

Capture of HIV-1 envelope protein gp120 using immobilized heparin

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Problem Statement

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:

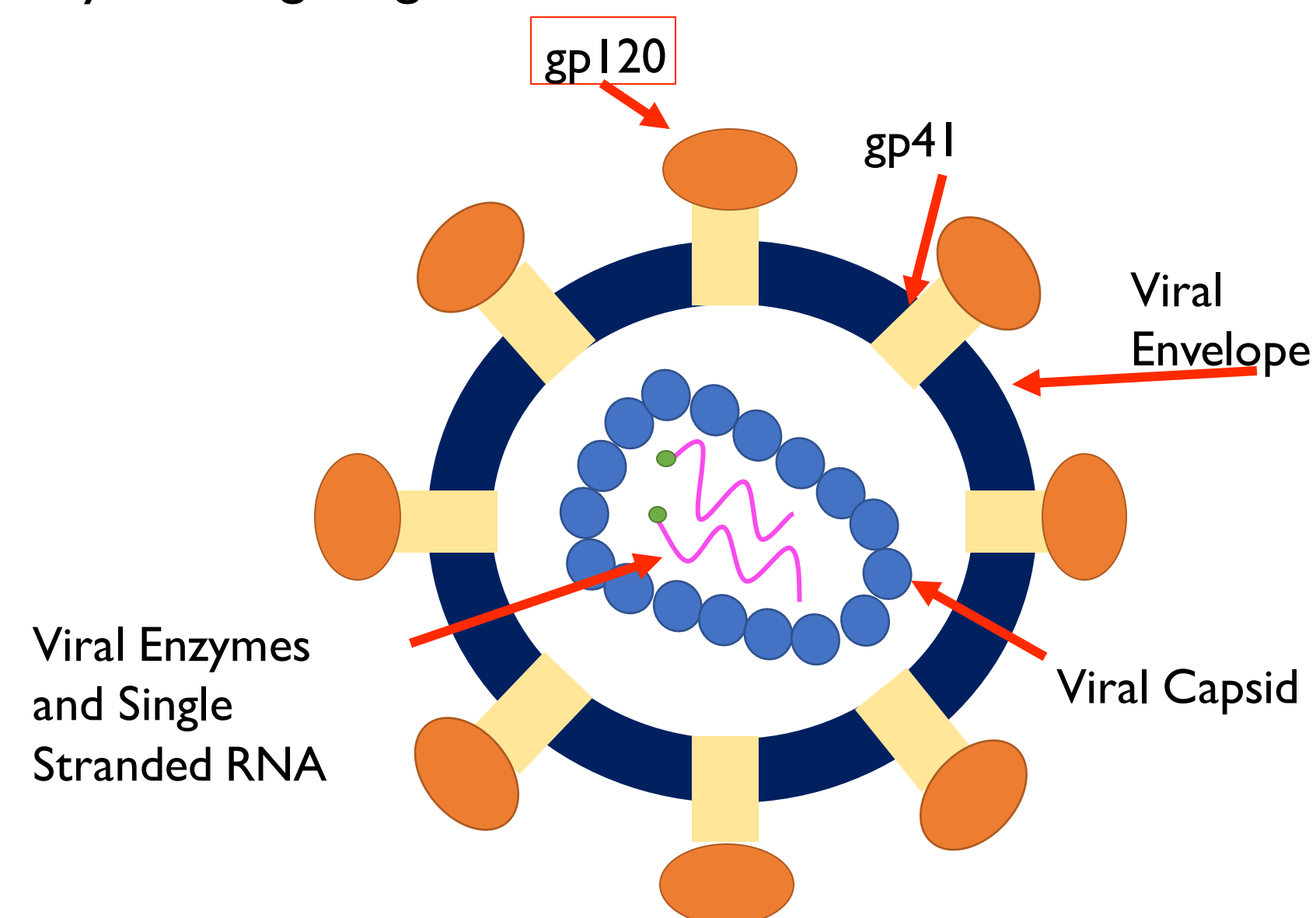
- Low Cost: less than \$10 per test
- Quick: Under 1 hour
- Accurate: Sensitivity of 1000 viruses/ml



