Development and evaluation of a multiplex filovirus serology panel

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Abstract# 6410

Background: Filovirus outbreaks, including Ebola and Marburg viruses, are sporadic but can be devastating when they occur. The proximity to wildlife reservoirs, inadequate healthcare infrastructure, and limited resources for outbreak response make the filovirus disease endemic in certain regions, posing a continuous threat to local populations. Additionally, global travel and trade contribute to the potential spread of filoviruses beyond endemic areas. Serosurveillance of filoviruses plays a crucial role in monitoring and understanding the prevalence of these viral infections within populations. By assessing the seroprevalence, researchers can gain insights into the extent of past and current infections, identify potential hotspots, and evaluate the effectiveness of public health interventions. We have developed a twelve plex fluorescent microsphere-based serology assay to monitor the IgG antibody response to glycoproteins (GP) antigens of six species of genus ebolavirus, namely Zaire ebolavirus (EBOV), Sudan virus (SUDV), Bundibugyo virus (BDBV), Reston virus (RESTV), Bombali virus (BOMV), and Tai Forest virus (TAFV). Additionally, we have included two GP antigens for Marburg virus disease causing Marburg virus (MARV) and Ravn Virus (RAVV) (Figure 1).

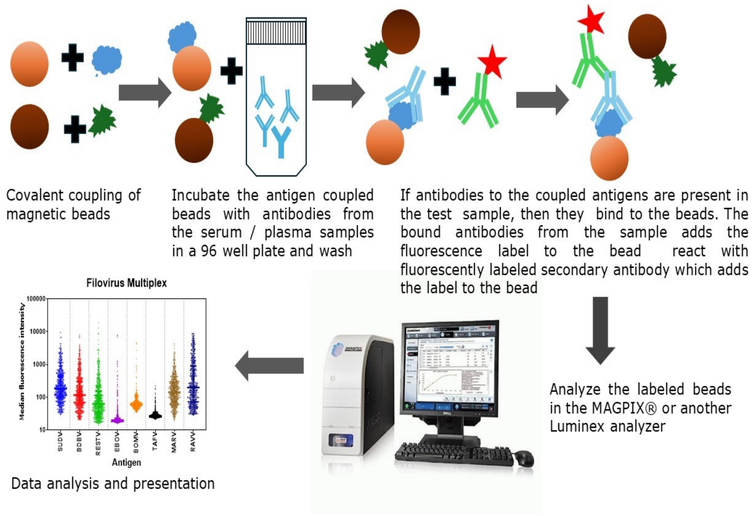


Figure 1. Schematic representation of a multiplex antigen detection assay using Luminex® xMAP® Technology.

Table 1. Description of 10 serum samples from NHPs used in this study.

ID	Vaccine	Challenge	Days after challenge
RESTV-NHP1	none	RESTV	28
RESTV-NHP2	none	RESTV	28
EBOV-NHP1	CMV-EBOV	EBOV	35
EBOV-NHP2	CMV-EBOV	EBOV	35
TAFV-NHP1	none	TAFV	28
TAFV-NHP2	none	TAFV	28
SUDV-NHP1	VSV-SUDV	SUDV	42
SUDV-NHP2	VSV-SUDV	SUDV	42
MARV-NHP1	VSV-MARV	MARV	42
MARV-NHP2	VSV-MARV	MARV	42

Materials and Methods: We have developed a 12-plex multiplex assay for detecting human IgG antibodies to filoviruses using Luminex® xMAP® technology. This assay contains 8 glycoprotein antigen coupled microspheres and 4 internal controls to monitor assay performance. The serum or plasma samples are mixed with the antigen-coated microspheres in wells of a 96-well plate and incubated for the antigen-antibody reaction. The antigen-specific antibodies get immobilized on the microspheres, and unbound material is washed away. The anti-human IgG-phycoerythrin (HIgG-PE) reporter conjugate detects antigen-captured human IgG antibodies on the microspheres that are resuspended in the buffer and analyzed using MAGPIX®.

We first evaluated this panel using hyperimmune rabbit serum or purified rabbit polyclonal antibodies for each antigen. Next, we evaluated the panel for anti-ZEBOV-GP using the human IgG panel from BEI resources (NR-52374) used as a reference for Filovirus Animal Nonclinical Group (FANG) anti-EBOV GP IgG ELISA. We also evaluated this panel using 10 non-human primate samples vaccinated and challenged with different filoviruses, as described in Table 2.

UC Davis group evaluated this panel using 768 human serum and plasma samples used for sero-survey in East Africa.

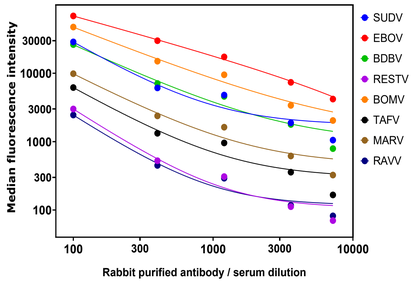


Figure 2. Evaluation of coupled antigens using rabbit polyclonal serum or purified antibodies

