accurate

Conclusions and Future Directions

- HbS and HbA can be distinguished in a single round of recombinase polymerase amplification with strategic primers
- Future work will incorporate the ability to detect HbC, and reduce the volume of the reaction, therefore reducing cost
- End goal to incorporate sample preparation steps and a custom fluorimeter for a true sample-to-answer test

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