Macha Mission Hospital

HIV-1 Structure

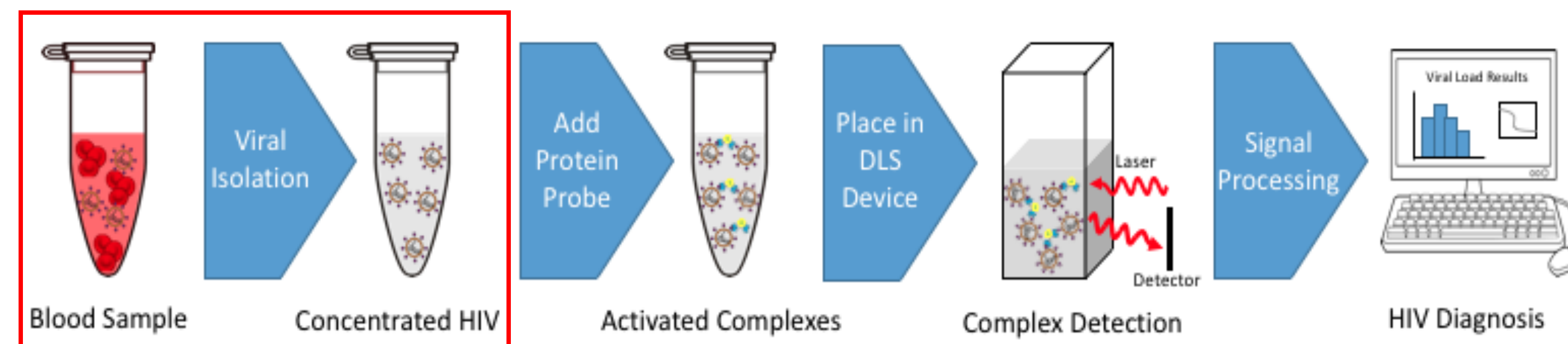
An estimated 36.7 million people are living with HIV/Aids globally. HIV/Aids Virus is a retrovirus that attacks immune cells preventing the body from fighting infections.



For this project, HIV's envelope protein gp120 is important in our diagnostic strategy.

