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DEVELOPMENT OF A STANDARDIZED MULTIPLEX FILOVIRUS AND SARS-COV2 ANTIBODY IMMUNOASSAY

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With the goal of producing multivalent recombinant subunit filovirus and SARS-CoV-2 vaccines, we develop formulations using surface glycoproteins of Ebola, Marburg and Sudan viruses or the Spike protein of the SARS-CoV-2 virus. In determining the potency of our formulations in generating an immune response in mice and non-human primates (NHP), serum antibody titers are used. Instead of using conventional antigen-binding ELISA assays for each antigen, we conduct testing by a custom multiplex immunoassay. This method uses regionally different magnetic beads coupled to purified recombinant antigens which are incubated with serum dilutions to simultaneously determine the antibody titers to the different immunizing antigens. After application of a secondary, fluorescently labeled antibody, values are normally shown as median fluorescent intensity or MFI.

By converting the MFI to an actual concentration, samples from different studies can more easily be compared. For this, standard curves using purified antigen-specific immunoglobulin G (IgG) to the three filovirus GP's or SARS-CoV2 spike protein are established with each assay. Standards were prepared passing high-titered mouse or NHP sera over a protein G column to isolate IgG, then purified further using affinity-chromatography columns with individual filovirus GP's or SARS-CoV-2 spike protein to select for antigen-specificity. The standards are quantified and curves are generated which will be run with each set of serum samples.

As antibody concentrations in the serum fluctuate widely from very low in control groups to very high in high-dose groups, sample dilutions may vary to allow proper interpolation on the standard curves. Our experience shows that dilutions of 1/100 to 1/10,000 typically yield MFIs that fall within the range of our standard curves that can then be interpolated to get an IgG concentration so that all values can be combined into one graph for filovirus and SARS-CoV-2 titers. This way of standardizing the antibody concentrations are used to measure multiple analytes to compare data from within the same but also between different studies.

Our standards range from 7.8 to 4000ng/ml of IgG. Serum dilutions should be adjusted so that the titers fall within the slope of the IgG curve. Normally, control (no antigen) groups will have titers below the limit of quantification while high-titered groups will have concentrations as high as 1 mg/ml.