THE UNIVERSITY OF YAOUNDE I

FACULTY OF SCIENCES

CENTRE FOR RESEARCH AND DOCTORATE TRAINING IN GRADUATE STUDIES IN LIFE, HEALTH AND ENVIRONMENTAL SCIENCES

RESEARCH AND DOCTORATE TRAINING UNIT OF GRADUATE STUDIES IN LIFE SCIENCES
P.O Box 3851, Messa Y'dé
Tel/Fax: 22 23 74 29
DEPARTMENT OF BIOCHEMISTRY

UNIVERSITE DE YAOUNDE I

FACULTE DES SCIENCES

CENTRE DE RECHERCHE ET DE FORMATION DOCTORALE EN SCIENCES DE LA VIE, SANTE ET ENVIRONNEMENT

UNITE DE RECHERCHE ET DE FORMATION DOCTORALE EN SCIENCES DE LA VIE
BP 3851, Messa Y'dé
Tel/Fax : 22 23 74 29
DEPARTEMENT DE BIOCHIMIE

NATIONAL BIOMEDICAL RESEARCH INSTITUTE (INRB)

LABORATORY FOR PUBLIC HEALTH RESEARCH BIOTECHNOLOGIES

(LAPHER-Biotech)

Singleplex gold-standard FANG ELISA versus Pan-filovirus Multiplex Immuno Assay analysis of EBOV GP IgG antibody reactivity in rVSV-EBOV-GP Vaccinated individuals in Beni, Democratic Republic of Congo

Dissertation presented in partial fulfilment of the requirements for the award of a Professional Master of Science degree in Biochemistry

Option: **Public Health Biotechnology**

Presented by:

AKANWUH CARINE NKEWOMOH

Registration Number: 10R1091

BSc. in Biochemistry (UYI)

Supervised

Nicole HOFF (PhD)
Senior Research Lead
UCLA-DRC

MBACHAM Fon Wilfred (ScD)
Professor of Public Health Biotechnology
University of Yaoundé 1

AYONG Lawrence (PhD)
Research Director
Centre Pasteur du Cameroun

Academic Year: 2023-2024

DEDICATION

To the NKEWOMOH's family

ACKNOWLEDGEMENTS

Firstly, my utmost gratitude goes to **God Almighty**. When I think about this my master's thesis, the whole process and the fact that I'm almost at the point of defending it. I see God in it all, it has been nothing short of God's grace, favor and wisdom at every point in time. I'm equally grateful to the **Holy Spirit** for his help and divine inspiration all along.

My heartfelt appreciation also goes to:

- **Pr. MBACHAM Wilfred** my supervisor and "great Prof" as we fondly call him for accepting to supervise my work despite his very tight schedules and numerous preoccupations.
- **Dr. ABIA Wilfred**, My co-supervisor and academic coach who believes so much in me and would constantly push me forward, advice, guide, direct, and check from time to time to make sure I'm making progress in this work.
- **Dr. AYONG Lawrence** who did not just introduce me into the research world during my internship in the Malaria Laboratory of Centre Pasteur du Cameroun (CPC) but also co-supervised my work serving as a pillar of support throughout this study.
- **Pr. Nicole HOFF** my able boss and country director (UCLA/DRC) who doubles as my career model for not just coining out this research topic but for allowing me to work in the Ebola project while doing my school work at the same time.
- **Dr. KOMPANY Jean Paul** (University of Kinshasa, DRC), my laboratory manager for his devotedness in overseeing and accompanying me all through this work.
- **Pr Anne Rimoin (UCLA School of Public Health), Pr Muyembe Jean Jacques (INRB), Pr Emile Okitolonda-Wemakoy (ESPK)** and **Dr Benoit Kebela Ilunga (Division of Disease Control)**, our co-investigators for making this work possible.
- **Bill and Melinda Gates Foundation (Seattle, Washington, USA); U.S. FDA (Washington DC, USA)** and **Stanford University (Palo Alto, California, USA);** our sponsors for being a pillar of financial support throughout this study.
- My loving Father **Pa NKEWOMOH Samson** and my caring Mother **Ma Rebecca SHIRRIMATAWAH** (of blessed memory who would have been very elated and proud of me for my growth/success hitherto) together with my wonderful siblings who put in so much to ensure that I succeed and for being a great pillar of all-round support all through these long years of education. Finally, my heart goes out with thanksgiving to all the participants who consented and took part in this study, providing a basis for this work. Not forgetting all those whose names have not been mentioned, I sincerely thank you for your respective contributions particularly towards the realization of this work.

TABLE OF CONTENTS

ABBREVIATIONS & ACRONYMS

Ab:	Antibody
BSA:	Bovine Serum Albumin
BSC:	Biosafety Cabinet
DSE:	Direction de Surveillance Epidémiologique (Epidemiological Surveillance Department)
DRC:	Democratic Republic of Congo
EHF:	Ebola Hemorrhagic Fever
EVD:	Ebola Virus Disease
EBOV:	Ebola Virus
ELISA:	Enzyme-linked Immunosorbent Assay
ESPK :	Ecole de Sante Publique de Kinshasa (Kinshasa School of Public Health)
FANG:	Filovirus Animal Nonclinical Group
FDA:	Food and Drug Administration
GP:	Glycoprotein
IgG:	Immunoglobulin G
IgM:	Immunoglobulin M
INRB:	Institut National de Recherche Biomédicale (National Biomedical research Institute)
LLOQ:	Low Limit of Quantification
MARV:	Marburg Virus
MIA:	Multiplex Immuno Assay
NC:	Negative Control
NHPs:	Non-Human Primates
NC:	Nucleocapsid
Nm:	Nanometre
NP:	Nucleoprotein
PBS:	Phosphate Buffered Saline
PEV:	Programme Elargi de Vaccination (Expanded Program on Immunization)
RC:	Republic of Congo
rGP:	Human Gingipain R

RNP:	Ribonucleoprotein
RS:	Reference Standard
rVSV-	Recombinant, live attenuated viral vector wherein the G
EBOV-GP:	Protein of Vesicular Stomatitis Virus (VSV) is replaced with Glycoprotein of EBOV.
SOPs:	Standard Operating procedures
SSA:	Sub-Saharan Africa
SUDV:	Sudan Virus
QCH:	Quality Control High
QCL:	Quality Control Low
UCLA:	University of California Los Angeles
VHF:	Viral Hemorrhagic Fevers
UK:	United Kingdom
USA:	United States of America
VP:	Viral protein
WHO:	World Health Organization
ODK:	Open Data Kit

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Between 2018 and 2020, the Democratic Republic of Congo (DRC) experienced an Ebola virus Disease (EVD) outbreak during which, the rVSV-ZEBOV vaccine was first used as an intervention strategy. However, no studies have been conducted on the duration of vaccine-induced antibodies (Ab). Again Ebola virus (EBOV) is a highly infectious pathogen, and its long-term consequences continue to be investigated. With its high fatality rate and potential for reinfection or latent infection, continued development of research tools is of utmost importance since not all immunoassays have been shown to detect a vaccine-induced immune response, and previous EBOV serosurveillance has been primarily conducted with singleplex technology, meanwhile MIA is an additional resource. We therefore aim to compare the gold-standard FANG ELISA and the pan-filovirus MIA in determining EBOV GP IgG antibody reactivity in rVSV-EBOV-GP Vaccinated individuals in Beni, DRC.

617 vaccine recipients were recruited from August 2018 to February 2019.A questionnaire was used to collect socio-demographic data and likely determinants of EVD exposure for each recruited individual. Subsequently, 6ml of blood was collected in dry tubes for Ebola glycoprotein IgG Ab testing. 9 had Ebola IgG antibodies at baseline. Follow-up visits at 21 days and 6 months after vaccination included 608 participants without anti-Ebola Ab at baseline. These samples collected were heat-inactivated and analyzed separately with the gold-standard FANG ELISA and Pan-filovirus MIA. In a total of 9 of 617 participants (1.5%), baseline Ebola Ab was elevated. Also, of the 608 participants expected at the time of the appointments, 90% (n=548) of the participants had responded by D21 after vaccination.71.4% (n=434) of the participants responded at the 6-month post-vaccination visit. Of these, 95.6% of the vaccinated participants had IgG anti-Ebola virus antibodies with significantly elevated geometric mean antibody concentrations of 1231 (1145 - 1324) EU/mL. Moreover, of the samples used for both FANG and MIA, 68% were MIA seroreactive and FANG non-seroreactive. This study shows that MIA could be an appropriate alternative to the singleplex FANG in assessing immunological responses to EBOV GP across a geographical area, especially in a low resource setting. In addition, this result shows that one dose of this vaccine can protect against EVD 6 months after use. However, further studies are recommended to understand and complement the data from this study.

Key-words: EVD, rVSV-ZEBOV vaccine, FANG ELISA, MIA, risk factors; DRC

RESUMÉ

Entre 2018 et 2020, la Republique Democratique du Congo (RDC) a connu une épidémie de Maladie à virus Ebola (MVE) au cours de laquelle le vaccin rVSV-ZEBOV a été utilisé pour la première fois comme stratégie d'intervention. Cependant, aucune étude n'a été menée sur la durée des Ac induits par le vaccin. Là encore, le virus Ebola (EBOV) est un agent pathogène hautement infectieux et ses conséquences à long terme continuent d'être étudiées. Avec son taux de mortalité élevé et son potentiel de réinfection ou d'infection latente, le développement continu d'outils de recherche est de la plus haute importance, car il n'a pas été démontré que tous les tests immunologiques détectent une réponse immunitaire induite par le vaccin et que la sérosurveillance précédente de l'EBOV a été principalement menée avec la technologie singleplex. en attendant, MIA est une ressource supplémentaire. Donc nous avons comme objectif de comparer le test FANG ELISA de référence et le pan-filovirus MIA pour déterminer la réactivité des anticorps IgG EBOV GP chez les individus vaccinés rVSV-EBOV-GP à Beni, RDC.

617 vaccinés ont été recrutés entre août 2018 et février 2019. Un questionnaire a été utilisé pour collecter des données sociodémographiques et des déterminants probables de l'exposition à la MVE pour chaque personne recrutée. Par la suite, 6 ml de sang ont été collectés dans des tubes secs pour le test des IgG Ab de la glycoprotéine Ebola. 9 avaient des anticorps IgG contre Ebola au départ. Les visites de suivi 21 jours et 6 mois après la vaccination ont inclus 608 participants sans Ab anti-Ebola au départ. Ces échantillons collectés ont été inactivés par la chaleur et analysés séparément avec l'étalon-or FANG ELISA et Pan-filovirus MIA. Chez un total de 9 participants sur 617 (1,5 %), l'Ebola Ac de base était élevé. Aussi, sur les 608 participants attendus au moment des rendez-vous, 90 % (n=548) des sujets avaient répondu à J21 après vaccination. 71,4 % (n = 434) des participants ont répondu lors de la visite post-vaccination 6 mois. Parmi eux, 95,6 % des participants vaccinés présentaient des anticorps IgG anti-virus Ebola avec des concentrations moyennes géométriques d'anticorps significativement élevées de 1 231 (1 145 - 1 324) UE/mL. De plus, les échantillons utilisés à la fois pour le FANG et le MIA, 68 % étaient séroréactifs pour le MIA et les FANG non séroréactifs. Cette étude montre que le MIA pourrait être une alternative appropriée au FANG simpleplex pour évaluer les réponses immunologiques à l'EBOV GP dans une zone géographique, en particulier dans un contexte à faibles ressources. De plus, ce résultat montre qu'une dose de ce vaccin peut protéger contre la MVE 6 mois après son utilisation. Mais, d'autres études sont recommandées pour comprendre et compléter les données de cette étude.

Mots-clés : MVE, vaccin rVSV-ZEBOV, FANG ELISA, MIA, facteurs de risque ; RDC.

INTRODUCTION

Ebola Virus Disease (EVD) earlier termed as Ebola Hemorrhagic Fever (EHF), is a severe disease, often a lethal ailment which primarily affects humans and non-human primates; characterized by coagulopathy, lymphopenia and multiple organ failure caused by infection with a virus of the *Filoviridae* family of non-segmented negative sense single stranded viruses, genus *Ebola virus* (Pinski & Messaoudi, 2020). The Filoviruses, Ebola Virus (EBOV) and Marburg (MARV), are virulent in nature and are amongst the deadliest viruses that cause disease in humans with reported case fatality rates varying from 25% up to 90% (average around 50%) in some outbreaks depending on the strain ((Rivera & Messaoudi, 2020).

In the past four decades, a total of 38 country-specific EVD outbreaks affecting 34626 cases and causing 15266 deaths were reported in 11 countries in Sub-Saharan Africa (SSA) since it was first recognized in 1976 (Barbiero, 2020). Of these 38 outbreaks, the Democratic Republic of Congo has had the most Ebola outbreaks, recording up to 14outbreaks (*WHO, 2022*) with the 10th Ebola epidemic outbreak which occurred between 2018-2020 in the Eastern Democratic Republic of Congo being the largest in DRC's history of Ebola outbreaks and the second largest in the world which caused 3,481 infections and 2,299deaths. Experts theorize that heavy forested areas containing infected fruit bats maybe to blame for the multiple outbreaks in DRC (Quattrochi *et al.,* 2023). It is worth noting that during the past four decades, no significant changes in EVD case fatality rates were observed according to a study conducted by (Rugarabamu *et al.,* 2020). However, EVD still remains a global public health menace due to the persistence of the virus in survivors, high virulence, ease of transmission, large immigrant population, possibility of use as bioterrorism agents in developed countries and the fact that there are currently no licensed therapeutics for EVD caused by some species of the Ebola virus like Sudan ebolavirus *(*Balmith *et al.*, 2017; Paladino *et al*., 2017) . However till date, EVD has not been diagnosed in Cameroon and there is little to no awareness of the disease in Cameroon (Wirsiy *et al.,* 2021). However, a serological survey conducted in 1983 in different areas of Cameroon suggested Ebola virus disease prevalence rates on the basis of indirect immunofluorescence (now known to be rather unspecific) ranging from 3.0% to 14.5%, depending on the ethnicity and location (Gonzalez *et al.,* 2005) and according to a study conducted on the serologic prevalence of Ebola in Equatorial Africa, 1.3% of seroprevalence was detected in a small set of samples from Southern Cameroon, which indicated a low risk for exposure in this region (Ste *et al*., 2020). Notwithstanding, Ebola virus (EBOV) is a highly

infectious pathogen, and its long-term consequences continue to be investigated. With its high fatality rate and potential for reinfection or latent infection, continued development of research tools is of utmost importance for earlier diagnosis and more effective treatment thereby increasing the chances of survival.

For decades, the gold-standard immunoassay for hemorrhagic fever detection has been the traditional ELISA. The reemerging epidemic illustrated the need for the development of novel serologic assays (like the pan-filovirus MIA using xMAP Microspheres) for their detection and surveillance. Again, most serological assays to detect anti-EBOV antibodies (Abs) target responses to the GP antigen, as this is the only external protein on the virion surface (Dube *et al.,* 2009) However, while the EBOV GP is presented as part of rVSV EBOV GP vaccination, anecdotal evidence has shown not all serological assays have been able to detect vaccine-elicited anti-GP responses (*Frontiers _ Designs and Characterization of Subunit Ebola GP Vaccine Candidates_ Implications for Immunogenicity*, n.d.; Coulborn *et al.,* 2024; Xu *et al.,* 2024). Further, while 21 days has been the established clinical follow-up time post-vaccination for EVD, studies have suggested that a later time point may be a more informative sampling time to assess seroconversion and development of a robust immune response (Bratcher *et al.,* 2022). Variations of enzyme-linked immunosorbent assays (ELISAs) have been developed to detect immunoglobulin G (IgG) antibodies specific to EBOV antigens such as GP (Logue *et al.,* 2018). Of these ELISAs, the Filovirus Animal Nonclinical Group, or FANG assay, is considered the gold standard and proxy for an immune response post-rVSV EBOV GP vaccination as it can detect a wide range of antibody (Ab) concentrations and has a lower background than commercially available alternatives such as the Alpha Diagnostics International (ADI) assay (Bramble *et al.,* 2018; Logue *et al.,* 2018; Rimoin *et al.,* 2018). The FANG assay has been FDA-approved for the detection of an anti-EBOV GP IgG response in human samples (Manno *et al.,* 2022). Also as an alternative to the singleplex FANG assay, a multiplex bead-based immunoassay (MIA) may provide a larger dynamic range, allowing for the reliable detection of IgG at varying concentrations using a single sample dilution. Previous literature suggests MIAs are more sensitive and specific in measuring Ab responses compared to an assay for Lassa Virus and EBOV, indicating a greater detection range for MIAs (Satterly *et al.,* 2017). The Magpix instrument can also measure up to fifty different analytes at the same time, reducing the blood sample quantity needed for multiple independent tests (*Diasorin | Molecular Diagnostics and Immunodiagnostic Specialist*, n.d.; *MAGPIX® System _ Multiplex Analyzer by Diasorin*, n.d.). Additionally, having multiple analyte detections in a large panel is

resource-efficient and economical–important considerations in limited resource settings. The use of MIA in lower- and middle-income countries (LMICs), like the DRC, which has also been the epicenter of endemic EBOV outbreaks and vaccination activites, can be important for improved detection of natural and vaccine-induced immune responses. Additionally, as a multiplex tool, the MIA broadens testing capacity as many filovirus targets can be assessed simultaneously. Although several studies including interlabolatory comparison of FANG ELISA has been carried out, little is known about the gold-standard FANG ELISA in comparison with the pan-filovirus MIA. We therefore, seek to do a comparison study between the gold-standard FANG ELISA and the pan-filovirus MIA using human samples.

RESEARCH QUESTIONS

- What is the range of IgG concentration in rVSV EBOV GP vaccinated individuals using the gold standard FANG ELISA?
- What is the range of IgG concentration in rVSV EBOV GP vaccinated individuals using the pan-filovirus multiplex Immuno assay (MIA)?
- How can these findings based on the above research questions be used in determining EBOV GP IgG reactivity especially in Low and Middle-income Countries (LMICs) like the DRC and Cameroon?

RESEARCH HYPOTHESIS

Pan-filovirus Multiplex Immuno Assay (MIA) is efficient and cost effective as compared to the gold standard Filovirus Animal Non-Clinical Group (FANG) ELISA.

OBJECTIVES

Overall Objective

To compare the gold-standard FANG ELISA and the pan-filovirus MIA in determining Ebola Virus Glycoprotein (EBOV GP) IgG antibody reactivity in rVSV EBOV GP Vaccinated individuals in Beni, DRC.

Specific Objectives

- To determine EBOV GP-IgG antibody reactivity using the gold-standard FANG ELISA
- To determine EBOV GP-IgG antibody reactivity using the pan-filovirus MIA
- To produce a manual of standard operating procedures for determining EBOV GP antibody reactivity in rVSV EBOV GP vaccinated individuals in Beni, DRC.

CHAPTER I: LITERATURE REVIEW

I.1 Origin and Discovery

EVD was first identified in 1976, during summer when two severe epidemics of hemorrhagic fever occurred almost simultaneously in Northern Zaire (now Democratic Republic of Congo) and Nzara (a small town in the Western Equatorial Province of southern Sudan, bordering the African rainforest). The former occurred in Yambuku (a small village in Mongala Province in northern DRC), near the Ebola River, from which the disease takes its name *(PDF) Ebola Virus Disease_ Past, Present and Future*, n.d.). Since these two countries are approximately 5miles (850km) apart, public health officials initially assumed these outbreaks were a single event associated with an infected person who traveled between the two locations. Scientists later discovered that the two outbreaks were caused by two genetically distinct viruses; *Zaire ebolavirus (ZEBOV,* now known as *Ebola virus*-EBOV*)* and *Sudan ebolavirus* (SUDV) respectively. After this discovery, scientists concluded that these viruses came from two different sources and spread independently to people in each of the affected areas. The outbreaks involved more than 500 cases and at least 400 deaths caused by clinical hemorrhagic fever. The outbreak in Zaire infected 318 people with extremely high mortality rate of 88%, while the outbreak of Sudan infected 284 people with a mortality rate of 53%. Medical centres were closed because of the high death toll among the healthcare staff, thus eliminating dissemination of infection through the use of unsterilized syringes and needles. The situation outside the clinics was controlled through the segregation of patients in the affected villages by proven methods of quarantine (Center for Disease Control and Prevention, 2024).