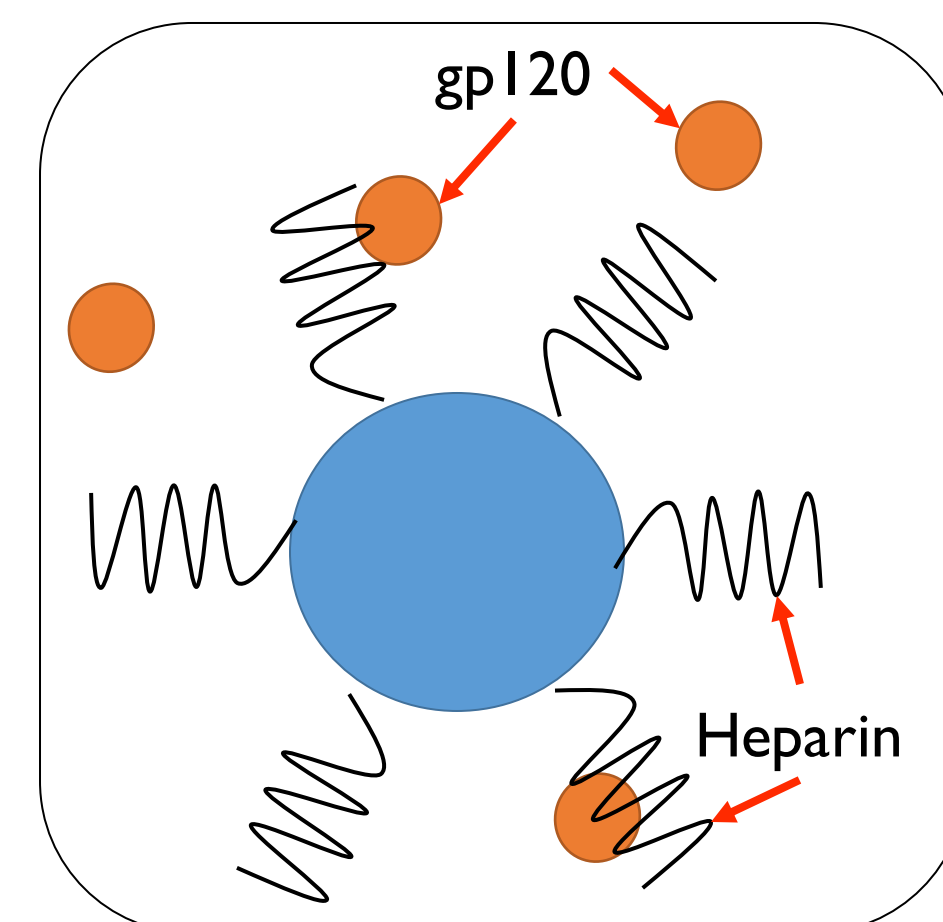
Diagnostic Strategy

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

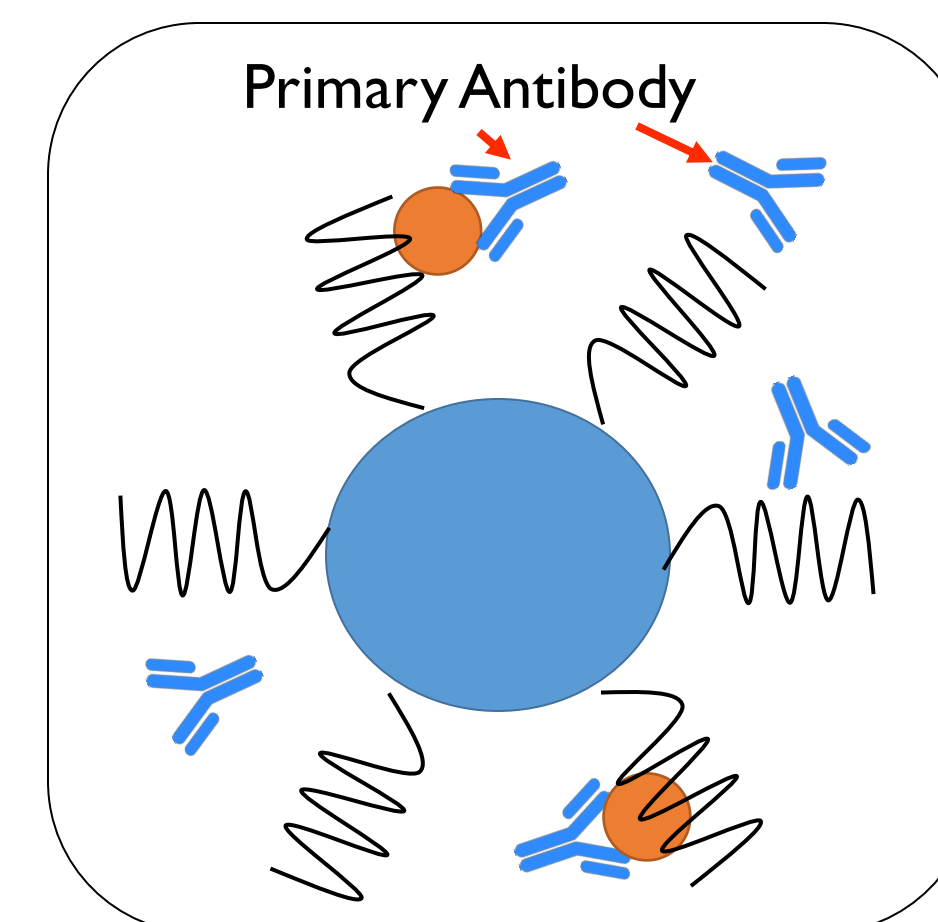


Viral isolation will be completed using a heparin filtering technique. A custom-immobilized heparin is known to collect 50% of the HIV present in a whole blood sample, and heparin is known to interact with gp120. Commercially produced porcine mucosal heparin is also shown to bind HIV-1. We are currently testing the ability of specific commercially-produced immobilized porcine mucosal heparin-agarose beads to capture free gp120 in our laboratory using antibody staining.

