An E-Junction Lateral Flow Immunoassay for Widespread Sickle Cell Screening

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Background

Sickle cell disease (SCD) is a life-threatening inherited blood disorder that causes red blood cells to become fragile and sickle shaped. Symptoms include anemia, pain episodes, organ damage, increased risk for infection and reduced life expectancy. The disease is especially prevalent in sub-Saharan Africa, where the mortality rate among children is estimated as high as 50-80%. In high-income countries, universal screening and early intervention programs have proven to be effective in reducing the fatality of SCD, but these methods rely on diagnostic methods that are expensive and resource heavy. As a result, SCD screening programs in sub-Saharan Africa has been limited to small-scale programs.[1] These programs are limited by factors including expense, test inaccuracy and the requirement of additional reagents for blood dilution, and affected individuals remain untreated or treated for false diagnoses.

A lateral flow immunoassay incorporating an E-junction allows for a diagnostic device that meets the economic, usability and reliability demands of a sickle cell screening device in low and middle-income countries (LMICs).

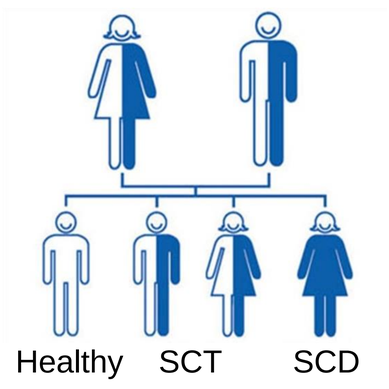

Figure 1. Trend for the genetic inheritance of SCD and sickle cell trait (SCT).

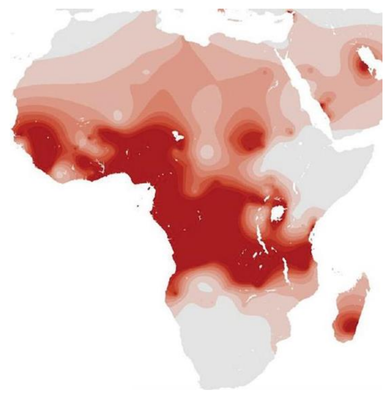

Figure 2. Frequency of the HbS (sickle cell hemoglobin) allele in Africa. The trait is most prevalent where the map is dark red.

Methods

The device incorporates an E-junction into a traditional direct-binding lateral flow test strip design. Through the E-junction design, the test strip can accept a whole blood sample without oversaturating the antibody binding sites, a phenomenon known as the "hook effect".[2] Each test progresses in the following order:

1. A drop of whole blood is deposited on arm 1
2. Arm 2 receives wash buffer (PBS + 0.1% Tween 20 + 0.1% BSA)
3. Detection beads are released from the conjugate pad on arm 3 with wash buffer.

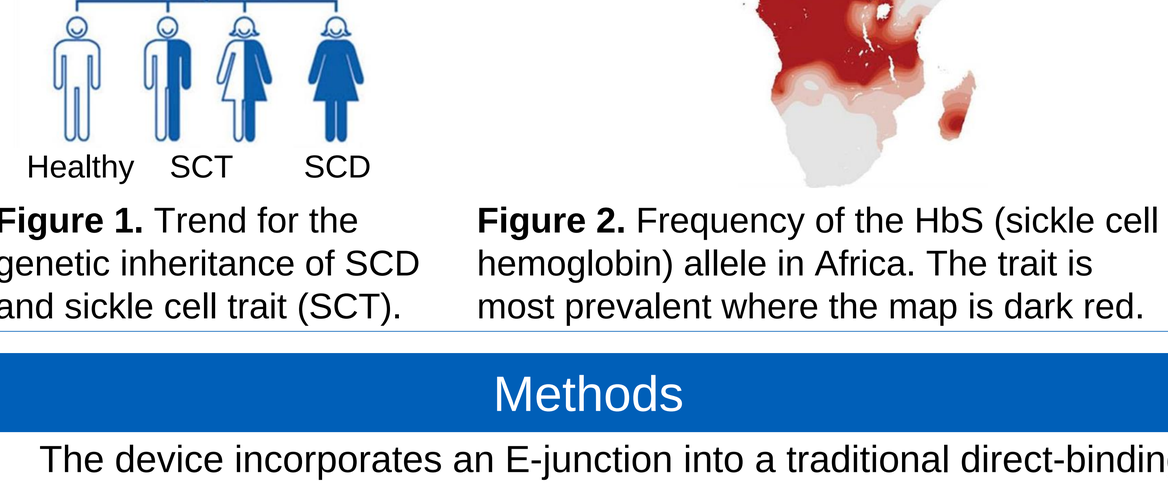

Figure 3. E-junction schematic with representation of the lateral flow binding mechanism at each E-junction phase.