In 1979, another Ebola epidemic occurred in Nzara, which is located in the South of Sudan. The reported number of human cases during this epidemic was 34, although the mortality rate climbed to 65%.

In 1989, Ebola appeared in a colony of Cynomolgus monkeys (*Macaca fascicularis*) imported from the Philipines into a primate facility in Reston, Virginia, outside of Washington DC. Subsequently it led to epidemic outbreaks in non-human primates in this and other facilities, namely Pennsylvania and Texas (US), and Sienna (Italy) through 1992 and recurred in 1996. Several research workers became infected with the virus during these outbreaks, but did not become ill and fortunately, no deaths among infected individuals were reported. Epidemiologic

studies conducted in connection with these incidents successfully traced the source of the outbreaks to an export facility in the Philippines, but how the facility was contaminated was not determined. Political instability at that time hampered the attempts to work in the remote areas where the monkeys were captured (*WHO, 1995*). Today, this virus is known as *Reston ebolavirus* (RESTV) and does not represent a threat to humans, although it is hazardous to primates (Feldmann *et al.*, 2003;Center for Disease Control and Prevention, 2024;)

After that, the Ebola virus had not been seen again in Africa until 1994, when in the period of just three years, five independent sites of viral transmission were recognized. Those were Ivory Coast in 1994, DRC in 1995 (precisely in Kikwit), and Gabon in 1994, 1995 and 1996. Alongside the previously known species, another distinct species was discovered during that period in Adrica which was named the Côte d'Ivoire ebolavirus (now known as *Taï Forest ebolavirus)*. A source of the virus was an infected ethnologist who performed a necropsy on a chimpanzee while working in the Taï Forest reserve in western Ivory Coast (Feldmann *et al.,* 2003).

Another large outbreak occurred in the Masindi, Mbarara and Gulu districts of Uganda at the turn of the century (2000-2001), infecting 425 people and carrying a mortality rate of 53%. The most important risks associated with this outbreak were attending the funerals of Ebola hemorrhagic fever patients, providing medical care to Ebola patients without using adequate personal protective measures, and having contact with family members of the diseased.

Ebola hemorrhagic fever remained a plague for the population of Africa during the 21st century. Almost all human cases during that period resulted due to the emergence or re-emergence of the *Sudan ebolavirus* in Sudan and Uganda, as well as the *Zaire ebolavirus* in regions of Gabon, Republic of Congo and DRC. However, in 2007, another species of the ebolavirus (Bundibugyo ebolavirus-BDBV) was discovered and was associated with two outbreaks (one in DRC and the other on the border of DRC and Uganda (Towner *et al.,* 2008)

Again, in 2008, RESTV was isolated from sick pigs in the Philippines. Several animal facility workers developed antibodies, but none reported any symptoms.

Also, in March 2014, an Ebola outbreak occurred across Guinea, Nigeria, northern Liberia, eastern Sierra Leone and was considered to be the largest one yet, often dubbed "the worst in history". The epidemic formally ended in March 2016, and a total of 28,616cases of the Ebola disease and 11,310deaths were reported in the African nations, with additional 36cases and

15deaths that occurred in countries outside of Africa (*Ebola Outbreak 2014-2016 - West Africa*, n.d.).

The world's second largest Ebola outbreak on record occurred on the 1st of august 2018 right up to 25 June 2020 in North Kivu of eastern DRC (Beni), Ituri, South Kivu and Equator province (Mbandaka) where it caused 3,481 infections and 2,299deaths. Few cases were also dictated in other parts of Central Africa like Uganda. Still in 2018, a sixth species of the Ebola virus was discovered in bats in Sierra Leone and named Bombali ebolavirus (BOMV). It is not yet known if these species is pathogenic to humans ((Breman et *al.,* 2016) .

Most recent outbreaks (reported in DRC and Uganda, involving the EBOV & SUDV strains respectively) have been small, but the virus captured the attention of the world due to death rates that can be as high as 90% as well as the visceral manner in which it kills (Breman *et al.,* 2016; Center for Disease Control and Prevention, 2024; *Ebola_uganda_ppe_2022812f1e301cc9494d8838778c984a7267*, n.d.)

I.2 Classification of Ebola Virus

The virus belongs to the Ebola virus genus, *Filoviridae* family. *Filoviridae* are non-segmented negative RNA viruses belonging to the order of *Mononegavirales* together with the families *Paramyxoviridae* and *Rhabdoviridae* (Baseler et al., 2017; Jain et al., 2021). The genus *Ebolavirus* consists of six identified species; *Zaire ebolavirus* (EBOV), *Reston ebolavirus* (RESTV), *Bundibugyo ebolavirus* (BDBV), *Taï Forest ebolavirus* (TAFV), *Sudan ebolavirus* (SUDV), and the newly identified *Bombali ebolavirus* (BOMV)- (Schindell *et al.,* 2018). Except for exclusive identification of RESTV in the Philippines, all the other species causes endemic West African EVD (Dong et *al.,* 2015).

Most of the members of the genus Ebolavirus, except Reston virus (RESTV) and Bombali virus (BOMV), cause severe and frequently fatal hemorrhagic fever in humans and non-human primates (NHPs). EBOV responsible for the EHF causes the highest human mortality (57%–90%), followed by SUDV (41%–65%) and Bundibugyo virus (40%). TAFV has caused only two nonlethal human infections to date, whereas RESTV is known to be pathogenic for humanized mice, though it causes asymptomatic human infections. In contrast, BOMV infects bats exclusively (Moghadam *et al.,* 2015;Escudero-Pérez *et al.,* 2019). The figure below shows the taxonomy of Ebola virus.

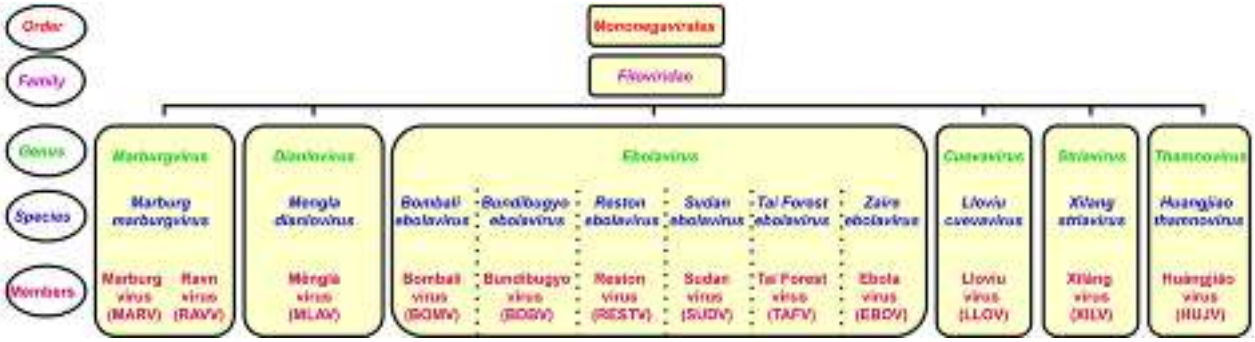


Figure 1: Taxonomical classification of ebolaviruses Source: (Jain et al., 2021)

I.3 Structure of the Ebola virion

Filoviruses are filamentous, enveloped particles with a non-segmented, negative sense, single-stranded RNA genome. The name Filovirus is derived from the Latin word 'filum', meaning 'thread', describing its thin, flexible, threadlike appearance. EBOV which is a member of the *Filoviridae* family has a ribbon or thread-like shape virion, which can be changed to circular or filamentous and appear to snake into distinctive shapes under a microscope (Geisbert & Jahrling, 1995). The filamentous shape could appear as long (measuring 800 nm length and 80 nm in diameter), short, branched, unbranched, or forming "6" and "U" configurations (Figure 2). Structural proteins associated with the nucleocapsid are the nucleoprotein (NP), VP30, VP35, and the polymerase (L) protein. Membrane-associated proteins are the matrix protein (VP40), VP24, and the GP (peplomer glycoprotein). The nucleocapsid is encapsulated by an outer viral envelope originating from the host cell membrane with characteristic 10 nm long viral glycoprotein (GP) spikes that initiates attachment. The viral genome is about 18-19kb long, linear, non-segmented negative sense (NNS), single-stranded RNA, encoding seven genes which include;

- GP- transmembrane glycoprotein that helps in attachment
- NP- nucleoprotein necessary for capsid assembly and packaging
- VP24- antiviral inhibitor, suppresses interferon production in the host cell
- VP35- inhibits interferon production or antiviral response to ds RNA.
- VP30- transcription anti-terminator
- VP40- maintaining the structural integrity of the virion, necessary for capsid assembly and budding
- L protein- viral polymerase (Bausch *et al.,* 2007; Grifoni *et al.,* 2016) -Figure 3.

Each gene, except GP, contains a single open reading frame (ORF). In contrast, the GP gene consists of three overlapping ORFs (Ikegami et *al.,* 2001; Mehedi *et al.,* 2011). During the assembly, viral RNA forms a ribonucleoprotein (RNP) complex with NP, L, VP30, VP35 and VP24 (*Dong S et al, 2015*), which appears as a helical nucleocapsid (NC) -(Bharat *et al.,* 2012; Chen *et al.,* 2019). NC protects the viral RNA from degradation by endonucleases and hosts immune response (Sun *et al.,* 2012).

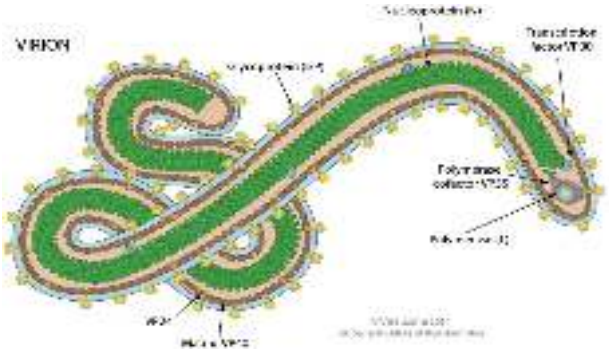


Figure 2: Structure of an Ebola virion Source: (Orthoebolavirus ~ ViralZone, n.d.)

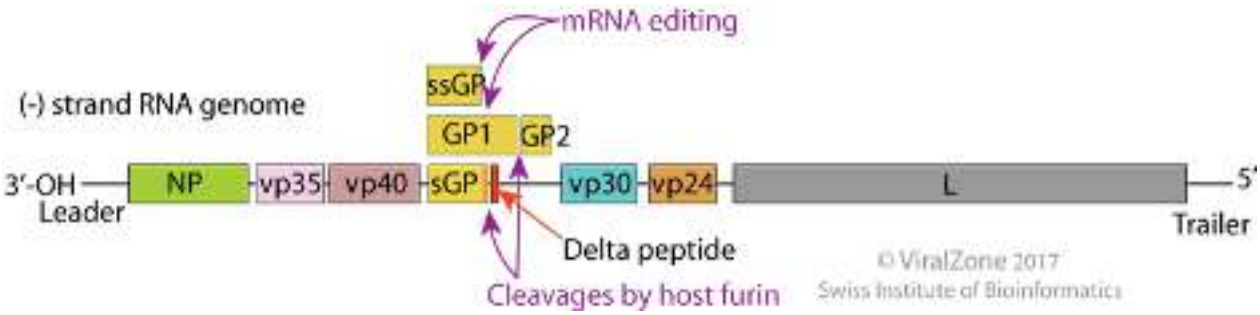


Figure 3: Ebola viral genome Source: (Orthoebolavirus ~ ViralZone, n.d.)

I.4 Ebola Virus Replication Cycle

Entry is mediated by attachment of virus to host receptors like DC-SIGN and DC-SIGNR through GP glycoprotein. The virion enters the cell by Macropinocytosis or Clathrin-mediated endocytosis (Bhattacharyya *et al.,* 2011) or caveolin-mediated endocytosis (Empig & Goldsmith, 2002). The internalization mechanism appears related to the shape of the virus. EBOV GP consists of two subunits; GP1 and GP2. After uptake, proteolysis of EBOV GP1 appears significant for viral entry (Groseth *et al.,* 2007). The mechanism of proteolysis varies depending on the host cell type (Valmas *et al.,* 2010). This EBOV GP1 proteolysis is essential for viral interaction with the obligate host receptor cholesterol transporter Niemann-PickC1 (NPC1), in late macropinosome and promotes fusion of virus membrane with the vesicle

membrane. The ribonucleocapsid (RNP) is then released into the cytoplasm (Carette *et al.,* 2011).

During transcription, the RNA genome is transcribed into seven monocistronic mRNAs whose length is determined by highly conserved start and stop signals. These start signals are predicted to form stable stem-loop structures. Just as in other negative sense RNA viruses, the transcription process begins with the binding of the polymerase complex to a single binding site located within the leader region of the genome. The complex then slides along the RNA template and sequentially transcribes the individual genes in their 3' to 5' order. However, the polymerase is released from the template following mRNA formation, so reinitiation at downstream genes is attenuated. Thus, the first gene, NP, is transcribed at the highest levels, whereas the last gene, L, is transcribed at the lowest. In sequential transcription, viral mRNAs are capped and polyadenylated by polymerase stuttering in the cytoplasm. VP30 is an important transcription activation factor for viral genome transcription, while VP24 is an inhibitor to this process. NP encapsidates both, filoviral genome, as well as anti-genome . NP associated RNA acts as a template for viral RNA transcription and replication (*Identification of a Small Molecule Inhibitor of Ebola Virus Genome*, n.d.). Replication presumably starts by binding of RNA dependent RNA polymerase complex to the leader sequence on the encapsidated (-) RNA genome. The antigenome is concomitantly encapsidated during replication. Accumulation of NP and other EBOV proteins results in the formation of inclusion bodies, which serve as additional sites for transcription and replication (Hoenen *et al.,* 2012; Kruse *et al.,* 2018; Receptor, n.d.).

At the late stage of transcription, the ribonucleocapsid complex, GP, and VP40 are transported to the cell surface via different mechanisms. Transport of ribonucleocapsid employs actin (Schudt *et al.,* 2015; Takamatsu *et al.,* 2018) while GP is carried to the cell surface via secretory pathway, where it is glycosylated as well as cleaved into GP1 and GP2 subunits (V *et al.*, 1998). The ribonucleocapsid interacts with the matrix protein VP40, and buds via the host ESCRT (endosomal sorting complex required for transport) complexes from the plasma membrane, releasing the virion. The released virion is very adaptable in evading host defenses and environmental change, can cause direct infection of tissues, immune dysregulation, hypovolemia, vascular collapse, electrolyte abnormalities, multiorgan failure, septic shock, disseminated intravascular coagulation (DIC), and coagulopathy. A schematic depiction of the viral life cycle is presented in Figure 4 below;

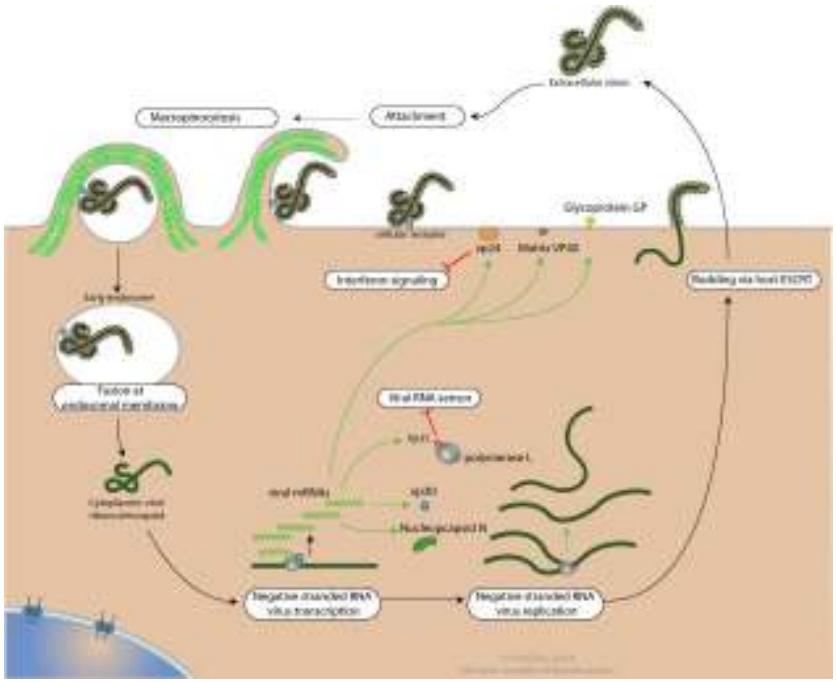


Figure 4: A schematic depiction of the Ebola virus life cycle Source: (Orthoebolavirus ~ ViralZone, n.d.)

I.5 EPIDEMIOLOGY OF EBOLA VIRUS

The average Ebola case fatality rate is around 50%. Case fatality rates have varied from 25-90% in past outbreaks, depending on circumstances and response. Results in the past four decades showed a total of 38 country-specific EVD outbreaks globally, affecting 34626 cases and causing 15266 deaths (Barbiero, 2020).

The impact this disease had on the world, and particularly West Africa, is significant. A total of 28,616 cases of EVD and 11,310 deaths were reported in Guinea, Liberia, and Sierra Leone. There were an additional 36cases and 15deaths that occurred when the outbreak spread outside of these three countries during the 2014-2016 outbreak which was the largest outbreak in the history of EVD (Bell et al., 2016). This lead the World Health Organization to declare EVD as a 'Public Health Emergency of International Concern (PHEIC) on August 8, 2014. Amongst the 38 global outbreaks, the Democratic Republic of Congo has had the highest number of outbreaks and is known as the country with the second largest outbreak on record which occurred in the 2018-2020 outbreak where it caused 3,481 infections and 2,299deaths (World Health Organization, 2020).

The table below shows the distribution of cases and deaths in countries affected by the different EVD outbreaks:

EVD outbreaks, virus type, morbidity, mortality and case fatality rate in affected countries					
Year	**Country/Town Affected**	**Virus**	**Cases**	**Death**	**Case Fatality Rate (%)**
Aug 1976	DRC (Yambuku)	EBOV	318	280	88
Jun-Nov1976	South Sudan (Nzara,Maridi)	SUDV	284	151	53
1977	DRC (Tandala)	EBOV	1	1	100
Aug-Sep 1979	South Sudan (Nzara,Maridi)	SUDV	34	22	65
1989-1990	Philippines	RESTV	3	0	0
1990	USA	RESTV	4	0	0
Dec1994-Feb1995	Gabon (Mekouka)	EBOV	52	31	60
1994	Côte d'Ivoire (Ivory Coast)	TAFV	1	0	0
May-Jul 1995	DRC (Kikwit)	EBOV	315	250	79
1995	Côte d'Ivoire (Ivory Coast)	Unknown	1	0	0
Jan1996	Gabon (Mayibou2)	EBOV	37	21	57
1996-Mar1997	Gabon (Booué)	EBOV	60	45	75
1996	South Africa	EBOV	2	1	50
Oct 2000-Jan 2001	Uganda (Gulu,Masindi)	SUDV	425	224	53
2001-2002	Gabon (Mekambo)	EBOV	65	53	82
Oct 2001-2002	RC (Mbomo)	EBOV	57	43	75
2002-Dec 2003	RC (Mbomo,Kelle)	EBOV	143	128	89
2003	RC (Mbomo)	EBOV	35	29	83
Apr-Jun 2004	South Sudan (Yambio)	SUDV	17	7	41
2004	Russia	EBOV	1	1	100
Apr-May2005	RC (Etoumbi)	EBOV	12	10	83
Aug-Nov2007	DRC (Mweka)	EBOV	264	187	71
Dec 2007-Jan 2008	Uganda	BDBV	149	37	25
2008	Philippines	RESTV	6	0	0
Dec 2008-Feb 2009	DRC (Kaluamba)	EBOV	32	15	46.8
2011	Uganda (Kibaale)	SUDV	1	1	100
Jun-Aug 2012	Uganda (Kibaale, Luwero)	SUDV	11	4	36.3
Jun-Nov 2012	DRC (Isiro)	BDBV	36	13	36.1
2012-2013	Uganda	SUDV	6	3	50
Aug-Nov2014	DRC (Boende)	EBOV	69	49	71
2014	Guinea	EBOV	3814	2544	66.7
2014	Liberia	EBOV	10 678	4810	45
2014	Sierra Leone	EBOV	14 124	3659	28
2014	Nigeria	EBOV	20	8	40
2014	Mali	EBOV	9	6	66.6
2014- Jun 2016	USA	RESTV	4	1	25
2014-2016	Spain	RESTV	1	0	0
2014-2016	UK	SUDV or EBOV	1	0	0
2014-2016	Italy	RESTV	1	0	0
2014-2016	Senegal	EBOV	1	0	0

2017	DRC (Bikoro)	EBOV	8	4	50
May-Jul 2018	DRC (Mbandaka)	EBOV	54	33	61
Aug 2018-Jun 2020	DRC (North Kivu-Ituri)	EBOV	3470	2280	66
May 2019-Nov 2020	DRC (Mbandaka)	EBOV	130	55	42
2019	Uganda	EBOV	4	4	0
Feb-May 2021	DRC (Beni)	EBOV	12	6	50
Feb-Jun 2021	Guinea	EBOV	23	12	52
Oct-Dec 2021	DRC (Mbandaka)	EBOV	11	9	82
2022	DRC (Mbandaka)	EBOV	5	5	100
Sept 2022-Jan 2023	Uganda	SUDV	164	77	47

Table 1: Distribution of cases and deaths in countries affected by the different 34 EVD outbreaks (Raj Kumar Singh et al 2017; Sima R et al, 2020; WHO 2023)

Figure 5: Countries affected by the EVD Outbreak Source: (Singh et al., 2017)

I.6 MODES OF TRANSMISSION AND PATHOGENESIS OF EBOLA VIRUS

I.6.1 Modes of Transmission

Following the discovery of ebolaviruses in 1976, scientists studied thousands of animals, insects and plants in search of the source, or reservoir host. It is believed that African fruit bats of *Pteropodidae* family, such as *Hypsignathus monstrous*, *Epomops franqueti*, and *Myonycteris torquat*a are likely involved in the spread of ebolaviruses and may even be the reservoir host though the means of local enzootic maintenance and transmission of the virus within bat populations remains unknown (Hasan *et al.,* 2019). Scientists continue to search for conclusive evidence of the bat's role in transmission of ebolaviruses. The most recent ebolavirus to be detected, Bombali virus, was identified in samples from bats collected in Sierra Leone.

