Viral Load Determination for HIV-1 for the Macha Research Hospital Using a Novel Recombinant Protein and Heparin-Affinity Protocol

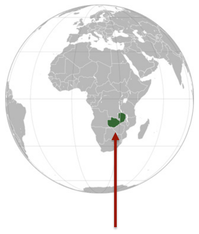
Lily Gaudreau, Daniel Haas, Danielle Reimer, Brianne Roper

The Need

An HIV positive infant will spend over $3,600 in a lifetime for viral load tests alone.

The Macha Hospital in Zambia requires an HIV viral load test that is:

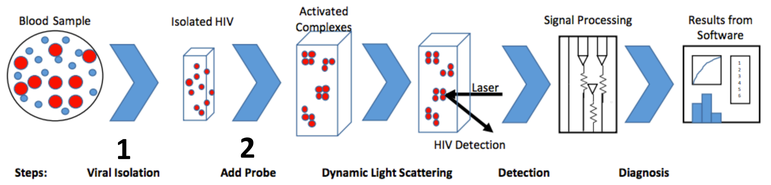
- Low Cost: less than $10 per test
- Quick: Under 1 hour
- Accurate: Sensitivity of 1000 viruses/ml



Macha Mission Hospital in Zambia

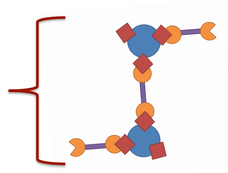
Diagnostic Strategy

The following Diagnostic Strategy has been proposed for HIV viral load Determination:



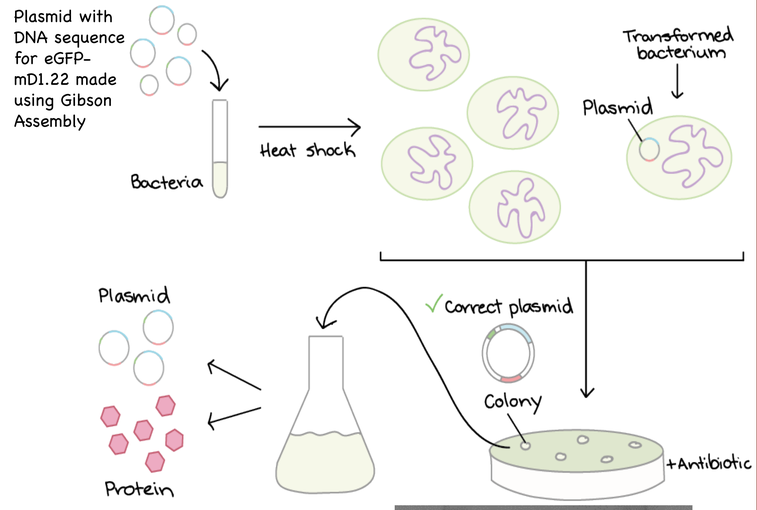
1. Viral isolation from whole blood will be carried out using heparin
2. The probe will be composed of an eGFP fluorescent tag and two HIV binding domains (mD1.22) connected by a linker

Goal: Activated complexes of HIV bound by mD1.22 protein dimer



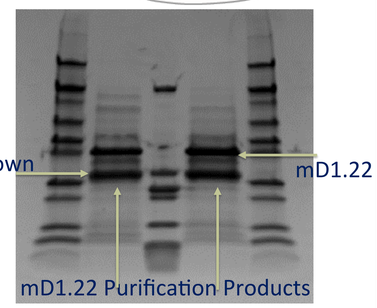
2. Probe Production

eGFP-mD1.22 Protein to bind to HIV



Protein purified with IMAC Columns

The identity of the protein product was confirmed using gel electrophoresis with 1 unknown impurity seen

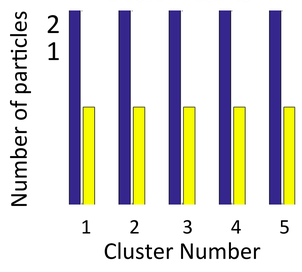


1. Viral Isolation

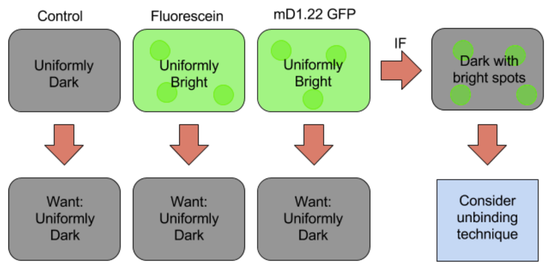
1 virus in a 1 ml container is the equivalent of 1 marble in space as large as two adjacent football stadiums. For our strategy, it would be comparable to 1000 marbles traveling around two football stadiums colliding and clustering together in order to get a diagnosis. Extremely high viral titers will be necessary for aggregation.

At a viral load of 5x10^{12} viruses/ml and dimer protein concentration of1.1 MM, the only aggregates present in solution were two proteins (blue) bound to each virus (yellow). No viral aggregates form.

Monte Carlo Simulation: Cluster Results



Concept Overview: Interaction of Heparin and Probe



Experimental Results: Comparing PBS and fluorescein in heparin solutions, it was determined that five 500 microliter washes of PBS will be needed to eliminate background fluorescence.

Conclusions

1) Viral Isolation: Five 500 microliter washes of PBS are required to remove fluorescence from heparin beads.
2) Probe Production: The plasmid sequence for the eGFP-mD1.22 has been verified and the protein has been produced with an unknown purity that needs to be removed.

Moving Forward: Begin testing of single eGFP-mD1.22 with heparin beads and produce eGFP-mD1.22 dimer.

Acknowledgements

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Please visit the "A Low-Cost Dynamic Light Scattering System for Detection of Viral Aggregates" poster to learn more about the optics portion of this project led by Lindsey Barner and Alex Roth

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