Human Hookworm Infection, a neglected tropical disease infects more than 600 million people around the world. Hookworms ingest hemoglobin containing erythrocytes and Neutrophil americanus Antropic Protease (APR-1) will target the hemoglobin. APR-1 vaccine was generated by vaccinating sixty Balb/c mice with 9.33µg vaccination and bleed schedule. Nutritional end products are absorbed by the hookworm's gut wall. Also, hemoglobin is toxic to hookworms upon digestion by hookworm's gut enzymes. The recombinant polypeptide monomeric APR-1wt was estimated using Cathepsin D Substrate, 7-methoxycoumarin-4-acetyl-GKPILF FRLK(dinitrophenyl)-d-Arg-amide (quencher) (fluorophor) and fluorescence (330 nm Excitation and 390nm Emission) was measured by a Fluorometer. To determine the neutralizing activity of the IgG, purified IgG from mouse IgG was used as a Negative control. Figure 1, shows the steps to manufacture the Na-APR-1 M74 drug product was fractionated by HPLC to determine the neutralizing activity of the IgG. One hundred mice were divided into 30 groups. Mice were vaccinated subcutaneously using the above dose and the following schedule and blood schedule.

### Results

The ELISA assay utilized a sigmoidal response or the achievement of a threshold of murine IgG against either Na-APR-1wt or Na-APR-1 M74 vaccine became 1.230 times more potent at time 7 month post manufacture respectively (Table 2). The upper 95% fiducial limits of the estimated relative potency at 7 and 9 months post manufacture was found to be 1.488 and 1.63 respectively.

### Acknowledgments

The authors wish to acknowledge Bruce J. McNeal and Jane Helmers for their assistance to the review and guidelines for the development of ELISA for a neglected tropical disease vaccine.

### References

Amar Rariwala, Xi Chen, Mark S. Pearson, Brian Keegan, Jill B. Breisford, Jordan L. Pleskait, Bin Zhian, Alex Loukas, Peter J. Hotez, Jeffrey M. Bethony.

Department of Microbiology, James XX University, Washington, DC, USA, Center for Bio-discovery and Molecular Medicine and Neurological Disorders, University of Washington, Seattle, WA, USA, Center for Tropical Medicine, University of California, San Francisco, CA, USA, Center for Tropical Medicine, Baylor College of Medicine, Houston, Texas, USA, Fundação Oswaldo Cruz, Instituto René Rachou, Belo Horizonte, Minas Gerais, Brazil.

Vaccination of BALB/c mice with the No-APR-1 M74 hookworm vaccine generates neutralizing antibodies and a potent immune response. A new vaccine which is currently under pre-clinical development. This vaccine is an APR-1wt vaccine containing the recombinant form of the APR-1. IFN-γ will neutralizing Na-APR-1 M74 and will block the initiation of the inflammatory response which will result in the hookworms from eggs to hookworms, leading to their death. Here, we report the results of the neutralizing activity of the IgG and potency (immunogenicity) of the Na-APR-1 M74 vaccine in BALB/c mice.