Like other viruses of it's kind, it is possible that the reservoir host animal does not experience serious illness despite being infected with the virus. Ebolaviruses are likely maintained in the environment by spreading from host to host or through intermediate hosts or vectors (organisms that can spread infection to humans). Infected animals carrying the virus can transmit the pathogens from infected animals to other living organisms (KANAAN AL-TAMEEMI & RAIAAN KABAKLI, 2019). Nonhuman primates may develop the infection when they get in precise or incidental contact with fruit bats by eating partly eaten fruits (previously eaten by fruit bats) and may also transmit it to other animals like apes, duikers (antelopes), monkeys (zoonotic transmission) through precise or incidental contact. Most infected animals will not get sick; however, ebolaviruses are known to cause severe illness in nonhuman primates (such as monkeys, gorillas and chimpanzees) similar to EVD in humans (Center for Disease Control and Prevention, 2024). Primary human infection occurs when humans come in close contact with body, fluids or contaminated organs/cells of infected/dead animals or bats through hunting, butchering, preparation/cooking, eating etc (Leroy *et al.,* 2005;Barrette *et al.,* 2009). Once ebolavirus has infected a person, secondary human to human transmission occurs through close bodily contact with the infected patients in the acute disease stages or their body fluids, contaminated tissue surfaces, and clothing from alive, infected or deceased individuals (Judson *et al.,* 2015). EVD dissemination has also been reported with hospital-acquired infections, particularly in areas with poor hygiene conditions. The infected needles usage was responsible for the 1976 EVD outbreak in Sudan and Zaire (Breman *et al.,* 2016). Improper hygiene and sterilization were the crucial factors for the 1976 Yambuku EVD outburst (Reichart *et al.,* 2016). Unsafe traditional burial practices also play a pivotal role in the disease transmission (Luo *et al.,* 2019). EVD dissemination may also occur through the inanimate materials with infected body secretions like fomites (Rewar & Mirdha, 2014).There is documented evidence regarding the sexual mode of disease transmission, although transmission through the air is unlikely (Petti *et al.,* 2016). The figure below shows the summary of Ebola virus transmission.

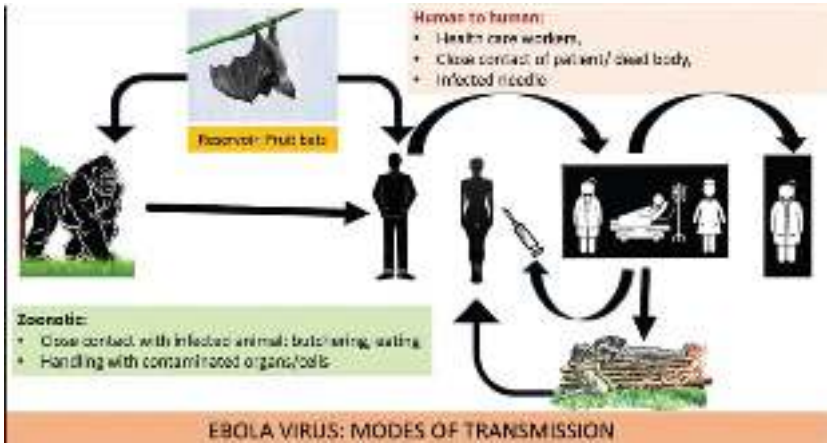


Figure 6: Modes of Ebola Virus Transmission Source: (Isha Mishra et al., 2017)

I.6.2 Pathogenesis of Ebola Virus

The Ebola virus enters the host through basic entry paths. It can enter through mucous membranes, skin lacerations/tear, close contact with infected patients/corpse, or by direct parental dissemination (Hofmann-Winkler *et al.,* 2012). It is capable of binding to membranes of essentially every human cell. The entry into the cell is controlled by a glycoprotein (GP) that is responsible for binding the virus to the cell receptors. After uptake of the viral particles by dendritic cells and macrophages, filovirus replication potently shuts down early innate immune responses by blocking interferon production and signaling (Prescott *et al.,* 2017;Escudero-Pérez *et al.,* 2019) . Dissemination probably occurs through the migration of dendritic cells to lymphoid tissues and release of virus into the circulation, leading to infection of fixed macrophages in the liver, spleen, and other tissues. Infection then spreads to adjacent hepatocytes, fibroblasts, and other cells (McElroy *et al.,* 2015;Baseler *et al.,* 2017) .

EBOV has a predilection to infect various cells of the immune system (dendritic cells, monocytes, and macrophages), endothelial and epithelial cells, hepatocytes, and fibroblasts where it actively replicates by gene modulation and apoptosis and demonstrate significantly high viremia (Bray & Geisbert, 2005; Martinez *et al.,* 2010). Disease is caused by direct effects of viral replication and host responses to infection (Baseler *et al.,* 2017). Viral replication leads to the formation of intracellular inclusion bodies, followed by cell lysis (Hoenen *et al.,* 2012; Martines *et al.,* 2015). The virus reaches the regional lymph nodes causing lymphadenopathy and hematogenous spread to the liver and spleen promoting an active inflammatory response (Fowler *et al.,* 2014). Release of chemical mediators of inflammation (cytokines and chemokines by infected dendritic cells, macrophages, and monocytes) causes a dysregulated

immune response by disrupting the vasculature system harmony, eventually causing disseminated intravascular coagulation and multiple organ dysfunction (Falasca *et al.,* 2015; Prescott *et al.,* 2017; Furuyama & Marzi, 2019). The specific cytokines the virus provokes are the proteins that prompt the natural inflammatory response. The inflammatory response induced by the infection of the virus is so drastic that it begins to cause damage to the host cells. By deregulating the cytokines, the virus is able to spread rapidly throughout the host. Proinflammatory mediators cause endothelial-cell dysfunction, followed by increased vascular permeability and fluid extravasation.18,26,28,29,32 Infected macrophages produce tissue factor with fibrin deposition in the spleen, lymphoid tissues, glomeruli, and renal proximal tubules. Consumption of clotting factors (from disseminated microthrombi), endothelial dysfunction, and inhibition of platelet function contribute to coagulopathy (Baseler *et al.,* 2017; Prescott *et al.,* 2017). Microvascular anomalies, hypovolemia, and further fluid losses through vomiting and diarrhea ultimately lead to tissue hypoperfusion and multiorgan failure (McElroy *et al.,* 2015;Leligdowicz *et al.,* 2016). These immune responses cause T-cell activation, which is rendered ineffective in severe or fatal cases because of T-cell exhaustion and apoptosis, followed by an impaired adaptive immune response.

Ebola virus has the capability to inhibit the synthesis of proteins that activate the response when the cell has been infected. A protein produced by the Ebola virus, VP35 is capable of inhibiting the synthesis of a specific INFs protein. Another way that the virus disrupts the INF response is through the production of protein VP24. A third way the virus is capable of defending itself from the antiviral response of the human body is through the glycoprotein inhibition of tetherin expression. When the virus spreads throughout the body, one of its targets are hepatic cells, or liver cells. The Ebolavirus is capable of inducing necrosis of the hepatic cells, which can result in multiple outcomes with a commensurate elevation in liver enzyme levels. When the virus is killing the hepatic cells, the liver can't synthesize enough coagulant proteins, allowing the hemorrhaging to happen. The necrosis doesn't only shut down the hemorrhaging proteins but also shuts down the liver functions, rendering the liver useless to the infected human. Other organs that undergo a similar attack from the virus are the spleen, thymus, and lymph nodes. Similar to the liver, these organs see lymphatic depletion and necrosis due to the infection. Renal tubular cells and glomerular epithelium are also affected, contributing to renal dysfunction. Myositis causes muscle aches and weakness, coupled with elevation of creatine kinase and aspartate aminotransferase levels (Heinz Feldmann *et al.,* 2020) The overall pathogenesis and pathology of Ebola virus include: Cell entry and tissue damage,

Gastrointestinal dysfunction, Systemic inflammatory response, Coagulation defects, and Impairment of adaptive immunity as illustrated in figure 5 below:

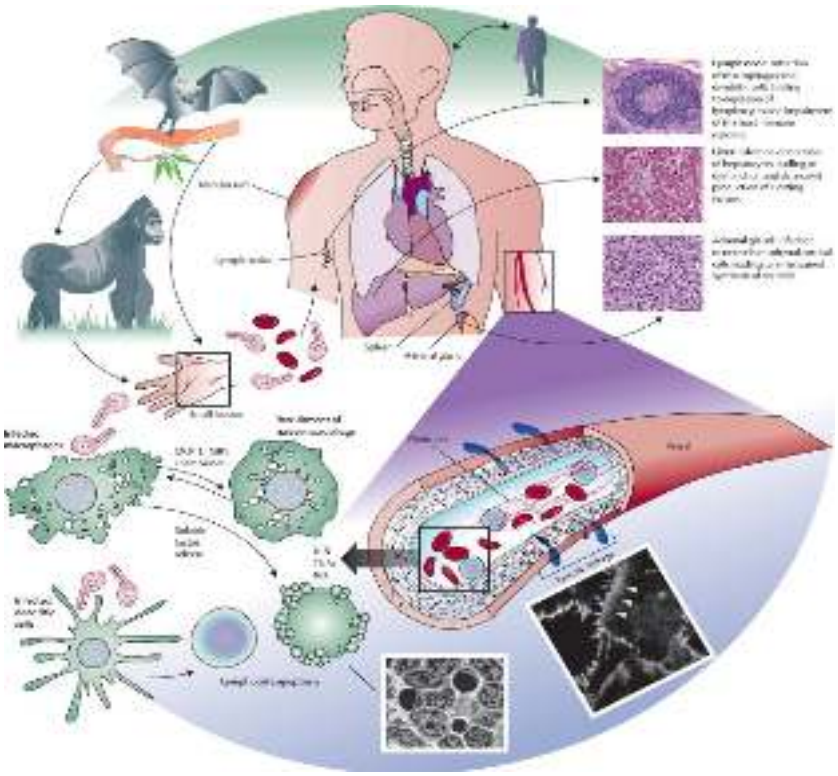


Figure 7: Pathogenesis of the Ebola virus Source: (Robot Space Brain, 2014)

I.7 Clinical Manifestations of Ebola Virus

Due to the bizarre and atypical manifestations in the initial phase, mimicking dengue fever, typhoid fever, malaria, meningococcemia, and other bacterial infections, EVD poses diagnostic dilemmas (Beeching *et al.,* 2014). The incubation period ranges from 2 to 21 days. However, symptoms usually develop 8–11 days following infection (Dallatomasina *et al.,* 2015).

The initial disease phase is represented by constitutional symptoms (Gostin & Friedman, 2015). High-grade fever of >38o C is the most frequently reported symptom (85–95%), followed by other vague symptoms such as general malaise, fatigue (85–95%), headaches, muscle pain (52–74%), dysphagia, arthralgia, myalgia, delirium, sore throat (56–58%), hiccups and dry cough (Meyers *et al.,* 2015;Balami *et al.,* 2017). The progressively advanced disease is accompanied by abdominal pain, chest/epigastric pain (62–68%), myalgia (50–79%), anorexia, nausea, vomiting, diarrhea (fluid losses can be substantial — up to 10 liters per day), oliguria, anuria, tachypnea, impaired kidney and liver functions, shortness of breath, rapid thread pulse, diminished consciousness or coma (84–86%)-(Balami *et al.,* 2017).

Variety of hemorrhagic manifestations forms an integral component of the late disease phase. Gastrointestinal tract bleeding manifests as petechiae or purpura (Rash or spots of blood under the skin), ecchymoses, hematuria, melena, red eyes/conjunctival bleeding, contusion, or intraperitoneal bleeding, nose bleeding, vomiting/coughing up of blood, blood in the stool. Mucous membrane and venipuncture site bleeding, along with excess clot formation may also occur. Laboratory finding include low white blood count (Leukopenia), low platelet count (which contributes to coagulopathy), lymphopenia, neutrophilia, thrombocytopenia and elevated liver enzymes. As the features advances with time, the patients experience dehydration, confusion, Convulsion, stupor, postural hypotension, severe metabolic disturbances and multiorgan dysfunction (multiorgan failure), resulting in fulminant shock and ultimately death (Joob & Wiwanitkit, 2014). Secondary infections like oral/esophageal candidiasis, persistent neurocognitive abnormalities can also characterize late complications of EVD.

Maculopapular exanthema constitutes a characteristic manifestation of all Filovirus infection, including EVD (Bwaka *et al.,* 1999). The rash usually appears during the 5th to 7th day of disease and occur in 25–52% of patients in the past EVD outbreaks (Kortepeter *et al.,* 2011).

The figure below shows clinical features of EVD in humans as observed in 2014 outbreak;

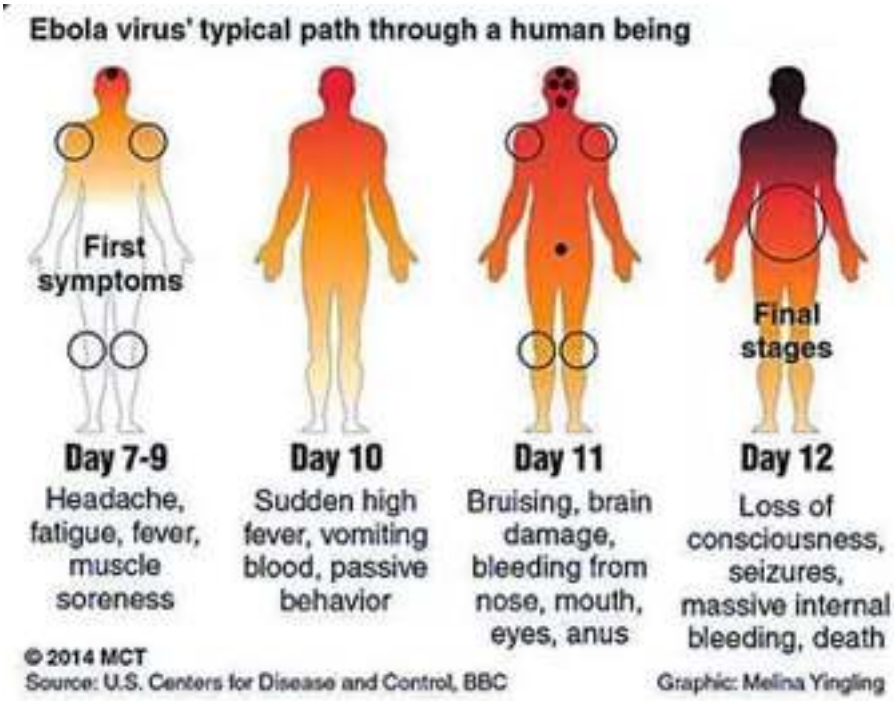


Figure 8: Clinical features of EVD in humans as observed in the 2014 outbreak Source: (CDC, 2014)

In addition, orofacial features like Gum bleeding, atypical mucosal lesions, and odynophagia comprise the distinctive oral manifestations of EVD. Epistaxis (nasal bleed), bleeding from venipuncture sites, conjunctivitis, and cutaneous exanthema are the other manifestations (Samaranayake *et al.,* 2015). Bleeding tendencies and gum bleeding is not seen in asymptomatic or initial EBOV patients reporting to the dental hospital.

EVD dissemination in the field of oral and dental health may appear nonsignificant; although, probable situations which may pose a risk to dental health professional have been appraised by (Samaranayake *et al.,* 2015). Between 25 to 90% of all clinically ill cases of EVD are fatal, depending on the virus species, patients' age and other factors.

The figure below outlines how a person's infectiousness changes over time, following infection with Ebola virus. When a person is displaying no symptoms, or early symptoms such as fever, the level of virus in the body is very low, and is likely to pose a very low risk to others. Once an individual becomes unwell with symptoms such as diarrhoea and vomiting, then all body fluids are infectious, with blood, faeces and vomit being the most infectious. When someone reaches the point at which they are most infectious, they are unlikely to be well enough to move or interact socially. Therefore, the greatest risk at this stage of infection is to people involved in their care. Skin is likely to be contaminated in the late stage of disease, because of the difficulty of maintaining good hygiene.

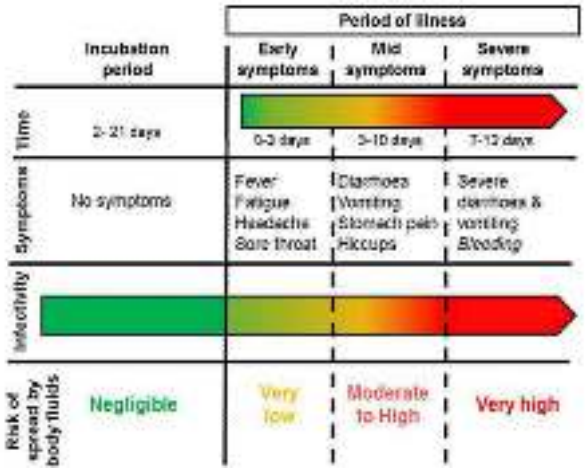


Figure 9: Schematic outline of a person's infectiousness changes over time Source: (Center for Disease Control and Prevention, 2024)

I.8 DIAGNOSIS, TREATMENT AND PREVENTION OF EBOLA VIRUS

I.8.1 Diagnosis

Multiple techniques have been established for laboratory diagnostic methods of filovirus detection including assays for the detection of viral genome, viral antigen, and host immune responses, even in field operations (Coarsey *et al.,* 2017). In the West African EBOV epidemic, on-site, high-end sequencing technology was implemented to improve outbreak response (Quick *et al.,* 2016) and simple bedside tests to detect viral antigen are also available (Shorten *et al.,* 2016).

However, EVD patients usually demonstrate altered laboratory parameters based on the stage of the disease. It is difficult to diagnose Ebola virus in the early stages due to nonspecific symptoms, which coexist in patients who are suffering from common diseases such as malaria and typhoid fever (Beeching *et al.,* 2014). Seroconversion of the EVD can be detected in the blood only when patient symptoms suggest a high level of virus load inside the body. This requires 3 days in order to reach for viral detectable levels. After 3 days of the symptoms, the Ebola virus usually reaches detectable levels in blood and cannot be ruled out earlier than 3 days by any test (Kaur *et al.,* 2017). The WHO (2014) recommended the sample collection of whole blood or body fluids, semen, or oral swabs at suitable centres called Ebola treatment centers (Balami *et al.,* 2017).