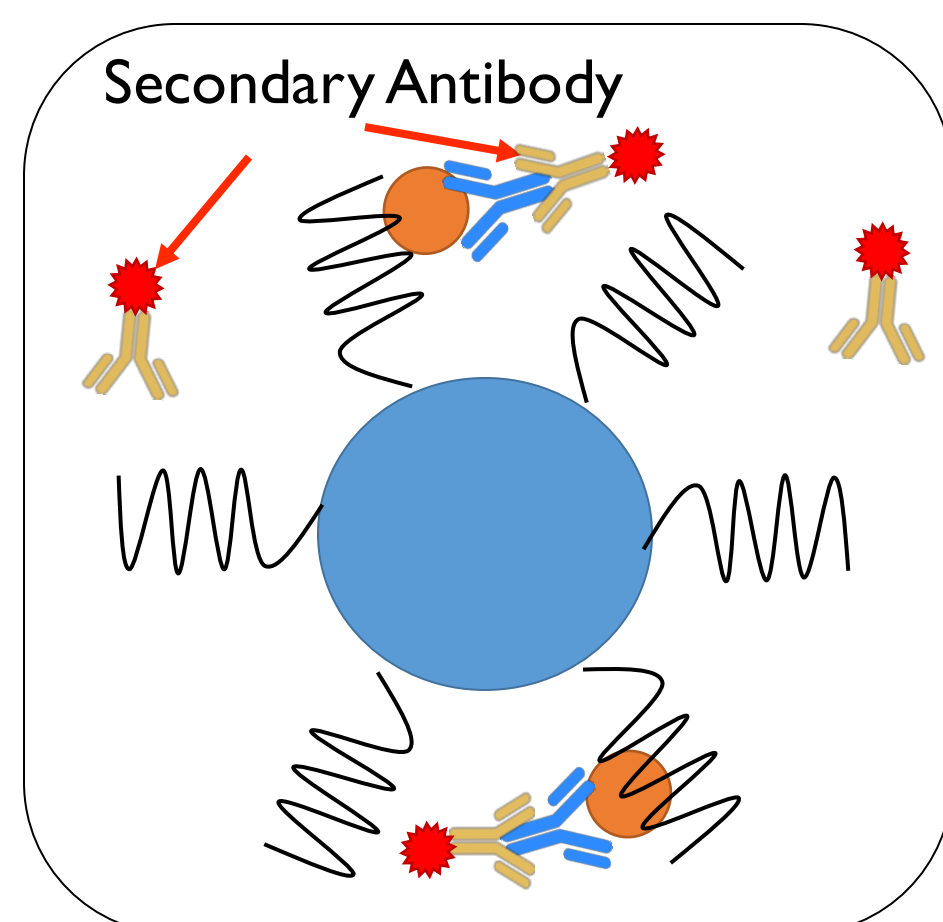
Heparin Testing Protocol



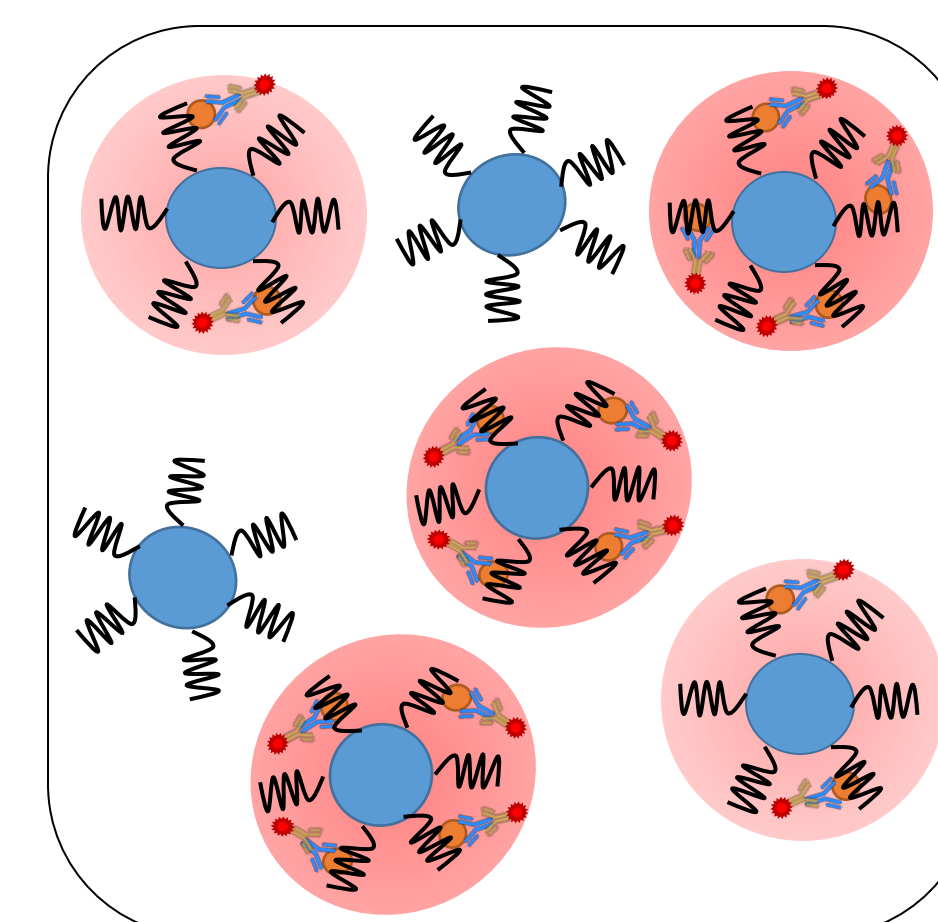
Step 1:
gp120 binds to heparin-agarose bead