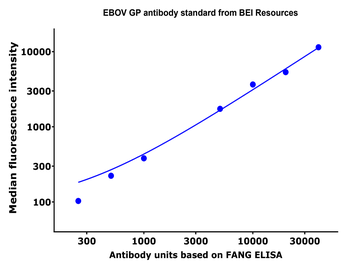


Figure 3. Evaluation of EBOV-GP standard from BEI resources

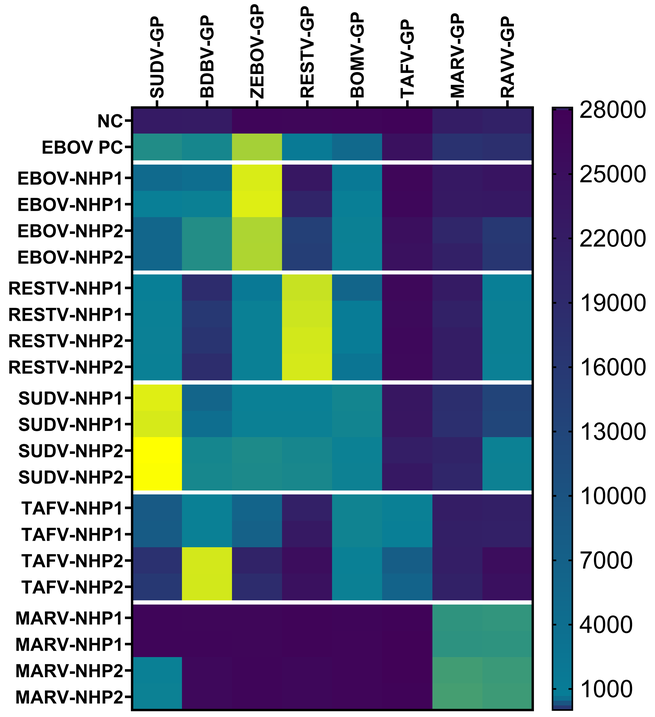
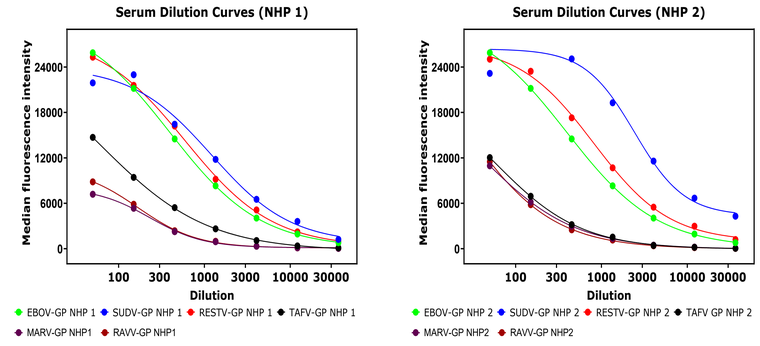


Figure 4. Reactivity pattern determination for 10 NHP samples as described in Table 1

Results and conclusions:

- The evaluation with rabbit serum or purified polyclonal antibodies showed that each microsphere showed expected reactivity. (Figure 2)
- The multiplex assay results were concordant with the FANG-ELISA. (Figure3)
- Next, we tested ten samples, two each of non-human primate (NHP) sera vaccinated against EBOV, SUDV, and MARV and then challenged with the same viruses. Also, two NHP sera samples were each not vaccinated but challenged with TAFV and RESTV. The reactivity pattern in each NHP was unique to the antigen they were vaccinated with or challenged with. (Figure4)
- The titration of NHP serum samples from 2 NHPs each showed IgG titers ranging from 4050 to greater than 36450 for different vaccinated and challenged sera for the respective viruses. Figures 5 & 6 show serum dilution curves for each of the set.
- The human serosurvey indicates positive reactivity of circulating antibodies to different filoviruses, as shown in the Figure 7. The data from internal controls was very useful to exclude the results that did not meet the acceptance criteria for controls. The controls were indicative of operator error, sample integrity and non specific binding.
- The UC Davis team is evaluating the significance of these results.
- In conclusion, this assay is easy to use and amenable to high throughput testing. The multiplex serology provides a much-needed cost and labor-saving tool for effective sero-surveillance of filovirus antibodies in endemic areas.



Figures 5 & 6: Serum dilution curves from each of the two NHPs as described in Table 1

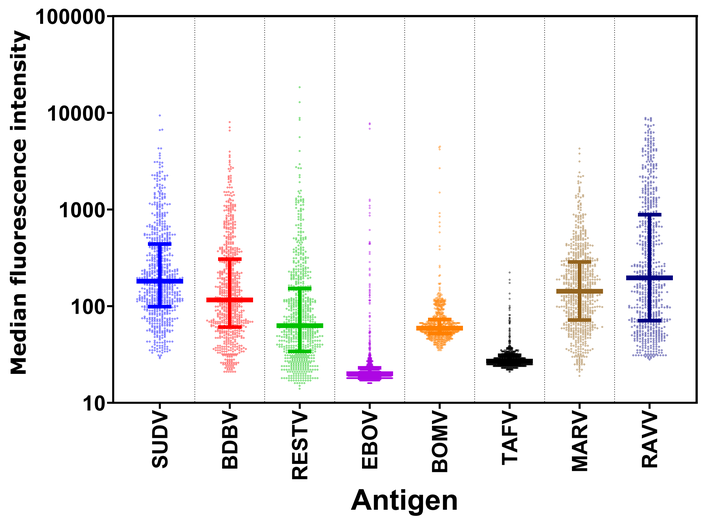


Figure 7. Cross-sectional survey of 719 serum/plasma samples tested in East Africa