EVD can be diagnosed using the following techniques:

- **Virus culture :** Used to isolate the virus in cell culture. It is the reference method by excellence for confirming the presence of the Ebola virus. This method generally uses kidney epithelial cells from African green monkeys (Vero and MA-104 monkey cell lines). The virus can be visualized directly by electron microscopy or indirectly by immunofluorescence microscopy within 5 days of inoculation. This method is reserved for biosafety level 4 laboratories (Yang *et al.,* 2019).
- **Electron microscopy:** Electron microscopy is an indispensable technique for the identification and detection of viral infections. Thanks to the characteristic shape of viral particles, this method is specific and rapid, but requires a large number of viral particles in the sample, specific and very expensive equipment, and qualified personnels. It is more suitable for research than diagnosis (Feldmann & Geisbert, 2011; Yang *et al.,* 2019).

- **Reverse-Transcription PolymerasePolymerase Chain Reaction (RT-PCR) :**

The most widely used technique to diagnose acute infections is a quantitative real-time polymerase-chain-reaction assay (qRT-PCR) using specific primers, preferably targeting two distinct genome locations to minimize false negative results due to evolving genome mutations. This test detects the viral genome in the patient's blood or tissues, and is specific if performed at least 72 hours after the onset of symptoms. Reverse transcriptase transforms viral RNA into DNA, which is then amplified by polymerase chain reaction for detection. Various tests exist using primers for different viral genes, and recently an automated multiplex PCR system has been developed (Geisbert & Feldmann, 2011).

The qRT-PCR assay is expected to be positive in symptomatic patients, with increasing viremia in fatal cases. Since the assay may be negative early in the disease course, however, follow-up testing is warranted in patients with initially negative tests who have continuing symptoms.

This is the reference method used during epidemics, as it is more sensitive, enabling virus detection 24 to 48 h before the antigen capture detection method, and is also positive for longer, up to 3 days after viral antigen elimination. Ebola virus RNA has even been detected up to 1 year after the onset of symptoms, in the semen of a surviving patient, making it possible to control possible sexual transmission of the virus.

Ebola virus. The main advantages of this technique are that it enables the progression of the disease to be monitored by quantifying the viral load using quantitative PCR (2log10 times higher in a non-surviving patient). DNA sequencing also enables the epidemic viral strain to be typed and the phylogeny to be studied, and PCR results are available within 1 to 6 hours (Geisbert & Feldmann, 2011).

- **ELISA technique (Enzyme-Linked Immunosorbent Assay):** Antibody-capture enzyme-linked immunosorbent assay (ELISA), is also one of the most frequently utilized test for laboratory affirmation of the EVD (Martinez *et al.,* 2010). qRT-PCR is capable of detecting viral RNA in the blood samples of infected patients immediately after the commencement of signs and symptoms (Meyers *et al.,* 2015), has a high sensitivity (up to 100%), and gives results within 1–2 days in cases of epidemics. This technique, which can detect viral antigens in blood, is a reliable method for diagnosing the acute phase of Ebola virus disease in symptomatic patients. This is because viral proteins generally accumulate to detectable levels within a few days of the onset of the disease. The ELISA test also detects IgM antibodies, which are detectable from the 2nd

day after the onset of symptoms and persist until the 30th to 168th day after infection, as well as IgG antibodies, which are detectable between the 6th and 18th day and remain positive for several years. It is used to determine seroprevalence in a given population (Leroy *et al.,* 2000; Yang *et al.,* 2019). However, the ELISA technique is not suitable for initial affirmation during an outbreak (Meyers *et al.,* 2015).

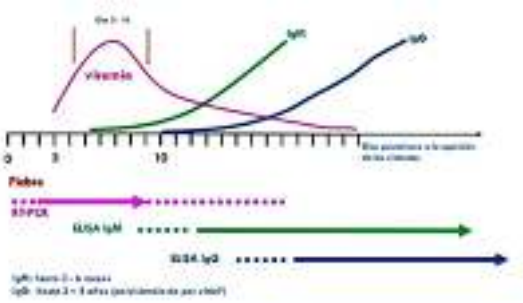


Figure 10: Kinetic curve for markers of Ebola virus infection (Source: Disease Control and Prevention)

- **Rapid diagnostic tests:** This test detects viral antigens through the presence of monoclonal antibodies that bind to viral proteins, using a drop of the patient's blood, plasma or urine. These tests are ideally suitable for the field, as they do not require sophisticated equipment or specialized personnel to use them, thus meeting the WHO criteria for rapid diagnostic tests (ReEBOV®, eZYSCREEN®, etc.) (Yang *et al.,* 2019).
- **Immunohistochemistry:** This is a diagnostic technique developed by the CDC and applied to formalin-fixed biopsy samples. It was used in 1995 to confirm the outbreak of Ebola virus disease in Kikwit, a town in the south-west of the DRC. It has the advantage of being safe for the technician, as once fixed in formalin the sample is no longer infectious, but it is a time-consuming technique and difficult to apply for field diagnosis in epidemic situations (Leroy *et al.,* 2000; Yang *et al.,* 2019).
- **Other serological methods**

The radio-immunoprecipitation test and the Western Blot (immunoassay of viral proteins separated by polyacrylamide gel electrophoresis) measure antibody responses to viral proteins, but in a slightly different way to the other methods mentioned above, as they enable the molecular specificity of the immune response to be demonstrated, but are not practical for use in epidemiological-scale studies (Leroy *et al.,* 2000; Yang *et al.,* 2019). Other diagnostic methods include; antigen-capture detection tests, serum neutralization test (*Infection of Ebola Virus - Microbewiki*, n.d.).

In addition, laboratory findings in EVD also include coagulation derangements such as prothrombin time (PT) and prolonged prothrombin time (PTT) prolonged, and leukopenia followed by neutrophilia, thrombocytopenia (50,000–100,000/mL range), and elevated liver enzyme: Elevation serum aspartate aminotransferase (AST) > alanine transferase (ALT) and renal defects that include proteinuria and increased creatinine. Early and well-regulated inflammatory response with elevated interlukin (IL)-6 concentration and IL-1beta presence in a symptomatic patient is indicative of a good outcome while a defective innate immune reaction with excessive macrophage/monocyte activation with release of interleukin-10, absent antibody response and elevated concentration of interleukin-1RA, and neopterin after a few days of the onset of disease are associated with a fatal outcome (Baize S., Leroy Km., Georges A.J., Georges Courbot M.C, Capron M., Bedjabaga L, Mavoungou E., 2002). According to a study, lymphoid depletion and lymphopenia associated with Zaire Ebola virus was most likely due to lymphocyte apoptosis via Fas/Fas ligand (FasL) interaction. The excessive macrophage/monocyte activation leads to a "cytokine storm" triggering disseminated intravascular coagulation, hypotension, and vascular dysfunction, resulting in multiple organ failure, vascular collapse, and shock (Wauquier *et al.,* 2010). According to a recent study, elevated thrombomodulin and ferritin levels have been associated with the death and hemorrhage in Ebola virus-infected patients (Lyon *et al.,* 2014).

I.8.2 Treatment

Treatment of patients has traditionally had three components: supportive care to maintain or restore normal physiology, treatment of discomfort or distress, and presumptive treatment of any concurrent, undiagnosed infections (Leligdowicz *et al.,* 2016).

EVD patients require intensive supportive therapy, including intravenous fluids or oral rehydration with solutions including electrolytes and maintenance of oxygen status and blood pressure (Figure 8). Rehydration, adequate nourishment, analgesics, and blood transfusion form a keystone supportive treatment of EVD patients (Fowler *et al.,* 2014). In advanced critical care settings, additional support may be used, such as renal-replacement therapy. Intravenous (IV) fluids and oral rehydration solution endow with proper electrolytes substitute and maintain the intravascular volume thereby stabilizing blood pressure. They also help in the treatment of specific symptoms like keeping pain manageable. Unrelenting vomiting and diarrhea are taken care of by the use of antiemetics and antidiarrheal drugs (Fowler *et al.,* 2014). Suspected cases of secondary bacterial infections and septicemia are best managed by the use of prophylactic

antibiotic regimen (third generation I.V. cephalosporins) Concurrent parasitic coinfections may also be seen and require prompt investigations and management (Fairley *et al.,* 2016). No less important is psychological support to help patients cope with anxiety, stress, and fear (Leligdowicz *et al.,* 2016).

Nonetheless, no definitive cure for EVD exist but there are two monoclonal antibody treatments for EVD caused by Zaire ebolavirus infections; REGN-EB3 (Inmazeb™) and mAb114 (Ebanga™). Inmazeb is a combination of three monoclonal antibodies and Ebanga is a single monoclonal antibody (DeSilva *et al.,* 2019). These Monoclonal antibodies work like the body's natural antibodies and help fight off the infection while the body builds its own defenses. The WHO's Therapeutics for Ebola virus disease guidance recommends the use of REGN-EB3 and mAb114 for patients with laboratory confirmed EVD infection and for neonates 7 days old or younger with unconfirmed EVD status, who are born to mothers with confirmed EVD (*Wikipedia: Western African Ebola Virus Epidemic*, n.d.).

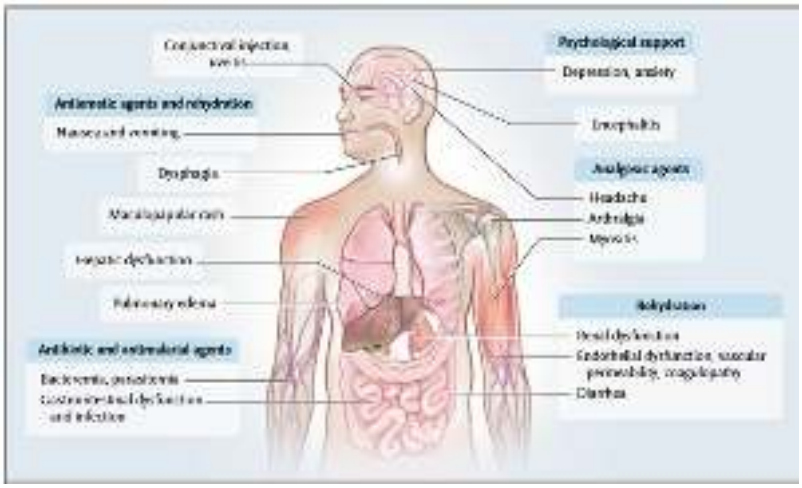


Figure 11: Key organs affected during Ebola virus infections, as well as treatment options for management of signs and symptoms Source: (Heinz Feldmann et al, 2020)

I.8.3 Prevention

I.8.3.1 General prevention measures

The most imperative strategy in EVD is to avert the vulnerable population from getting infected and limit the transmission. These preventive strategies entail intensive and rigorous endeavors from the Government, public health amenities, medical units, and personnels.

The most essential aspect to curb EVD person-to-person transmission is to avert direct bodily contact with infected individuals and their body fluids (such as urine, feaces, vomit, saliva,

sweet, semen, vaginal fluids and breast milk). Therefore, great care needs to be taken when nursing patients to avoid contact with infected bodily fluids (Samaranayake *et al.,* 2015). Follow up of contacts of infected patients for a period of 21days (Maximum incubation period) is also essential. Not forgetting community engagement and public perception (Wilkinson *et al.,* 2017).

Health caregivers are extremely vulnerable and experience an augmented professional threat for EVD (Matanock MD *et al.,* 2014). Thus, scrupulous adherence to the universal infection control measures is fundamental in all the hospitals, laboratories, and other health care services (Katz & Tobian, 2014) that is; patients should be isolated, and strict barrier nursing techniques should be used, including wearing masks, gloves and gowns. Invasive procedures such as the placing of intravenous lines, handling of blood, bodily secretions, catheters and suction devices are a particular risk and strict infection control is essential. Non-disposable protective equipment must be properly disinfected before re-use. Other infection control measures include proper use, disinfection, and disposal of instruments and equipment used in caring for patients. The U.S. CDC has advocated the appropriate use of various personal protective equipment as a mandate for health care professionals. The risk of rapid importation of Ebola virus into human beings can be prevented by averting the direct bush meat and bats contact (Dixon *et al.,* 2015).

Unsafe traditional burial procedures of those who died of EVD, especially in the African continent significantly contributed to the EVD transmission. Hence, it is essential to practice safe and guarded funeral rituals, burial or cremation (while respecting cultural values) to prevent the disease spread and this includes disinfection of the environment (e.g. at funerals), contaminated by infected persons and deceased bodies (Nielsen CF *et al,* 2014;Poliquin PG *et al,* 2016).

WHO recommends the implementation of safe sex practices to combat the sexual transmission of EVD. Strict abstinence or proper and regular condom use in male EVD survivors at least for a period of 12 months of the symptom onset or until their semen has twice tested negative should be followed (Den Boon *et al.,* 2019).

I.8.3.2 Vaccination

Three vaccines are licensed for the prevention of EVD:

- **rVSV-ZEBOV (Merck & Co, USA):** The rVSV-ZEBOV vaccine (ERVEBO®) which is a single-shot, live-attenuated, vectored vaccine based on a recombinant vesicular

stomatitis virus (VSV) expressing the Zaire ebolavirus glycoprotein (rVSV-ZEBOV-GP [ERVEBO, Merck]). That is, the G protein of the VSV envelop is replaced with Glycoprotein (GP) of the EBOV virus envelop (Pinski & Messaoudi, 2020).

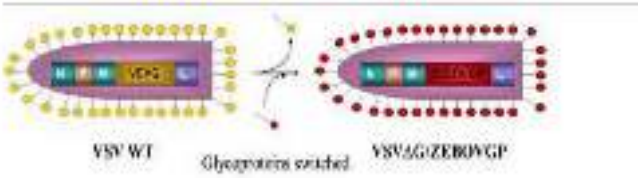


Figure 12: Protocol for use of the rVSV-ZEBOV vaccine against Ebola virus disease - 08/08/2019 version.

rVSV-ZEBOV vaccine is available as a 1ml injectable ampoule and is used for adults over 18 years old. It is an intramuscular vaccine and can be injected around the shoulder or thigh. This vaccine can be stored between -60°C and -80°C. rVSV-ZEBOV can be used as part of the response to an EVD outbreak caused by Zaire ebolavirus as only a single dose is required to elicit an immune response (Henao-restrepo et al., n.d.; Kelly *et al.,* 2021). In the context of an outbreak, the Strategic Advisory Group of Experts (SAGE) advising the WHO, recommends that individuals who have already been vaccinated more than 6 months earlier, should be revaccinated if they are among the contacts, or contacts of contacts, of a confirmed case *(WHO, 2020).*

- **Ad26.ZEBOV + MVA-BN-Filo (Johnson & Johnson, USA and Bavarian Nordic, Denmark):** This is a heterogenous vaccine regimen composed of Ad26.ZEBOV, a monovalent vaccine that provides immunity to the Ebola virus and MVA-BN-Filo, a multivalent vaccine that provides immunity to the Ebola virus, Sudan virus, Marburg virus, and Tai Forest virus. It is often delivered in 2 doses. The first dose, Ad26.ZEBOV-GP (Zabdeno), is given followed by a second dose, the heterologous prime–boost regimen containing the Janssen AdVac for priming, and Bavarian Nordic modified vaccinia Ankara, MVA-BN-Filo (Mvabea) technologies for boosting (Johnson & Johnson), 8 weeks later. The 2 doses are administered as injections into the muscle (intramuscular) around the shoulder or thigh. Ad26.ZEBOV-GP and MVA-BN-Filo can be used in adults and children over one year old. The vaccine requires 2 doses and is therefore not suitable for use in outbreak response where immediate protection is necessary. People who have previously received Ad26.ZEBOV-GP and MVA-BN-Filo injections more than 4 months earlier and are at immediate risk of infection can receive a booster dose

of Ad26.ZEBOV-GP (Callendret et al., 2018; Manno et al., 2023; Mclean et al., 2023; McLean et al., 2023)

- **ChAd3-EBO-Z/cAd3-EBO (GlaxoSmithKline, UK) with or without MVA-BN Filo (Bavarian Nordic, Denmark):** This is a viral vectored vaccine (made from viruses which are modified so that they cannot multiply in humans). This vaccine comes in two forms: one composed of a monovalent recombinant chimpanzee adenovirus type-3 vector expressing the wild-type glycoprotein (GP) of the Ebola virus (ChAd3-EBO-Z), and the other composed of a bivalent recombinant adenovirus type-3 expressing the wild-type glycoprotein (GP) of the Ebola! virus and/or the Sudan virus (cAd3-EBO) (Leroy et al., 2000; Mutua *et al.,* 2019).
- **Other Vaccines**

The Russian and Chinese vaccines are based on the Ebola virus strain ("Makona" variant) responsible for the 2013-2016 epidemic in West Africa. The Russian vaccines use rVSV and AdS expressing the glycoprotein of the Makona variant Ebola virus as vectors. The Chinese vaccine is lyophilized and uses the Ad5 vector expressing the glycoprotein of the Ebola virus variant Makona. It was approved for use in emergency situations following phase 1 clinical trials in China and phase 2 trials in Sierra Leone, which showed that the vaccine was highly immunogenic, especially when a booster dose was administered a few months after the first dose, and no major adverse events were recorded (Leroy *et al.,* 2000). Other candidate vaccines using other types of vector are certainly being studied or evaluated in clinical trials, but the results are not yet available (Henao-restrepo et al., n.d.; Leroy et al., 2000; Huttner et al., 2015).

There are currently no licensed vaccines for the prevention of EVD caused by Sudan ebolavirus. According to WHO, current evidence shows that ZEBOV vaccine (which is highly effective against Zaire ebolavirus), does not provide cross protection against Sudan ebolavirus. At least 6 candidate vaccines against Sudan ebolavirus are in different stages of development, including 3 vaccines with Phase 1 data (safety and immunogenicity data in humans)- (Feldmann *et al.,* 2018).

I.9 Gold-standard Filovirus Animal Nonclinical Group (FANG) anti-Ebola virus GP IgG enzyme-linked immunosorbent assay (ELISA) and the Pan-filovirus Multiplex Immuno Assay (MIA).

To coordinate the development of biodefense mechanisms, the Filovirus Animal Non-Clinical Group (FANG), was established in 2011 to support the development of vaccines and

therapeutics to be licensed using non-traditional pathways such as the FDA's AR.(Kilgore et al., 2015; Taylor *et al.,* 2022). This FANG ELISA is currently being used in many laboratories and the interlaboratory comparison of FANG ELISA has been done (Id *et al.,* 2020). The development, qualification, and validation of the Filovirus Animal Nonclinical Group anti-Ebola virus glycoprotein immunoglobulin G enzyme-linked immunosorbent assay for human serum samples (Rudge *et al.,* 2019; Id *et al.,* 2020) further enhanced the use of FANG ELISA as one of the assays use to evaluate the immunogenicity of Ebola vaccines (Hoff *et al.,* 2021; Manno *et al.,* 2023)

A study carried out where Filovirus Animal Non-Clinical Group assay and the Alpha Diagnostics International assay, were evaluated for detection of immunoglobulin G against Ebola virus glycoprotein. Results showed that the Filovirus Animal Nonclinical Group assay produced a wider range of relative antibody concentrations, higher assay precision, larger relative accuracy range, and lower regional background. Additionally, it was found that to sufficiently power a vaccine trial, use of the Filovirus Animal Nonclinical Group assay would require one third the number of participants than the Alpha Diagnostics International assay. This reduction in needed study participants will require less money, fewer man hours, and much less time to evaluate vaccine immunogenicity (Logue *et al.,* 2018).

Another study compared the MIA with a traditional ELISA for IgM and antigen detection of infections from Lassa and Ebola viruses (LASV and EBOV, respectively). For IgM detection in nonhuman primate samples, the MIA was 5 and 25 times more sensitive in detecting LASV and EBOV, respectively, compared to that with ELISA. For antigen detection in buffer, the MIA was 25 and 2.5 times more sensitive in detecting lower levels of LASV and EBOV,respectively. In both IgM and antigen detection assays, the MIA demonstrated excellent reproducibility at the lower limit of detection (LLOD)- (Satterly *et al.,* 2017).

