A Novel Approach for Detecting HIV-1 in Infants
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INTRODUCTION

The majority of children with HIV are located in sub-Saharan Africa, yet most of the HIV research conducted is based on research priorities identified by the developed world. In contrast, we have worked with our partner, Dr. Thumma of the Macha Research Centre in Zambia to identify two primary areas of HIV-1 research that are lacking for the developing world:

1. Identifying timing of mother-to-child HIV-1 transmission
2. Reducing the time to diagnosis for infants born to HIV+ mothers.

Our Goal: To create a diagnostic method that is
- Quick—under 30 minutes
- Accurate—returns quantitative value of HIV particles present
- Low Cost

Currently, Dr. Thumma sends blood samples to another hospital where it can be tested for DNA based results. It can take up to 6 months for him to receive the results, which in most cases is far too late to be helpful. Quick methods for diagnosis available to Dr. Thumma are anti-body based so the results he receives can be inaccurate.

He desperately needs a new method, our method, to improve his research and help save lives.

HIV STRUCTURE

The HIV-1 virus attacks the immune system by destroying Helper T-cells. The virus attaches to the CD4 receptor on Helper T-cells using the envelope protein gp120 and enters the cell. It then inserts its DNA into the cell's DNA. The cell then becomes a virus producing factory, sending out multiple copies of the virus into the body before eventually dying. This weakens the immune system and leaves the body more susceptible to infection.

The gp120 protein is the primary focus for our detection method using a soluble CD4 protein to attack to the virus.

DIAGNOSTIC STRATEGY

Ultimately, the detection method should go as follows:

1) A blood sample is received and then is filtered to isolate only virus particles
2) The probe is added to create clusters (complexes) of the viral particles
3) The complexes will be detected and a concentration of HIV in the blood will be reported using Dynamic Light Scattering.

FILTER DESIGN

A 3-D printed microchip filter with a membrane and double adhesive will be used to filter HIV viruses from a blood sample. The membrane filter goes between the two plastic layers. It requires a 40 µL blood sample and 200µL of PBS (phosphate buffered saline) to be pipetted into the inlet which drives the particles through the filter. Total Cost < $1

PROTEIN DESIGN

Using standard gene manipulation techniques (PCR and Gibson Assembly), we can produce a protein vector by inserting the DNA that produces our desired protein into a bacterial plasmid. We can then place this plasmid into E. coli bacteria, where the bacteria will act as protein producing factories. This method of creating a probe is inexpensive and contributes to making the entire diagnostic process low cost.

The Domain 1.22 of the soluble CD4 protein has a high binding affinity for gp120. By including two of these domains in our probe we have two different sites for binding which will allow for the creation of large complexes of attached viruses in solution. Accurate detection can then occur with the use of Dynamic Light Scattering.

RESULTS

A Monte Carlo simulation of probe-virus interactions was run in order to obtain pre-experimental results on the viability of our design. It is important that enough activated complexes of probes and viruses are made that Dynamic Light Scattering can detect the concentration of the virus.

The simulation was run for an 200 µl sample with 20 viruses/µl and 22.5 proteins/µl with random motion for a probe with a length of 20 nm.

Particles moved throughout a 3-D container in random directions for 100 time steps. The results showed that nearly 97% of complexes formed were large enough to be detectable with dynamic light scattering and that the average size of complexes increases over time and eventually reaches an equilibrium.

CONCLUSIONS

We currently have a theoretical diagnostic strategy from the reception of a blood sample to detection of HIV-1 that is predicted to be quick, low cost, and accurate. Our inexpensive filter will isolate HIV particles from the blood. Our easily produced protein should create large enough complexes to accurately diagnose HIV using Dynamic Light Scattering. The projected results indicate that this is a viable method for quickly and accurately diagnosing HIV in infants at a low cost. The entire technique should report a viral concentration in under an hour.

Our next steps are to produce the detection protein and to test our filter.

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