Results

The wash step in Arm 2 ensures that blood samples with 0.1-1000 µg of hemoglobin can be read by the test strip. Confirmatory testing with whole blood samples has determined this dynamic range to be adequate for device function.

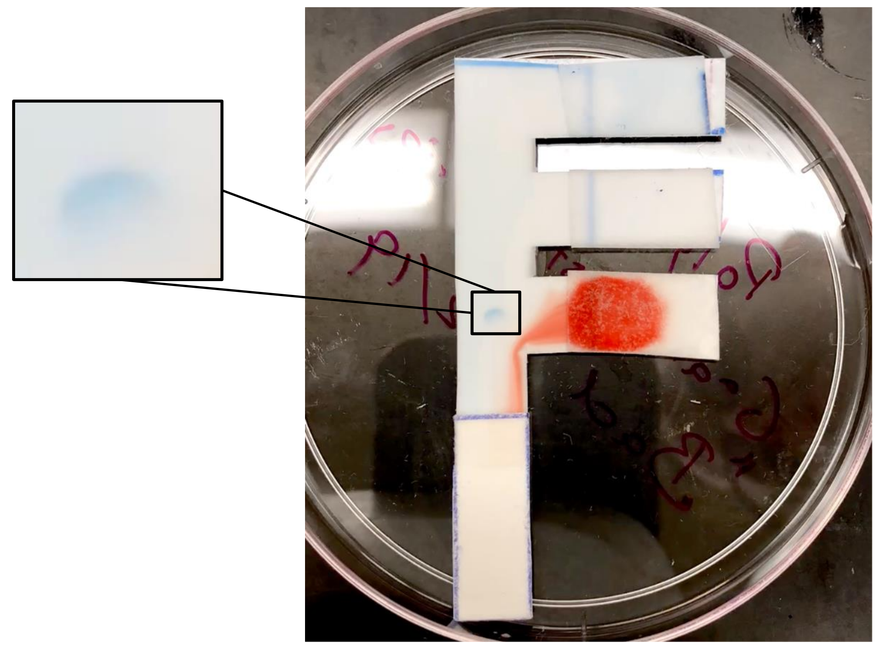

Figure 4. An E-junction test run with a whole blood sample. The HbA test line appears as a blue dot, with a zoomed in view provided.

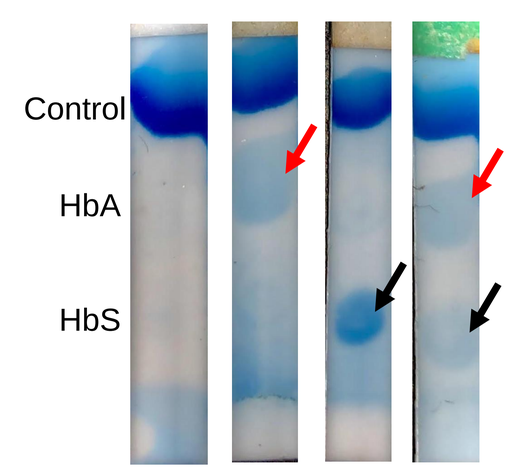

Figure 5. Mock-up test strips with all three antibodies printed run under four potential conditions: (A) no antigen (B) healthy individual (C) SCD individual (D) SCT individual. Black arrows indicate a positive HbS test and red arrows indicate a positive HbA test. All test strips develop a control line.

Conclusions

An operational test is ready for continued testing with blood samples of all sickle cell types and under preparation for alpha testing in Sierra Leone in August 2020. Further developments will continue to reduce the test strip cost, which is currently $2.98/test strip, and improve test line intensities.

References

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Figure 1: What is Sickle Cell Trait? (2019, October 21). Retrieved February 23, 2020, from https://www.cdc.gov/ncbddd/sicklecell/traits.html
Figure 2: Aneke, J. C., & Okocha, C. E. (2016). Sickle cell disease genetic counseling and testing: A review. *Archives of Medicine & Health Sciences*, 4(1), 50–57. doi: 10.4103/2321-4848.183342

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