The findings above demonstrates that FANG ELISA and the MIA are viable diagnostic tools for hemorrhagic fevers including Ebola virus. However, little is known about the gold-standard FANG ELISA in comparison with the pan-filovirus MIA using human samples amongst others. We therefore seek to do a comparison study between the gold-standard FANG ELISA and the pan-filovirus MIA using human samples from Beni (North Kivu) in the Democratic Republic of Congo.

CHAPTER II: METHODOLOGY

II.1 STUDY FRAMEWORK, TYPE AND PERIOD

This study is part of a collaboration between ”Ecole de Santé Publique de Kinshasa (ESPK)”, “Institut National de Recherche Biomédicales (INRB)”, “Programme Elargi de Vaccination (PEV)”, “Direction de Surveillance Epidémiologique (DSE)” of the Democratic Republic of Congo (DRC) and the Fielding School of Public Health at the University of California Los Angeles (UCLA), USA.

This is a longitudinal cohort study running from August 2018 to February 2019 and took place in eight Health Zones (Zs) in the province of North Kivu, namely:

Beni, Oicha, Mutwanga, Kamango, Kyondo, Vuhovi, Mabalako and Kalunguta.

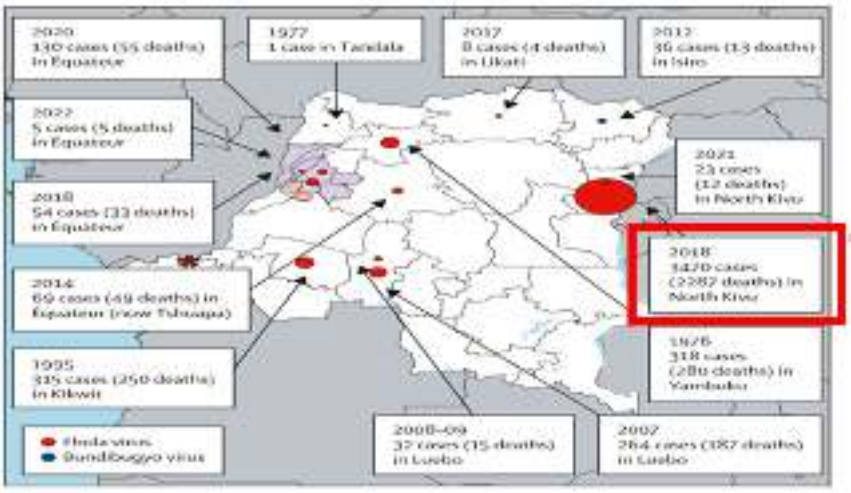


Figure 13: Study Area (Source: Kinganda-Lusamaki, E et al, 2024)

This study was conducted during the EVD vaccination campaign, during the 10th EVD epidemic and our sampling was based on convenience for the three visits (Day 0, Day 21 and Month 6).

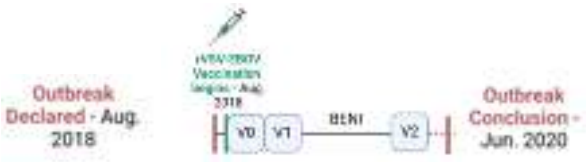


Figure 14: Collection Timeline

II.1.1 Ethical Considerations

This study received ethical approval from the Institutional Review Boards (IRBs) at the school of Public Health of the University of Kinshasa in Kinshasa, DRC (ESP/CE/022/2017) and at the University of California, Los Angeles (IRB no. 16–001346). Additionally, the study was approved by the Scientific Committee for Ebola Research during an outbreak at the National Institute of Biomedical Research under the Ministry of Health. Before any study-related procedures were conducted, consent and assent were administered in the local language for each participant. The confidentiality of the information obtained was scrupulously respected and maintained.

II.2 STUDY POPULATION

The study population consisted of all rVSVZEBOV vaccine recipients residing in Beni, DRC precisely in the health zones targeted during the study period.

II.2.1 Inclusion criteria

Any person vaccinated during the epidemic period with negative IgG anti-Ebola serology whose consent or written and verbal assent was obtained for participation in this study.

II.2.2 Exclusion criteria

In this study, all non-vaccinated persons with IgG-positive anti-Ebola serology, all participants with other infections and participants with a history of having taken another Ebola virus vaccine during the study period were excluded.

II.2.3 Non-inclusion criteria

Anyone with a history of taking Ebola vaccine prior to the day of inclusion

II.3 DATA COLLECTION

a. **Sociodemographic data:** Included age, gender, education level, marital status, profession/occupation.

b. **Exposure data**: Included information related to exposure to risk factors for EVD, a contact or a case (confirmed, suspected or probable). A unique barcode was assigned to each enrolled subject, linking the samples to personal information.

The study data was collected from the vaccine recipient or a family member by consent/assent interview, using a pre-developed and pre-loaded questionnaire on Asus® model K008 tablets using ODK collect v1.117.2 software.

II.4 SAMPLE COLLECTION, PROCESSING, TRANSPORTATION AND STORAGE

Blood samples were collected from the participants in our study during three visits: day 0 (before vaccination), day 21 and 6 months after vaccination.

Six milliliters of blood were collected using a Vacutainer® into tubes without anticoagulant from all vaccinated participants and at other appointments. Samples were transported from the collection site to the field laboratory in indigo at 4°C by motorcycle or vehicle, where they were centrifuged using a Rotina Hettich® 320 centrifuge at 3,500 rpm for 10 minutes. The sera obtained were decanted using a 1000µL micropipette and aliquoted into three previously identified cryotubes, then stored at -80°C in a Westech® brand portable freezer. These aliquots were flown to INRB, heat-inactivated and stored at -80°C in the biobank until the laboratory analyses were scheduled.

Figure 15: Collecting, processing, storing and transporting samples from the field

II.5 SEROLOGICAL ANALYSIS

The biological analysis of samples in the laboratory was carried out in 3 phases: pre-analytical, analytical and post-analytical.

II.5.1 Pre-analytical phase

Blood samples were accompanied by a shipping (dispatching) slip. Each tube was marked with a barcode corresponding to the number assigned to the sample at the time of collection on site.

The quality control of the samples to be analyzed was carried out by comparing each sample with its corresponding bar code to ensure conformity prior to analysis.

II.5.2 Analytic phase

The samples were analyzed using the gold-standard FANG ELISA on one hand and the pan-filovirus MIA on the other hand

II.5.2.1 FANG ELISA

Filovirus Animal Nonclinical Group ELISA (FANG) enzyme-linked immunosorbent assay is an indirect, quantitative ELISA technique developed by the Battelle Memorial Institute of the US Department of Defense (DOD) and subsequently approved by the Food and Drug Administration (FDA) at the National Institut of Health (NIH) in the USA (Rudge et al., 2019). This test was used in the INRB laboratory to measure the level of antibody (Ab), precisely IgG before vaccination, 21 days and 6 months after use of the rVSV-ZEBOV vaccine.

a. **Principle**: FANG ELISA detects the presence of an antibody in a sample by using the principle of enzyme-linked immunosorbent assay (ELISA)-Indirect Sandwiched ELISA that is, it relies on antigens (EBOV) to detect a target antibody (anti-Ebola IgG) using highly specific antibody-antigen interactions and this generally takes place on flat surfaces in multi-well plates.

   This implies, Anti-Ebola IgG (primary antibody) present in positive samples bind to EBOV GP (antigen) pre-coated on the polystyrene microplate wells. This binding forms an 'antibody-antigen' immunocomplex. A second antibody conjugated with peroxidase enzyme (labeled antibody) also binds to a different epitope on the 'antibody-antigen' immunocomplex to form an antibody-antigen-antibody(enzyme) 'sandwich' immunocomplex. A coloured product is formed upon addition of TMB substrate (Chromogen Substrate). The intensity of the colour measured at 450nm is proportional to the amount of antibody captured.

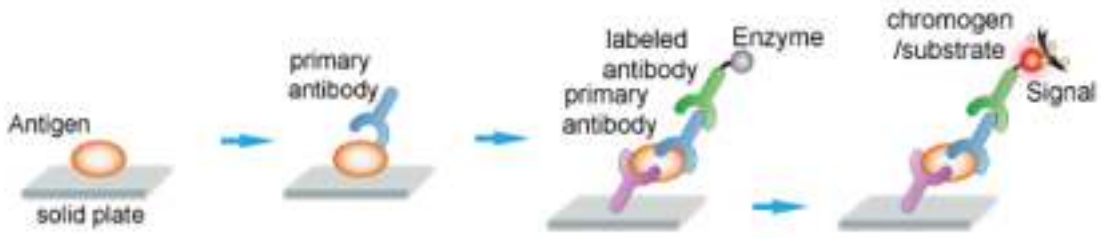


Figure 16: Principle of ELISA (Source: Golden Bio technologies-GBT)

b. **Procedure**

- FANG reagents: ELISA Diluent (ED), wash solution (WS), Phosphate Buffered Saline 1X (PBS1X) and EBOV rGP, were prepared.
- Plates were coated with 100µl EBOV rGP and sealed with adhesive plate seals then wrapped with aluminum foil. Followed by Incubation in the fridge at 4°C for 24 hours,
- Plates were washed with 300µl of wash solution three times and tapped dry on a tissue paper.
- Samples (sera), Reference Standard (RS) and controls (NC, QCH, QCL) were diluted with ELISA Diluent in a dilution box.
- 100µl each of diluted samples, RS and controls were distributed in duplicates into the washed plate, then incubated at 37°C for 1 hour.
- Plates were washed three times with 300µl of wash solution, tapped dry on tissue paper and 100µl of peroxidase-conjugated secondary antibody (prepared 15minutes in advance) was added to the plate, then incubated for 1 hour at 37°C
- Plates were washed five times with 300µl of wash solution and tapped dry on tissue paper.
- 100µl of chromogenic substrate-TMB (placed at room temperature 15minutes in advance) was then distributed into the plate and incubated at room temperature in an opaque box protected from light for 10 minutes.
- 100µl reaction stop solution was added to stop the Ab-Ag reaction.
- Absorbance was read at 450 nm using a Molecular Diagnostic Spectramax 384 plus Spectrophotometer.

	1	2	3	4	5	6	7	8	9	10	11	12
A	RS 1	RS 2	RS 3	RS 4	RS 5	RS 6	RS 7	RS 8	RS 9	RS 10	RS 11	NC
B	RS 1	RS 2	RS 3	RS 4	RS 5	RS 6	RS 7	RS 8	RS 9	RS 10	RS 11	NC
C	S1 1:50	S1 1:50	S2 1:50	S2 1:50	S3 1:50	S3 1:50	S4 1:50	S4 1:50	S5 1:50	S5 1:50	QCH 1:50	QCH 1:50
D	S1 1:1,000	S1 1:1,000	S2 1:1,000	S2 1:1,000	S3 1:1,000	S3 1:1,000	S4 1:1,000	S4 1:1,000	S5 1:1,000	S5 1:1,000	QCH 1:1,000	QCH 1:1,000
E	S1 1:4,000	S1 1:4,000	S2 1:4,000	S2 1:4,000	S3 1:4,000	S3 1:4,000	S4 1:4,000	S4 1:4,000	S5 1:4,000	S5 1:4,000	QCH 1:4,000	QCH 1:4,000
F	S6 1:50	S6 1:50	S7 1:50	S7 1:50	S8 1:50	S8 1:50	S9 1:50	S9 1:50	S10 1:50	S10 1:50	QCL 1:50	QCL 1:50
G	S6 1:1,000	S6 1:1,000	S7 1:1,000	S7 1:1,000	S8 1:1,000	S8 1:1,000	S9 1:1,000	S9 1:1,000	S10 1:1,000	S10 1:1,000	QCL 1:1,000	QCL 1:1,000
H	S6 1:4,000	S6 1:4,000	S7 1:4,000	S7 1:4,000	S8 1:4,000	S8 1:4,000	S9 1:4,000	S9 1:4,000	S10 1:4,000	S10 1:4,000	QCL 1:4,000	QCL 1:4,000

Figure 17: FANG plate dilution ratio (Source: FANG ELISA procedure, see Appendix

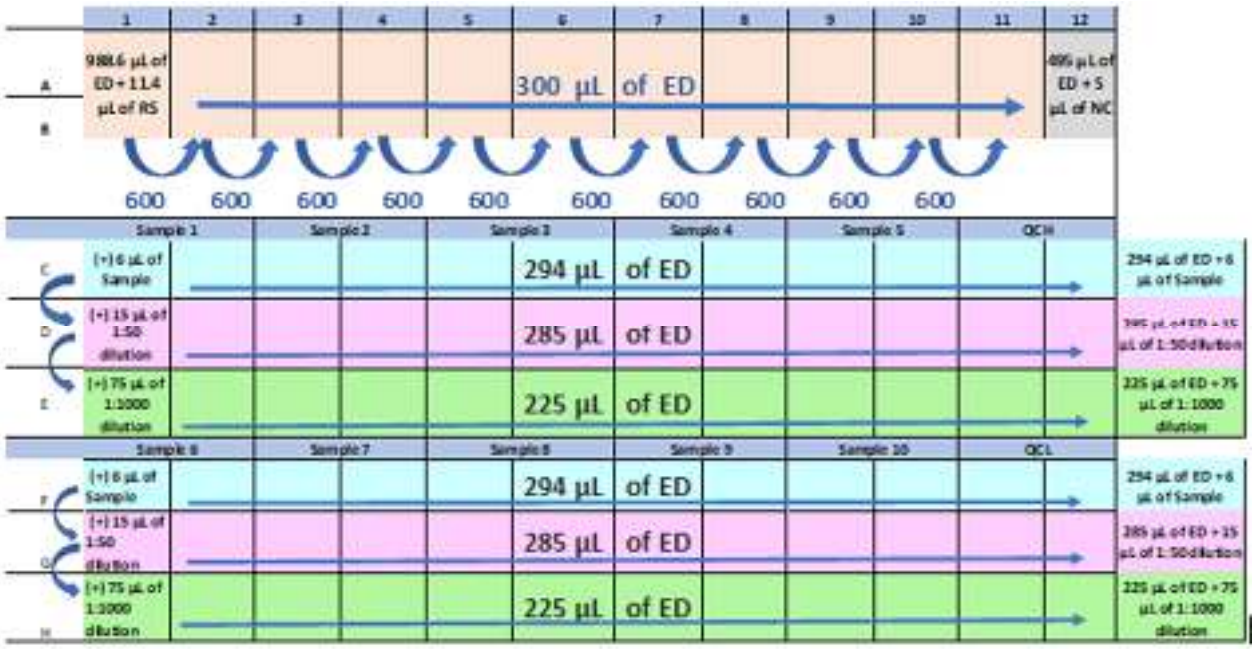


Figure 18: Layout diagram for standard curve, controls and samples (Source: FANG ELISA Procedure, see Appendix 2)

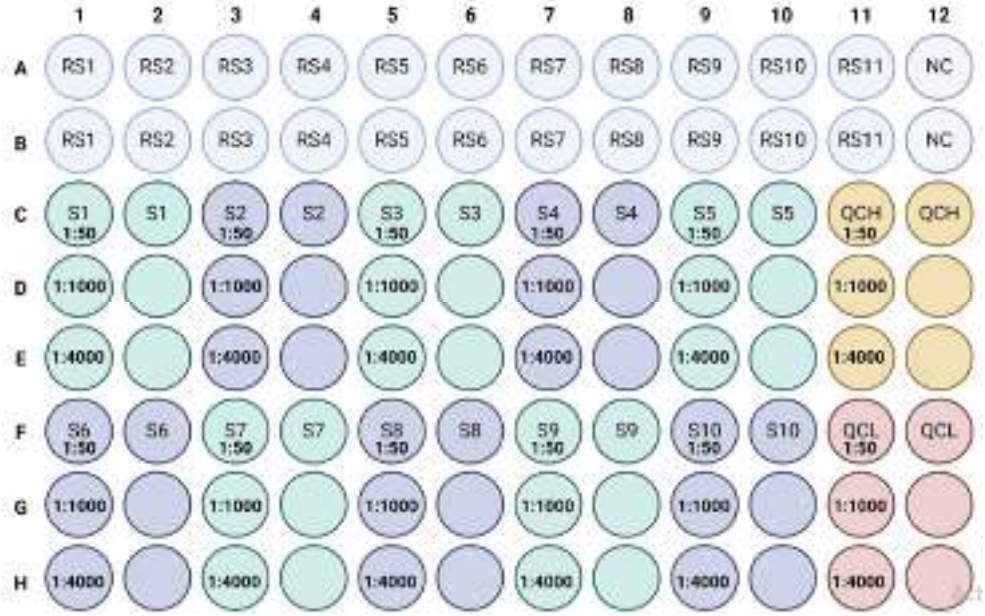


Figure 19: FANG Plate Layout (Source: FANG ELISA Procedure, see Appendix2)

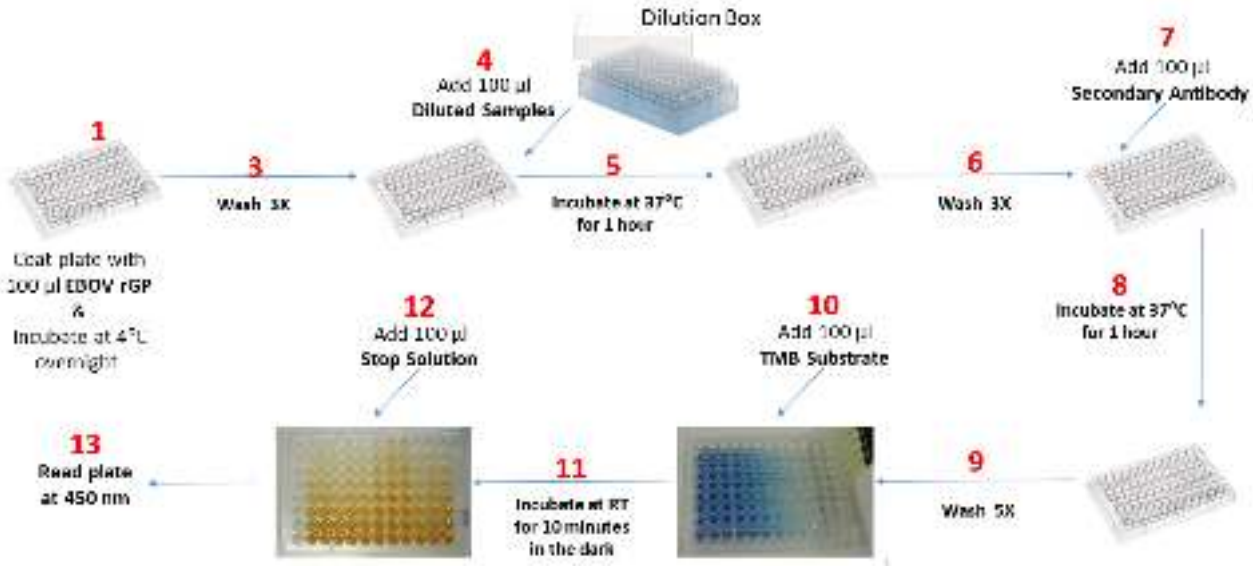


Figure 20: FANG ELISA Flow Chart (Source: FANG ELISA Procedure, see Appendix 2)

c. Interpretation of Results

Interpretation of result took into account the validity criteria for Elisa plates, that is;

- The mean value of the negative control (CN) should be < 0.2
- QCH value should be between 293.01- 687.73
- QCL value should be between 109.28-255.26

- An 11-point reference standard (RS), providing sufficient resolution for quantitative antibody titers expressed in EU/ml.

Samples with a 20% discrepancy in geometric mean antibody concentrations were selected and re-analyzed.

A sample with geometric mean Ab concentrations between 66.96 - 607 EU/ml was considered positive; and one with geometric mean Ab concentrations below 66.96 EU/ml was considered negative (95% CI).

All results generated were sent to the National Institut of Health (NIH) for quality control (see Appendix 2).

II.5.2.2 Multiplex Immuno Assay (MIA)

The MIA is a luminex assay, precisely a bead-based immunoassay that measures multiple analytes in a single microplate well.

a. **Principle**

It captures targets onto spherical beads in suspension that is, the sample (serum with Anti-Filovirus IgG) is added to a mixture of color-coded beads, pre-coated with analyte-specific capture antigens (Filovirus Antigen). The antibodies (Anti-Filovirus IgG) bind to the analytes of interest (Filovirus Antigen).