Step 2:
Primary Antibody binds to gp120



Step 3:
Secondary Antibody binds to Primary Antibody



Step 4:
Image results for red fluorescence indicating gp120 capture

Results

Primary→Secondary↓	4.5 µg/ml	5 µg/ml	5.5 µg/ml
1:100		X	
1:500	X	X	X
1:1000	X	X	X
1:2000	X	X	X

Table I: Antibody staining tests
The antibody tests shown were completed using a concentration of 16.7 ng/mL of gp120, each run in parallel with a control without gp120 present.

Figure 1: Antibody staining results

The images were collected using the highlighted concentrations in Table I. These do not show a difference in red fluorescence of the heparin-agarose beads with or without the presence of gp120, suggesting that gp120 did not bind to the heparin beads.

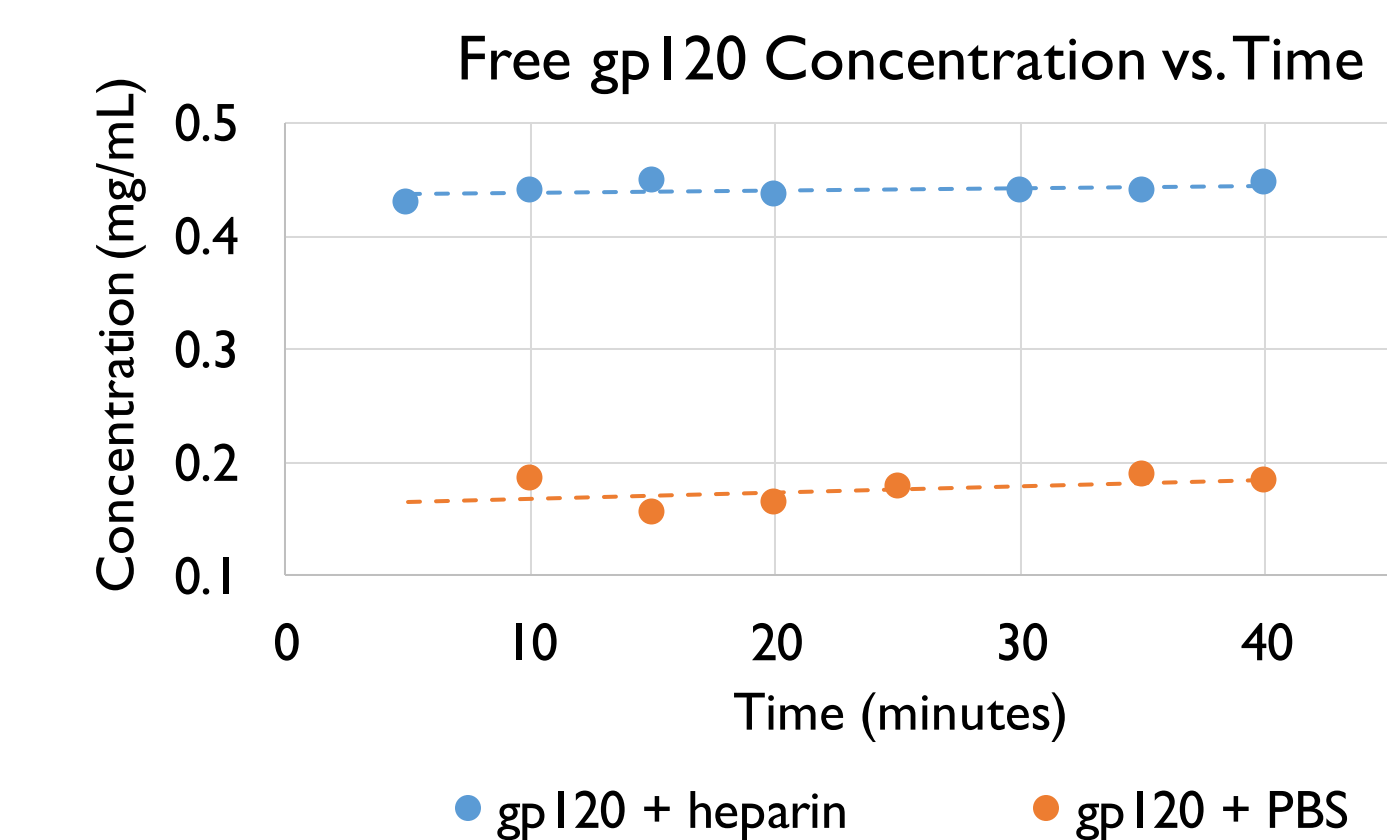