Biotinylated detection antibodies specific to the analytes of interest (anti-human IgG) are added and form an antibody-antigen sandwich. Phycoerythrin (PE)-conjugated streptavidin is added. It binds to the biotinylated detection antibodies (since biotin increases the signals for sensitivity).

Beads are read on a dual-laser flow-based detection instrument, such as the Luminex 200™ Analyzer (Magpix Luminex Machine).

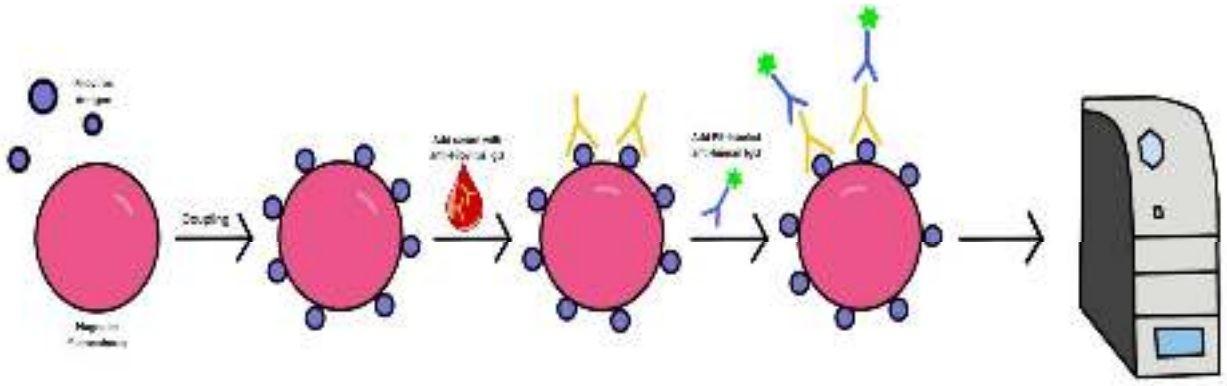


Figure 21: MIA Principle (Source: Luminex® assay)

b. **Procedure**

- Magnetic MagPlex microspheres (Luminex Corporation, Austin, TX) were coupled covalently with purified recombinant glycoproteins (EBOV GP, SUDV GP, Marburg Virus (MARV) GP, BDBV GP, and bovine serum albumin (BSA) as control).
- PBS 1X/BSA Tween-20 solution was prepared by adding 5g of BSA to 500ml of PBS 1X and after keeping it on a magnetic stirrer for 10minutes (time for BSA to dissolve), 100µl of Tween-20 was added and this was stored in the refrigerator at 4ºC.
- The bead mix was prepared by diluting the master bead mix (after vortexing for 10seconds) in PBS 1X/BSA Tween-20 solution (1:30dilution). The tube containing the bead mix was then wrapped with Aluminium foil to protect from light.

N.B: The beads are very sensitive to light, reason why black plates are used for the experiment and the whole experiment takes place in a dark room (lights turned off).

- The negative control and samples were vortexed for 10minutes each and diluted in a dilution box by adding 2µl of negative control and sample respectively to 198µl of PBS1X/BSA Tween-20
- The positive controls (EBOV, SUDV, MARV, BDBV) were equally vortexed for 10minutes each and diluted in a dilution box by adding 3µl of each in 191µl of PBS1X/BSA Tween-20.
- 50µl of the bead mix was distributed in each well on a plate.

- 50µl of each sample was then distributed into each well in the plate and this was done in duplicates (following this order A1, B1, C1, D1, E1, F1, G1, H1, A2 ....) then followed by the distribution of 50µl of negative control and finally positive control (still in duplicates and same order).
- The plate was then covered with Aluminum foil and incubated at a temperature of 37ºC on a shaker set at 700tr/min for 3hours.
- Just before the end of the 3hours incubation, anti-human IgG phycoerythrin conjugate (secondary antibody) was diluted in PBS 1X/BSA Tween-20. For 100 wells: 5000 µL PBST: 25 µL secondary antibody (1:200 dilution).
- After 3 hours incubation, the plates were placed on the magnetic plate for 2 minutes and after 2 minutes, the liquid was discarded in a tray containing 10% bleach solution.
- The plate was washed by adding 200ul PBS1X/BSA Tween-20 to each well using a P200 multichannel pipette, then placed on the shaker for 10 to 15 seconds (Still protected from light). The plate was removed from shaker and placed on magnetic plate for 2 minutes and after 2 minutes, the liquid was discarded into a tray containing 10% bleach solution. This wash process was repeated for a total of two washes.
- 50 µl of diluted secondary antibody was distributed into the respective wells in the direction of the plate then plate was covered with aluminum foil and incubated in a 37°C incubator for 1 hour at 700 rpm on the plate shaker.
- After 1 hour incubation, the plates were placed on the magnetic plate for 2 minutes and after 2minutes, the liquid was discarded in a tray containing 10% bleach solution.
- The plate was washed by adding 200ul PBS1X/BSA Tween-20 to each well using a P200 multichannel pipette, then placed on the shaker for 10 to 15 seconds (Still protected from light). The plate was removed from shaker and placed on magnetic plate for 2 minutes and after 2 minutes, the liquid was discarded into a tray containing 10% bleach solution. This wash process was repeated for a total of two washes.
- 120 ul of MAGPIX drive fluid was added to each plate well and the plate was covered with aluminum foil and kept on the shaker at 700 rpm for 10-15 seconds at room temperature.

- A batch was created using the "Liberia Human Project" protocol on magpix. The plate was then taken from the shaker and read on the Magpix Luminex Machine.
- Finally, the number of beads and MFI for all wells were checked.

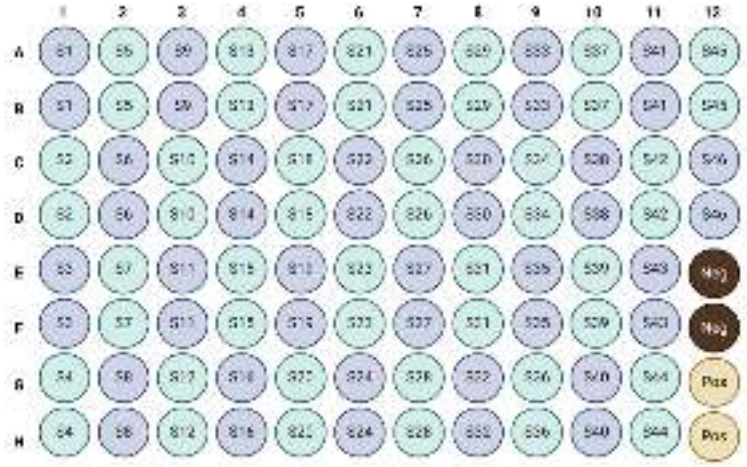


Figure 22: MIA Plate Layout (Source: HAWAII Procedure, see Appendix)

c. **Results Interpretation**

Results were interpreted by estimating the Median Fluorescence Intensity (MFI) based on the reactivity cut offs. Reactivity cut offs were calculated using an assumed-naïve cohort of Kinshasa individuals (n = 407) at baseline – *mean MFI for each Antigen (Ag) + 3SD.*

*These cut offs are variable and can change with the population used for reactivity calculations.

Antigen	Mean	Standard Dev.	CUT OFF
EBOV GP	659.7	973.7	3580.8
EBOV VP40	3488.5	3092.5	12766
EBOV NP	1345.6	1547.2	5987.2
MARV GP	1224.4	1585.6	5981.2
MARV VP40	2623.6	2608.4	10448.8
SUDV GP	2534.6	2796.8	10925
BDBV GP	1464.8	1547.9	6108.5

Table 2: MIA Mean Fluorescence Index (MFI) Cutoff values for each analyte

II.5.3 Post Analytic Phase

II.5.3.1 Filovirus Animal Non-clinical Group Enzyme-linked Immunosorbent Assay (FANG ELISA)

Preliminary results were reported directly to the health authorities.

Operational definitions:

- **Seropositivity** is defined by the presence of geometric mean Ab concentrations > 66.96 EU/ml in a participant prior to vaccination.

- S**eroconversion**: the appearance of IgG antibodies directed against the Ebola virus in a seronegative participant at baseline after an injection of rVSV -ZEBOV vaccine.

- **Geometric concertation of Ab**, was defined as a ≥four-fold or greater increase in antibody titers from one point to another compared with their value at a starting point.

II.5.3.2 Multiplex Immuno Assay (MIA)

Preliminary results were reported directly to the health authorities.

Operational definitions:

- **Seropositivity:** For this analysis, a cut-off value of 2967 MFI was calculated for the EBOV-GP target of the MIA.

-S**eroconversion**: the appearance of IgG antibodies directed against the Ebola virus in a seronegative participant at baseline after an injection of rVSV -ZEBOV vaccine.

II.6 STATISTICAL ANALYSIS

II.6.1 Filovirus Animal Non-Clinical Group Enzyme-Linked Immuno-Sorbent Assay (FANG ELISA)

Vaccinees' sociodemographic and biological data were entered on the Asus® tablet model K008 using Open Data Kit (ODK) software and exported to official Excel 2013 software. Statistical analyses were performed using SAS software (version 9.4, Cary, NC) and R (version 3.2.3, R Foundation for Statistical Computing, Vienna, Austria).

-Numerical data were summarized as mean and standard deviation, categorical data as proportion or percentage.

-Geometric mean CA concentrations were measured in EU/ml.

-The association between socio-demographic data, EVD exposure factors and concentration was investigated using simple and mixed linear regression, including all factors and calculating the ratio of Ab (Odds CI 95%). The results are presented in tables and figures.

II.6.2 Multiplex Immuno Assay (MIA)

All statistical analysis were performed using SAS 9.4 and R (version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria). It is worth noting that the same samples used for the FANG analysis were used for the MIA analysis. Therefore the statistical analysis for MIA was the same as that of FANG as described above.

CHAPTER III: RESULTS/DISCUSSIONS

III.1 RESULTS

III.1.1 Filovirus Animal Non-Clinical Group Enzyme-Linked Immuno-Sorbent Assay (FANG ELISA)

III.1.1.1 Description of the General Study Population

Between August 2018 and February 2019, 617 vaccinated participants were enrolled. The average age of vaccinated participants was calculated at 35 years, with extremes ranging from 12 to 82 years. The male gender was more preponderant with 388/608 (63.8%). The most common age groups were 20-29 and 30-39, with 25.7% and 32.6% respectively. Only 346/608 (57.5%) were married, and 248/608 (40%) had a primary school education. Non-healthcare providers were more represented 363/208 (59.4%) and reported no exposure to EVD risk factors 59/608 (59.1).

Socio-demographic characteristics and EVD exposure factors are shown in Table 2.

Socio-demographic data		Number	%
Gender	Male	388	63.8
	Female	220	36.2
Age	12–19	60	9.9
	20-29	156	25.7
	30-39	198	32.6
	40-49	112	18.4
	50-82	82	13.5
Level of Education	Primary	248	40.9
	University	177	29.2
	Secondary	160	26.4
	Analphabetic	22	3.6
	ND	1	0.2
Civil Status	Married	346	57.3
	Single	243	40.2
	Divorced/Widow (er)	15	2.5
	ND	4	0.7
Health Care Worker (i.e Doctor, nurse, assistant, hygiene)	Yes	245	40.6
	No	363	59.4
Contact with a case (confirmed-probable-suspect)			
Yes		176	28.9
No		359	59.1
ND		73	10

Table 3: Socio-demographic data and EVD exposure factors in the general study population

***Key**: (ND) Not Determined*

III.1.1.2 Serological profile of the study population

Nine out of six hundred and seventeen, or 1.5% of participants, had high titers of Ab at baseline compared with the threshold set by the study, and were excluded from the analyses at 21 days and 6 months post-vaccination. The follow-up visits therefore included 608 participants with no anti-Ebola Ab at baseline (Figure 23).

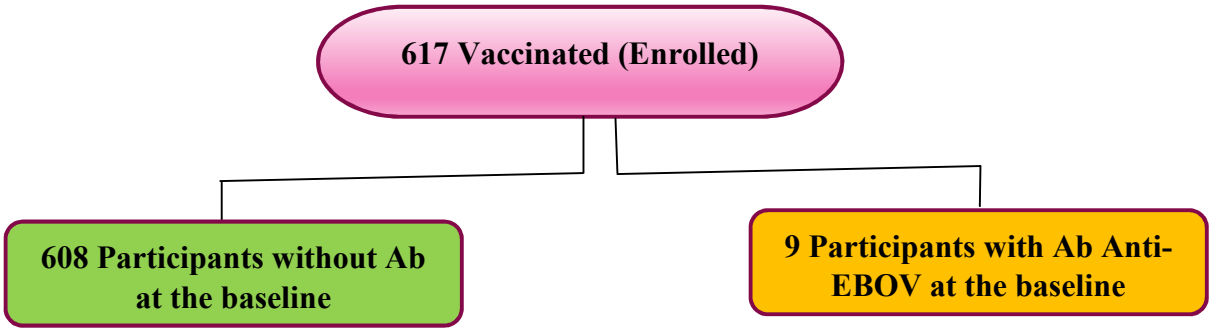


Figure 23: Description of the Study Population

A total of 608 participants enrolled without anti-Ebola Ab at the baseline, 548 Participants had been reviewed at 21 days post-vaccination, i.e. a 90% participation rate. Of these, 87.2% had synthesized IgG anti-Ebola antibodies 21 days post-vaccination, with geometric mean titers significantly elevated at 900 (826-981) EU/mL. 434 participants were present at the 6-month post-vaccination visit, representing a 71.4% participation rate. Of these, 95.6% had IgG antibodies to the Ebola virus glycoprotein, with geometric mean antibody concentrations of 1231 EU/mL (1145-1324 EU/mL) 6 months after a dose of rVSV-ZEBOV vaccine . (Table 4)

	rVSV-ZEBOV (n)
Variable	
At the baseline	
Number of participants	608
Geometric mean concentration of Ab (CI at 95%) – EU/mL	9 (8-10)
At 21days	
Number of participants	548
Geometric mean concentration of Ab (CI at 95%) – EU/mL	900 (826-981)
Seroconversion (CI at 95%) – %	87.2 (84.1 -89.9)
At 6Months	
Number of participants	434
Geometric mean concentration of Ab (CI at 95%) – EU/mL	1231 (1145 - 1324)
Seroconversion (CI at 95%) – %	95.6 (93.3 – 97.3)

Table 4: Seroconversion rates at 21 days and 6 months among participants (rVSV-ZEBOV)

***Key**: (CI) Confidence Interval*

Tables 5 and 6 illustrates the sociodemographic factors and potential risk factors associated with immune response and Ab persistence in the vaccinated population.

In this study, we looked at the sociodemographic data and exposure factors associated with a good immune response at 21 days and 6 months in the vaccinated population.

Female gender, young age and unmarried status were the socio-demographic factors associated with a good immune response 21 days after vaccination and 6 months after use of a dose of rVSV-ZEBOV vaccine, using univariate and multivariate statistical analyses as shown in Tables 5 and 6. However, no association was found between potential risk factors for transmission of EVD linked to immune response and persistence of Ab 6 months after vaccination.

21 Days			**6 Months**	
Ratio of Ab Titer		**CI at 95%**	**Ratio of Ab Titer**	**CI at 95%**
Sex				
Male	*Reference*		*Reference*	
Female	1.11	0.93-1.32	1.35	1.15-1.58
Age				
12-19	*Reference*		*Reference*	
20-29	0.72	0.54-0.96	0.67	0.51-0.90
30-39	0.65	0.49-0.87	0.65	0.49-0.86
40-49	0.56	0.41-0.75	0.75	0.56-1.01
50-82	0.66	0.47-0.93	0.84	0.61-1.17
Level of Education				
Analphabetic	0.66	0.43-1.03	0.77	0.52-1.14
Primary	*Reference*		*Reference*	
Secondary	1.05	0.83-1.31	0.89	0.74-1.07
University	0.87	0.71-1.06	0.96	0.81-1.14
Civil Status				
Married	1.22	1.03-1.46	1.07	0.93-1.25
Single	*Reference*		*Reference*	
Divorced/Widow (er)	1.13	0.63-2.05	1.25	0.77-2.03
Health Care Worker				
Yes	0.83	0.70-0.99	0.98	0.85-1.14
No	*Reference*		*Reference*	
Contact with a case (confirmed-probable-suspect)				
Yes	0.87	0.72-1.05	0.96	0.81-1.14
No	*Reference*		*Reference*	
Don't Know	0.91	0.66-1.27	1.05	0.81-1.36

Table 5: Univariate analysis factors associated with antibody response 21 days and 6 months after rVSV-ZEBOV vaccination

***Key**:(CI) Confidence Interval*

21 Days				6 Months	
		Ratio of Ab Titer	**CI at 95%**	**Ratio of Ab Titer**	**CI at 95%**
Sex	Male	*Reference*		*Reference*	
	Female	1.11	0.92-1.33	1.36	1.15-1.62
Age	12-19	*Reference*		*Reference*	
	20-29	0.73	0.53-1.01	0.69	0.49-0.97
	30-39	0.68	0.48-0.97	0.62	0.44-0.89
	40-49	0.59	0.40-0.86	0.71	0.48-1.05
	50-82	0.71	0.47-1.07	0.80	0.54-1.19
Level of Education	Analphabetic	0.72	0.46-1.12	0.80	0.53-1.21
	Primary	*Reference*		*Reference*	
	Secondary	1.15	0.90-1.46	0.99	0.81-1.21
	University	1.00	0.80-1.25	1.10	0.91-1.34
Civil Status	Single	1.00	0.79-1.25	0.94	0.77 – 1.14
	Married	*Reference*		*Reference*	
	Divorced/Widow (er)	1.18	0.67-2.10	1.03	0.63-1.70
Health Care Worker					
Yes		0.90	0.74-1.09	1.06	0.90-1.26
No		*Reference*		*Reference*	
Contact with a case (confirmed-probable-suspect)					
Yes	0.91		0.74-1.12	0.94	0.79-1.13
No	Reference			Reference	
ND	1.03		0.68-1.55	1.08	0.77-1.51

Table 6: Multivariate analysis of factors associated with antibody response 21 days

***Key**: (CI) Confidence Interval*
(ND) Not Determined

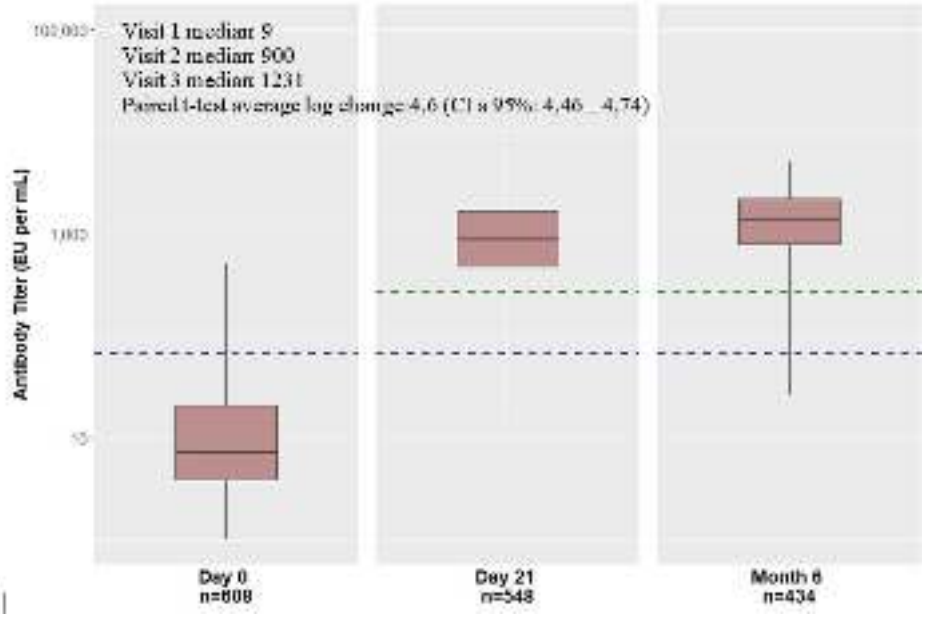


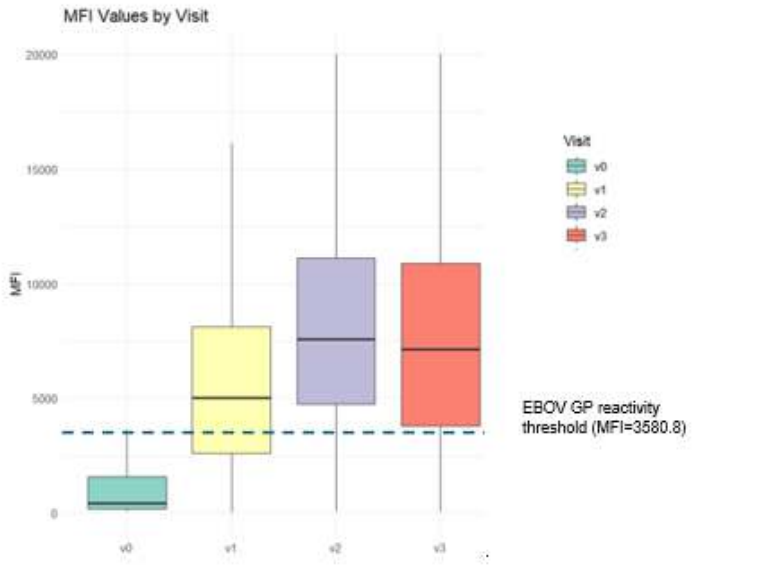
Figure 24: Antibody titers following rVSVΔG-ZEBOV-GP vaccination among 608 participants in Beni, DRC. The blue dashed line indicates 66.96 EU/mL, the LLOQ for FANG ELISA. The green dashed line indicates 267.85 EU/mL, four times the LLOQ for FANG ELISA, or the lower limit for which our participants could be considered positive for antibody response or persistence. Box plots show the median, interquartile range, and range of antibody titers at each time point for individuals who did not have an elevated titer at baseline.

The increase in antibody concentration from baseline (7.04) to 21 days (922.51) and 6 months (1334.94) after vaccination meets the operational definition of geometric mean antibody concentration (t-test log10 changed from 4.46 - 4.74) with a mean of 4.6).

III.1.2 Multiplex Immuno Assay (MIA)

The same samples were used for both FANG and MIA analysis separately. Therefore the study population and demographic data for MIA is the same as that of FANG described above. However, it is worth noting that 166 individuals were seroactive at V0 (Visit zero) and these seroreactive individuals at V0 were not included in the denominator nor as "seroconverters".Again, at:

- **D1:** MFI Value is > 4x Visit 0 MFI **and** > Kin. Naïve Mean
- **D2 :** MFI Value is > Kin. Naïve Mean + 3SD (EBOV GP Cut Off)
- **D3 :** At specific visit, Mean Fluorescence index (MFI) is considered reactive by **D1** or **D2**



D1	77.1	84.2	87.8
D2	63.4	82.9	77.1
D3	84.2	90.8	89.4

** Variable denominators with each visit

Figure 25: MFIs of participants injected with rVSV-ZEBOV vaccine

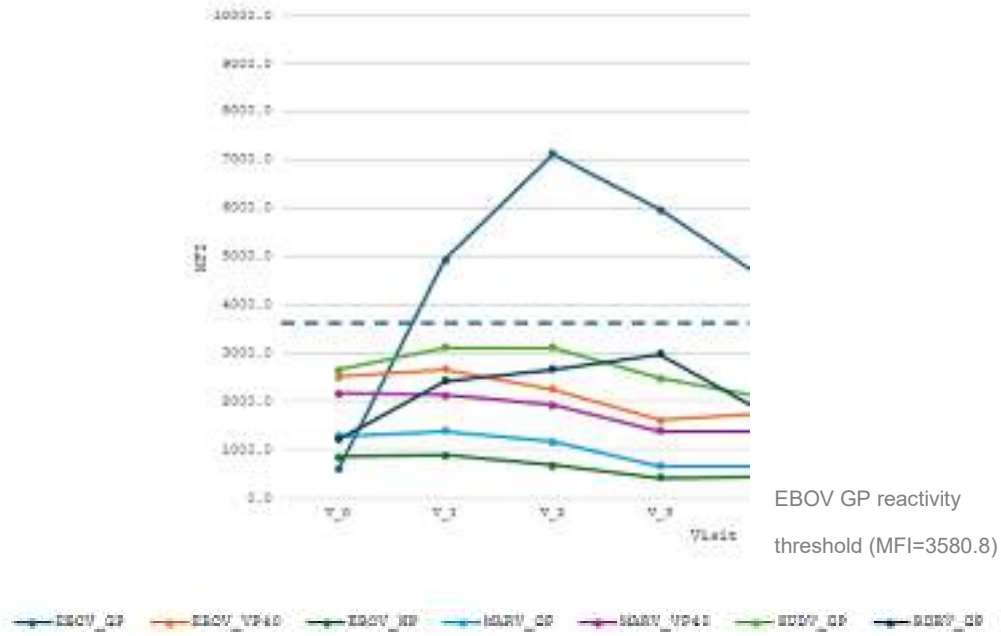


Figure 26: Overall reactivity with the Pan-filovirus MIA

III.1.3. Golden Standard FANG ELISA And Pan-Filovirus MIA

Parameters	FANG ELISA	MIA
Reagents and consumables	More	Less
Assay time	More than 24hours (24hours+3hours 15minutes)	Less than 24hours (5-6hours maximum)
Number of samples	10 per microplate	46 per microplate
Quantity of sample	More sample for test (6ml)	Less sample for test (2ml)
Results	Single target results	Multi-target results
Cut off	well defined as 607 EU/mL	Cut-off can vary greatly based on the parameters chosen to define seroreactivity

Table 7: Comparison between FANG ELISA and MIA

III.2 DISCUSSION

The main objective of this study was to compare the gold-standard FANG ELISA and the pan-filovirus MIA in determining EBOV GP IgG antibody reactivity in rVSV-EBOV-GP Vaccinated individuals in Beni, DRC. In order to achieve this, we enrolled 617 participants from whom we collected blood samples before, 21 days and 6 months after vaccination. The main results show the prevalence of IgG Ab (1.5%) before vaccination, seroconversion of 87.2% (21 days) and 95.6% (6 months) after vaccination. The geometric mean Ab concentrations calculated in vaccinated participants without IgG Ab against the Ebola virus at baseline were 9 EU/ml before vaccination, 900 EU/ml and 1231 EU/ml 21 days and 6 months after vaccination (use of the rVSV-ZEBOV vaccine). Female gender and young age were sociodemographic factors associated with a good immune response 21 days and 6 months after the vaccine. No differences were observed between sex and occupation in seroreactivity post vaccination. Several authors have estimated that the use of rVSV-ZEBOV is one of the major strategies in the management of EVD epidemics because it induces Ab levels early from the 7th day and 14th day. This Ab rate increases very significantly 21 days and 6 months after use with vaccine effectiveness estimated at 100% in vaccinated people (Henao-restrepo *et al.,* n.d.; Huttner *et al.,* 2015; Richards *et al.,* 2018).

Moreover, of the samples used for both FANG and MIA, 68% were MIA seroreactive and FANG non-seroreactive, which may indicate that the MIA is a more sensitive assay in the

context of assessing vaccine-induced Ab responses and may therefore also be beneficial in determining the waning of immunity over time. This discordance in seroreactivity was particularly significant with samples collected at day-21 following rVSV-EBOV-GP vaccination. This variability in results between the FANG and MIA assays suggests that day 21 may, in fact, be more of an immunological turning point than a definitive time point at which all vaccinees have mounted an immune response.

Uniquely, these data shows that both the MIA and FANG assays are capable of reliably detecting anti-EBOV-GP IgG responses up to six months following vaccination. The MIA can assess reactivity of these samples and shows correlation with FANG results particularly for higher Ab titers. These data further suggest that there is higher detected seroreactivity at 6 months following vaccine-induced IgG production than at day 21 post immunization. This is aligned with previous findings which assert that an increased follow-up interval maybe beneficial for detecting the rVSV-EBOV-GP-elicited humoral immune response (Bratcher *et al.,* 2022; Shaffer *et al.,* 2022). These results also have implications for determining timelines for when administration of booster doses may be most beneficial at the population level.

The MIA is also unique in its design to detect multiple targets simultaneously. With this aspect of the MIA, samples deemed "sticky" or potentially compromised were identified by analyzing reported responses to other filoviral targets included in the panel. Using the definition of a "sticky" sample, ten extreme cases were identified where reactivity to BSA was greater than 10,000 MFI. When these samples were excluded from analysis, correlation was nominally improved between the assays. Of these ten samples, eight were also EBOV-GP Ab seroreactive by the FANG assay. Further investigation into the molecular or biological causes of the "sticky" sera, as well as codified definitions of samples that are multi-target reactive as opposed to "sticky" are important for improving MIA result interpretation. Additionally, as 8/10 samples were considered reactive on both assays and only identified as problematic due to the broader scope of the MIA, this underscores the value of a multiplex approach in identifying individuals which may potentially skew reactivity profiles and ultimately accuracy in serosurveillance. Ultimately, these analyses demonstrate that the MIA could be an appropriate alternative to the singleplex FANG in assessing immunological responses to EBOV GP across a geographical area, especially in a low resource setting. Notwithstanding, in choosing serological assessments, it is important to tailor assays for suitability to specific study context - the MIA is not a perfect match to the FANG standard, but it affords multi-target results, may be more sensitive to vaccine-induced Abs, and testing can be conducted entirely in a low and medium income

country (LMIC) such as DRC. However, FANG and MIA assay results are most correlated among “high-reactive” groups – those month 6 post-vaccination.

Again, from a logistical perspective, the MIA also uses less sample per test; an important consideration in the collection and transportation of samples from hard-to-reach populations. This in-country capacity of the MIA is yet another facet to consider in choosing assays to deploy in comparable LMICs or with expansion to filovirus hot zones for broader serosurveillance potential.

CHAPTER IV: CONCLUSION/PERSPECTIVES

IV.1 CONCLUSION

This study was aimed at comparing the gold-standard FANG ELISA and the pan-filovirus MIA in determining EBOV GP IgG antibody reactivity in rVSV-EBOV-GP Vaccinated individuals in Beni, DRC. On the basis of the results of our findings and discussions, we can therefore conclude that;

- FANG assays are capable of reliably detecting anti-EBOV-GP IgG responses up to six months following vaccination since the geometric mean concentrations of Ab calculated 6 months after vaccine use are close to those found by (Huttner et al., 2018):1837.9 EU/ml, 1600 EU/ml, 1359.8 EU/ml and1392.6 EU/ml.
- Seroreactivity was greater than 2967 MFI for MIA. Also, the Pan-filovirus MIA is cost effective than the gold-standard FANG ELISA and MIA could be an appropriate alternative to the singleplex FANG in assessing immunological responses to EBOV GP across a geographical area, especially in a low resource setting.
- 16 SOPs (02 SOPs for hygiene and biosafety; 05 SOPs for research documents and reception of participants; 03 SOPs for sample collection, transportation and storage; 03 SOPs for sample analysis with FANG and 03 SOPs for sample analysis with MIA) were produced and documented in a manual.

IV.2 STUDY LIMITATIONS

This study was carried out in the eastern part of DRC, precisely in Beni, North Kivu. In North Kivu, security constraints did not allow for a larger, randomized sampling in order to compare immunity within two types of populations. Also, the reduced sampling associated with the low participation rate during the various visits after vaccination would limit the generation of this result on the entire population. Moreover, there were on-going outbreaks during study period – thus also potentially increasing exposure.

In addition, there is a need for larger discussion on determination of cut-off for reactivity versus cutoff for indication post vaccination of elevated antibody response (4-fold difference) and it is worth noting that while seroreactivity can serve as a proxy for protection, there is still more to understand on how these correlate for better interpretation of results.

IV.3 PERSPECTIVES

This study was one of the first to evaluate immunity within the population vaccinated with the rVSV-ZEBOV vaccine during the EVD epidemic in the DRC.

- The results generated in this study constitute a significant supplement on the future of the immunity of this vaccine and can provide scientific arguments on the management of future epidemics of EVD. However, other studies are recommended to understand and complete the data from this study.
- We therefore look forward to carrying out a similar research in the near future not just comparing the serological methods but different populations across different geographical locations that have been affected with EVD.

REFERENCES

(PDF) Ebola virus disease_ Past, present and future. (n.d.).

Baize S., Leroy Km., Georges A.J., Georges Courbot M.C, Capron M., Bedjabaga L, Mavoungou E., L.-S. . 1. M. . (2002). lnllaml11atory responses in Ebola virus-infected patients. Clin. *Clinical and Experimental Immunology*, *128*(1), 163–168.

Balami, L. G., Ismail, S., S. M., S., & S. H., G. (2017). Ebola virus disease: epidemiology, clinical feature and the way forward. *International Journal Of Community Medicine And Public Health*, *4*(5), 1372. https://doi.org/10.18203/2394-6040.ijcmph20171744

Balmith, M., Faya, M., & Soliman, M. E. S. (2017). Ebola virus: A gap in drug design and discovery - experimental and computational perspective. *Chemical Biology and Drug Design*, *89*(3), 297–308. https://doi.org/10.1111/cbdd.12870

Barbiero, V. K. (2020). Ebola: A hyperinflated emergency. *Global Health Science and Practice*, *8*(2), 178–182. https://doi.org/10.9745/GHSP-D-19-00422

Barrette, R. W., Metwally, S. A., Rowland, J. M., Xu, L., Zaki, S. R., Nichol, S. T., Rollin, P. E., Towner, J. S., Shieh, W. J., Batten, B., Sealy, T. K., Carrillo, C., Moran, K. E., Bracht, A. J., Mayr, G. A., Sirios-Cruz, M., Catbagan, D. P., Lautner, E. A., Ksiazek, T. G., … Mcintosh, M. T. (2009). Discovery of swine as a host for the reston ebolavirus. *Science*, *325*(5937), 204–206. https://doi.org/10.1126/science.1172705

Baseler, L., Chertow, D. S., Johnson, K. M., Feldmann, H., & Morens, D. M. (2017). The Pathogenesis of Ebola Virus Disease∗. *Annual Review of Pathology: Mechanisms of Disease*, *12*, 387–418. https://doi.org/10.1146/annurev-pathol-052016-100506

Bausch, D. G., Towner, J. S., Dowell, S. F., Kaducu, F., Lukwiya, M., Sanchez, A., Nichol, S. T., Ksiazek, T. G., & Rollin, P. E. (2007). Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. In *Journal of Infectious Diseases* (Vol. 196, Issue SUPPL. 2). https://doi.org/10.1086/520545

Beeching, N. J., Fenech, M., & Houlihan, C. F. (2014). Ebola virus disease. *BMJ (Online)*, *349*(December), 1–15. https://doi.org/10.1136/bmj.g7348

Bell, B. P., Damon, I. K., Jernigan, D. B., Kenyon, T. A., Nichol, S. T., O'Connor, J. P., & Tappero, J. W. (2016). Overview, Control Strategies, and Lessons Learned in the CDC Response to the 2014-2016 Ebola Epidemic. In *MMWR supplements* (Vol. 65, Issue 3, pp. 4–11). https://doi.org/10.15585/mmwr.su6503a2

Bharat, T. A. M., Noda, T., Riches, J. D., Kraehling, V., Kolesnikova, L., Becker, S., Kawaoka, Y., & Briggs, J. A. G. (2012). Structural dissection of Ebola virus and its assembly determinants using cryo-electron tomography. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(11), 4275–4280. https://doi.org/10.1073/pnas.1120453109

Bhattacharyya, S., Hope, T. J., & Young, J. A. T. (2011). 基因的改变NIH Public Access. *Virology*, *419*(1), 1–9. https://doi.org/10.1016/j.virol.2011.07.018.Differential

Bramble, M. S., Hoff, N., Gilchuk, P., Mukadi, P., Lu, K., Doshi, R. H., Steffen, I.,

Nicholson, B. P., Lipson, A., Vashist, N., Sinai, C., Spencer, D., Olinger, G., Wemakoy, E. O., Illunga, B. K., Pettitt, J., Logue, J., Marchand, J., Varughese, J., … Cavet, G. (2018). *Pan-Filovirus Serum Neutralizing Antibodies in a Subset of Congolese Ebolavirus Infection Survivors*. *218*, 1929–1936. https://doi.org/10.1093/infdis/jiy453

Bratcher, A., Hoff, N. A., Doshi, R. H., Gadoth, A., Halbrook, M., Mukadi, P., Musene, K., Ilunga-Kebela, B., Spencer, D., Bramble, M. S., McIlwain, D., Kelly, J. D., Mukadi, D., Kingebeni, P. M., Ahuka, S., Okitolonda-Wemakoy, E., Muyembe-Tamfum, J. J., & Rimoin, A. W. (2022). Correction: Zoonotic risk factors associated with seroprevalence of Ebola virus GP antibodies in the absence of diagnosed Ebola virus disease in the Democratic Republic of Congo. *PLoS Neglected Tropical Diseases*, *16*(5), 10465. https://doi.org/10.1371/journal.pntd.0010465

Bray, M., & Geisbert, T. W. (2005). Ebola virus: The role of macrophages and dendritic cells in the pathogenesis of Ebola hemorrhagic fever. *International Journal of Biochemistry and Cell Biology*, *37*(8), 1560–1566. https://doi.org/10.1016/j.biocel.2005.02.018

Breman, J. G., Heymann, D. L., Lloyd, G., McCormick, J. B., Miatudila, M., Murphy, F. A., Muyembé-Tamfun, J. J., Piot, P., Ruppol, J. F., Sureau, P., Van Der Groen, G., & Johnson, K. M. (2016). Discovery and Description of Ebola Zaire Virus in 1976 and Relevance to the West African Epidemic during 2013-2016. *Journal of Infectious Diseases*, *214*(Suppl 3), S93–S101. https://doi.org/10.1093/infdis/jiw207

Brokowski C, A. M. (2019). HHS Public Access. *Physiology & Behavior*, *176*(5), 139–148. https://doi.org/10.1038/s41564-018-0227-2.Discovery

Bwaka, M. A., Bonnet, M. J., Calain, P., Colebunders, R., De Ann, R., Guimard, Y., Katwiki, K. R., Kibadi, K., Kipasa, M. A., Kuvula, K. J., Mapanda, B. B., Massamba, M., Mupapa, K. D., Muyembe-Tamfum, J. J., Ndaberey, E., Peters, C. J., Rollin, P. E., & Van Den Enden, E. (1999). Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: Clinical observations in 103 patients. *Journal of Infectious Diseases*, *179*(SUPPL. 1), 1–7. https://doi.org/10.1086/514308

Callendret, B., Vellinga, J., Wunderlich, K., Rodriguez, A., Steigerwald, R., Dirmeier, U., Cheminay, C., Volkmann, A., Brasel, T., Carrion, R., Giavedoni, L. D., Patterson, J. L., Mire, C. E., Geisbert, T. W., Hooper, J. W., Weijtens, M., Hartkoorn-Pasma, J., Custers, J., Pau, M. G., … Zahn, R. (2018). A prophylactic multivalent vaccine against different filovirus species is immunogenic and provides protection from lethal infections with Ebolavirus and Marburgvirus species in non-human primates. *PLoS ONE*, *13*(2), 1–24. https://doi.org/10.1371/journal.pone.0192312

Carette, J. E., Raaben, M., Wong, A. C., Herbert, A. S., Obernosterer, G., Mulherkar, N., Kuehne, A. I., Kranzusch, P. J., Griffin, A. M., Ruthel, G., Cin, P. D., Dye, J. M., Whelan, S. P., Chandran, K., & Brummelkamp, T. R. (2011). Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. *Nature*, *477*(7364), 340–343. https://doi.org/10.1038/nature10348

Center for Disease Control and Prevention. (2024). Outbreak History | Ebola | CDC. In *Outbreaks*. https://www.cdc.gov/ebola/outbreaks/index.html

Chen, J., He, Z., Yuan, Y., Huang, F., Luo, B., Zhang, J., Pan, T., Zhang, H., & Zhang, J. (2019). Host factor SMYD3 is recruited by Ebola virus nucleoprotein to facilitate viral mRNA transcription. *Emerging Microbes and Infections*, *8*(1), 1347–1360. https://doi.org/10.1080/22221751.2019.1662736