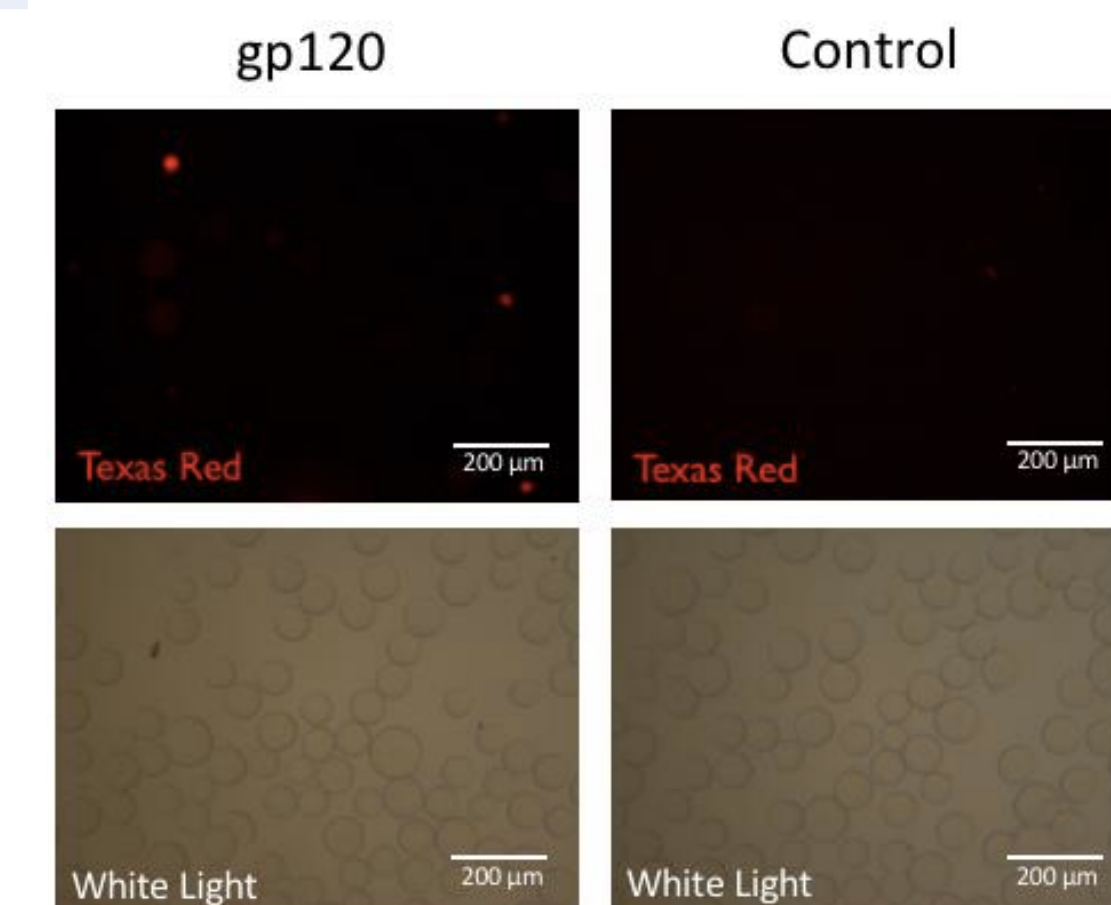


Figure 2: Free gp120 vs. Time
The concentration of gp120 in PBS did not change with time when incubated with heparin-agarose beads. These data show that these beads are not removing gp120 from solution.

Conclusion

The specific commercially-produced porcine mucosal Type I heparin-agarose beads purchased do not efficiently capture gp120.

Going Forward:

- Alternatively processed heparin sulfate beads will be investigated for gp120 capture using Nanodrop protein concentration measurement and antibody staining.
- The biomedical engineering lab's recently configured Fast Protein Liquid Chromatography instrument will be used to analyze gp120 binding to a heparin column.

Acknowledgements

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