Coarsey, C. T., Esiobu, N., Narayanan, R., Pavlovic, M., Asghar, W., Science, C., Atlantic, F., Raton, B., States, U., Raton, B., Raton, B., States, U., States, U., & States, U. (2017). *HHS Public Access*. *43*(6), 779–798. https://doi.org/10.1080/1040841X.2017.1313814.Strategies

Coulborn, R. M., Bastard, M., Peyraud, N., Gignoux, E., Luquero, F., Guai, B., Bateyi Mustafa, S. H., Mukamba Musenga, E., & Ahuka-Mundeke, S. (2024). Case fatality risk among individuals vaccinated with rVSVΔG-ZEBOV-GP: a retrospective cohort analysis of patients with confirmed Ebola virus disease in the Democratic Republic of the Congo. *The Lancet Infectious Diseases*, *24*(6), 602–610. https://doi.org/10.1016/S1473-3099(23)00819-8

Dallatomasina, S., Crestani, R., Sylvester Squire, J., Declerk, H., Caleo, G. M., Wolz, A., Stinson, K., Patten, G., Brechard, R., Gbabai, O. B. M., Spreicher, A., Van Herp, M., & Zachariah, R. (2015). Ebola outbreak in rural West Africa: Epidemiology, clinical features and outcomes. *Tropical Medicine and International Health*, *20*(4), 448–454. https://doi.org/10.1111/tmi.12454

Den Boon, S., Marston, B. J., Nyenswah, T. G., Jambai, A., Barry, M., Keita, S., Durski, K., Senesie, S. S., Perkins, D., Shah, A., Green, H. H., Hamblion, E. L., Lamunu, M., Gasasira, A., Mahmoud, N. O., Djingarey, M. H., Morgan, O., Crozier, I., & Dye, C. (2019). Ebola virus infection associated with transmission from survivors. *Emerging Infectious Diseases*, *25*(2), 240–246. https://doi.org/10.3201/eid2502.181011

DeSilva, M. B., Styles, T., Basler, C., Moses, F. L., Husain, F., Reichler, M., Whitmer, S., McAuley, J., Belay, E., Friedman, M., Muoghalu, I. S., Swaray, P., Ströher, U., & Redd, J. T. (2019). Introduction of ebola virus into a remote border district of Sierra Leone, 2014: Use of field epidemiology and RNA sequencing to describe chains of transmission. *Epidemiology and Infection*, *147*. https://doi.org/10.1017/S0950268819000104

Diasorin | Molecular Diagnostics and Immunodiagnostic Specialist. (n.d.). https://int.diasorin.com/en

Dixon, M. G., Taylor, M. M., Dee, J., Hakim, A., Cantey, P., Lim, T., Bah, H., Camara, S. M., Ndongmo, C. B., Togba, M., Touré, L. Y., Bilivogui, P., Sylla, M., Kinzer, M., Coronado, F., Tongren, J. E., Swaminathan, M., Mandigny, L., Diallo, B., … Dahl, B. (2015). Contact tracing activities during the ebola virus disease epidemic in Kindia and Faranah, Guinea, 2014. *Emerging Infectious Diseases*, *21*(11), 2022–2028. https://doi.org/10.3201//eid2111.150684

Dong, S., Yang, P., Li, G., Liu, B., Wang, W., Liu, X., Xia, B., Yang, C., Lou, Z., Guo, Y., & Rao, Z. (2015). Insight into the Ebola virus nucleocapsid assembly mechanism: crystal structure of Ebola virus nucleoprotein core domain at 1.8 Å resolution. *Protein and Cell*, *6*(5), 351–362. https://doi.org/10.1007/s13238-015-0163-3

Dube, D., Brecher, M. B., Delos, S. E., Rose, S. C., Park, E. W., Schornberg, K. L., Kuhn, J. H., & White, J. M. (2009). The Primed Ebolavirus Glycoprotein (19-Kilodalton GP 1,2): Sequence and Residues Critical for Host Cell Binding . *Journal of Virology*, *83*(7), 2883–2891. https://doi.org/10.1128/jvi.01956-08

ebola_uganda_ppe_2022812f1e301cc9494d8838778c984a7267. (n.d.).

Ebola outbreak 2014-2016 - West Africa. (n.d.).

Empig, C. J., & Goldsmith, M. A. (2002). Association of the Caveola Vesicular System with Cellular Entry by Filoviruses. *Journal of Virology*, *76*(10), 5266–5270. https://doi.org/10.1128/jvi.76.10.5266-5270.2002

Escudero-Pérez, B., Ruibal, P., Rottstegge, M., Lödtke, A., Port, J. R., Hartmann, K., Gómez-Medina, S., Möller-Guhl, J., Nelson, E. V., Krasemann, S., Rodríguez, E., & Munoz-Fontela, C. (2019). Comparative pathogenesis of Ebola virus and Reston virus infection in humanized mice. *JCI Insight*, *4*(21), 1–12. https://doi.org/10.1172/jci.insight.126070

Fairley, J. K., Kozarsky, P. E., Kraft, C. S., Guarner, J., Steinberg, J. P., Anderson, E., Jacob, J. T., Meloy, P., Gillespie, D., Espinoza, T. R., Isakov, A., Vanairsdale, S., Baker, E., & Wu, H. M. (2016). Ebola or not? Evaluating the ill traveler from Ebola-affected countries in West Africa. *Open Forum Infectious Diseases*, *3*(1), 1–7. https://doi.org/10.1093/ofid/ofw005

Falasca, L., Agrati, C., Petrosillo, N., Di Caro, A., Capobianchi, M. R., Ippolito, G., & Piacentini, M. (2015). Molecular mechanisms of Ebola virus pathogenesis: Focus on cell death. *Cell Death and Differentiation*, *22*(8), 1250–1259. https://doi.org/10.1038/cdd.2015.67

Feldmann, H. and, & Geisbert, T. . (2011). Ebola haemorrhagic fever. Author Manuscript. *NIH Public Acess Lancet Author Manuscript*, *377*(9768), 849–862. https://doi.org/10.1016/S0140-6736(10)60667-8.Ebola

Feldmann, H., Feldmann, F., & Marzi, A. (2018). Ebola: Lessons on Vaccine Development. *Annual Review of Microbiology*, *72*, 423–446. https://doi.org/10.1146/annurev-micro-090817-062414

Feldmann, H., Jones, S., Klenk, H., & Schnittler, H. (2003). *Nri1154*. *3*(August).

Fowler, R. A., Fletcher, T., Fischer, W. A., Lamontagne, F., Jacob, S., Brett-Major, D., Lawler, J. V., Jacquerioz, F. A., Houlihan, C., O'Dempsey, T., Ferri, M., Adachi, T., Lamah, M. C., Bah, E. I., Mayet, T., Schieffelin, J., McLellan, S. L., Senga, M., Kato, Y., … Bausch, D. (2014). Caring for critically ill patients with Ebola virus disease: Perspectives from West Africa. *American Journal of Respiratory and Critical Care Medicine*, *190*(7), 733–737. https://doi.org/10.1164/rccm.201408-1514CP

Frontiers _ Designs and Characterization of Subunit Ebola GP Vaccine Candidates_ Implications for Immunogenicity. (n.d.).

Furuyama, W., & Marzi, A. (2019). Ebola Virus: Pathogenesis and Countermeasure Development. *Annual Review of Virology*, *6*, 435–458. https://doi.org/10.1146/annurev-virology-092818-015708

Geisbert, T. W., & Feldmann, H. (2011). Recombinant vesicular stomatitis virus-based vaccines against Ebola and marburg virus infections. *Journal of Infectious Diseases*, *204*(SUPPL. 3), 1075–1081. https://doi.org/10.1093/infdis/jir349

Geisbert, T. W., & Jahrling, P. B. (1995). Virus Research ELSEVIER Differentiation of filoviruses by electron microscopy 1. *Virus Research*, *39*, 129–150.

Gonzalez, J. P., Herbreteau, V., Morvan, J., & Leroy, É. M. (2005). Ebola virus circulation in Africa: A balance between clinical expression and epidemiological silence. *Bulletin de La Societe de Pathologie Exotique*, *98*(3), 210–217.

Gostin, L. O., & Friedman, E. A. (2015). A retrospective and prospective analysis of the west

African Ebola virus disease epidemic: Robust national health systems at the foundation and an empowered WHO at the apex. *The Lancet*, *385*(9980), 1902–1909. https://doi.org/10.1016/S0140-6736(15)60644-4

Grifoni, A., Lo Presti, A., Giovanetti, M., Montesano, C., Amicosante, M., Colizzi, V., Lai, A., Zehender, G., Cella, E., Angeletti, S., & Ciccozzi, M. (2016). Genetic diversity in Ebola virus: Phylogenetic and in silico structural studies of Ebola viral proteins. *Asian Pacific Journal of Tropical Medicine*, *9*(4), 337–343. https://doi.org/10.1016/j.apjtm.2016.03.016

Groseth, A., Feldmann, H., & Strong, J. E. (2007). The ecology of Ebola virus. *Trends in Microbiology*, *15*(9), 408–416. https://doi.org/10.1016/j.tim.2007.08.001

Hasan, S., Ahmad, S. A., Masood, R., & Saeed, S. (2019). *Ebola virus : A global public health menace : A narrative review*. https://doi.org/10.4103/jfmpc.jfmpc

Henao-restrepo, A. M., Camacho, A., Longini, I. M., Watson, C. H., Edmunds, W. J., Egger, M., Carroll, M. W., & Dean, N. E. (n.d.). *Effi cacy and eff ectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease : fi nal results from the Guinea ring vaccination , open-label , cluster-randomised trial (Ebola Ça Suffi t !)*. 505–518. https://doi.org/10.1016/S0140-6736(16)32621-6

Hoenen, T., Shabman, R. S., Groseth, A., Herwig, A., Weber, M., Schudt, G., Dolnik, O., Basler, C. F., Becker, S., & Feldmann, H. (2012). Inclusion Bodies Are a Site of Ebolavirus Replication. *Journal of Virology*, *86*(21), 11779–11788. https://doi.org/10.1128/jvi.01525-12

Hoff, N. A., Bratcher, A., Mukadi, P., Ahuka, S., Kabamba, M., Musene, K., Halbrook, M., Dzogong, C., Ngoie, G., Mbala, P., Kelly, J. D., Paul, J., Tambu, M., Kaba, D., Kebela-ilunga, B., Muyemebe-tamfum, J. J., & Rimoin, A. W. (2021). Increasing Ebola transmission behaviors 6 months post-vaccination : Comparing vaccinated and unvaccinated populations near 2018 Mbandaka Ebola outbreak in the Democratic Republic of Congo. *Vaccine*, *39*(51), 7464–7469. https://doi.org/10.1016/j.vaccine.2021.10.071

Hofmann-Winkler, H., Kaup, F., & Pöhlmann, S. (2012). Host cell factors in filovirus entry: Novel players, new insights. *Viruses*, *4*(12), 3336–3362. https://doi.org/10.3390/v4123336

Huttner, A., Agnandji, S. T., Combescure, C., Fernandes, J. F., Bache, E. B., Kabwende, L., Ndungu, F. M., Brosnahan, J., Monath, T. P., Lemaître, B., Grillet, S., Botto, M., Engler, O., Portmann, J., Siegrist, D., Bejon, P., Silvera, P., Kremsner, P., Siegrist, C. A., … ROTHENBERGER, S. (2018). Determinants of antibody persistence across doses and continents after single-dose rVSV-ZEBOV vaccination for Ebola virus disease: an observational cohort study. *The Lancet Infectious Diseases*, *18*(7), 738–748. https://doi.org/10.1016/S1473-3099(18)30165-8

Huttner, A., Dayer, J., Yerly, S., Combescure, C., Auderset, F., Desmeules, J., Eickmann, M., & Finckh, A. (2015). *The eff ect of dose on the safety and immunogenicity of the VSV Ebola candidate vaccine : a randomised double-blind , placebo-controlled phase 1 / 2 trial*. *15*(October). https://doi.org/10.1016/S1473-3099(15)00154-1

Id, M. S. A., Niemuth, N. A., Sabourin, C. L., Badorrek, S., Bounds, C. E., Rudge, T. L., & Id, J. (2020). *Interlaboratory comparison for the Filovirus Animal Nonclinical Group (*

FANG) anti-Ebola virus glycoprotein immunoglobulin G enzyme- linked immunosorbent assay. *10*, 1–15. https://doi.org/10.1371/journal.pone.0238196

Identification of a small molecule inhibitor of Ebola virus genome. (n.d.).

Ikegami, T., Calaor, A. B., Miranda, M. E., Niikura, M., Saijo, M., Kurane, I., Yoshikawa, Y., & Morikawa, S. (2001). Genome structure of Ebola virus subtype Reston: Differences among Ebola subtypes. In *Archives of Virology* (Vol. 146, Issue 10, pp. 2021–2027). https://doi.org/10.1007/s007050170049

Infection of ebola virus - microbewiki. (n.d.).

Jain, S., Martynova, E., Rizvanov, A., Khaiboullina, S., & Baranwal, M. (2021). *Structural and Functional Aspects of Ebola Virus Proteins*. 1–29.

Joob, B., & Wiwanitkit, V. (2014). Ebola virus 2014 and pregnancy. *Asian Pacific Journal of Reproduction*, *3*(4), 320–321. https://doi.org/10.1016/S2305-0500(14)60047-6

Judson, S., Prescott, J., & Munster, V. (2015). Understanding Ebola virus transmission. *Viruses*, *7*(2), 511–521. https://doi.org/10.3390/v7020511

KANAAN AL-TAMEEMI, & RAIAAN KABAKLI. (2019). Ebola Virus: an Overview Disease and Treatment. *Asian Journal of Pharmaceutical and Clinical Research*, *12*(10), 57–62. https://doi.org/10.22159/ajpcr.2019.v12i10.35005

Katz, L. M., & Tobian, A. A. R. (2014). Ebola virus disease, transmission risk to laboratory personnel, and pretransfusion testing. *Transfusion*, *54*(12), 3247–3251. https://doi.org/10.1111/trf.12913

Kaur, G., Sachdeva, S., Jha, D., & Sulania, A. (2017). *Ebola virus disease in the light of epidemiological triad*. *January*. https://doi.org/10.4103/1119-0388.198096

Kelly, J. D., Wannier, R., Sinai, C., Moe, C. A., Hoff, N. A., Blumberg, S., Selo, B., Mossoko, M., Chowell-puente, G., Jones, J. H., Okitolonda-wemakoy, E., Rutherford, G. W., Lietman, T. M., Muyembe-tamfum, J. J., Rimoin, A. W., Porco, T. C., & Richardson, E. T. (2021). *The Impact of Different Types of Violence on Ebola Virus Transmission During the 2018 – 2020 Outbreak in the Democratic Republic of the Congo*. *94158*, 2021–2029. https://doi.org/10.1093/infdis/jiaa163

Kilgore, P. E., Grabenstein, J. D., Salim, A. M., & Rybak, M. (2015). Treatment of Ebola virus disease. *Pharmacotherapy*, *35*(1), 43–53. https://doi.org/10.1002/phar.1545

Kortepeter, M. G., Bausch, D. G., & Bray, M. (2011). Basic clinical and Laboratory features of filoviral hemorrhagic fever. *Journal of Infectious Diseases*, *204*(SUPPL. 3). https://doi.org/10.1093/infdis/jir299

Kruse, T., Biedenkopf, N., Hertz, E. P. T., Dietzel, E., Stalmann, G., López-Méndez, B., Davey, N. E., Nilsson, J., & Becker, S. (2018). The Ebola Virus Nucleoprotein Recruits the Host PP2A-B56 Phosphatase to Activate Transcriptional Support Activity of VP30. *Molecular Cell*, *69*(1), 136-145.e6. https://doi.org/10.1016/j.molcel.2017.11.034

Leligdowicz, A., Fischer, W. A., Uyeki, T. M., Fletcher, T. E., Adhikari, N. K. J., Portella, G., Lamontagne, F., Clement, C., Jacob, S. T., Rubinson, L., Vanderschuren, A., Hajek, J., Murthy, S., Crozier, I., Ibrahima, E., Lamah, M. C., Schieffelin, J. S., Brett-Major, D., Bausch, D. G., … Fowler, R. A. (2016). Ebola virus disease and critical illness. *Critical Care*, *20*(1). https://doi.org/10.1186/s13054-016-1325-2

Leroy, E. M., Baize, S., Volchkov, V. E., Fisher-Hoch, S. P., Georges-Courbot, M. C., Lansoud-Soukate, J., Capron, M., Debré, P., McCormick, J. B., & Georges, A. J. (2000). Human asymptomatic Ebola infection and strong inflammatory response. *Lancet*, *355*(9222), 2210–2215. https://doi.org/10.1016/S0140-6736(00)02405-3

Leroy, E. M., Kumulungui, B., Pourrut, X., Rouquet, P., Hassanin, A., Yaba, P., Délicat, A., Paweska, J. T., Gonzalez, J. P., & Swanepoel, R. (2005). Fruit bats as reservoirs of Ebola virus. *Nature*, *438*(7068), 575–576. https://doi.org/10.1038/438575a

Logue, J., Tuznik, K., Follmann, D., Grandits, G., Marchand, J., Reilly, C., Sarro, S., Pettitt, J., Stavale, E. J., Fallah, M., Olinger, G. G., Bolay, F. K., & Hensley, L. E. (2018). *Use of the Filovirus Animal Non-Clinical Group (FANG) Ebola virus immuno-assay requires fewer study participants to power a study than the Alpha Diagnostic International assay*. 84–90. https://doi.org/10.1016/j.jviromet.2018.02.018.Use

Luo, D., Zheng, R., Wang, D., Zhang, X., Yin, Y., Wang, K., & Wang, W. (2019). Effect of sexual transmission on the West Africa Ebola outbreak in 2014 : a mathematical modelling study. *Scientific Reports*, *December 2018*, 1–11. https://doi.org/10.1038/s41598-018-38397-3

Lyon, G. M., Mehta, A. K., Varkey, J. B., Brantly, K., Plyler, L., McElroy, A. K., Kraft, C. S., Towner, J. S., Spiropoulou, C., Ströher, U., Uyeki, T. M., & Ribner, B. S. (2014). Clinical Care of Two Patients with Ebola Virus Disease in the United States. *New England Journal of Medicine*, *371*(25), 2402–2409. https://doi.org/10.1056/nejmoa1409838

MAGPIX® System _ Multiplex Analyzer by Diasorin. (n.d.).

Manno, D., Ayieko, P., Ishola, D., Afolabi, M. O., Rogers, B., Baiden, F., Serry-bangura, A., Bah, O. M., Köhn, B., Swaray, I., Owusu-kyei, K., Otieno, G. T., Kowuor, D., Tindanbil, D., Smout, E., Robinson, C., Keshinro, B., Foster, J., Gallagher, K., … Watson-jones, D. (2022). *Ebola Virus Glycoprotein IgG Seroprevalence in Community Previously Aff ected by Ebola , Sierra Leone*. *28*(3), 0–4.

Manno, D., Bangura, A., Baiden, F., Kamara, A. B., Ayieko, P., Kallon, J., Foster, J., Conteh, M., Connor, N. E., Koroma, B., Njie, Y., Borboh, P., Keshinro, B., Lawal, B. J., Kroma, M. T., Otieno, G. T., Deen, A. T., Choi, E. M. L., Balami, A. D., … Watson-Jones, D. (2023). Safety and immunogenicity of an Ad26.ZEBOV booster dose in children previously vaccinated with the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen: an open-label, non-randomised, phase 2 trial. *The Lancet Infectious Diseases*, *23*(3), 352–360. https://doi.org/10.1016/S1473-3099(22)00594-1

Martines, R. B., Ng, D. L., Greer, P. W., Rollin, P. E., & Zaki, S. R. (2015). Tissue and cellular tropism, pathology and pathogenesis of Ebola and Marburg viruses. *Journal of Pathology*, *235*(2), 153–174. https://doi.org/10.1002/path.4456

Martinez, O., Johnson, J., Manicassamy, B., Rong, L., Olinger, G. G., Hensley, L. E., & Basler, C. F. (2010). Zaire Ebola virus entry into human dendritic cells is insensitive to cathepsin L inhibition. *Cellular Microbiology*, *12*(2), 148–157. https://doi.org/10.1111/j.1462-5822.2009.01385.x

Matanock MD, A., Arwady MD, M. A., Ayscue DVM, P., Forrester MD, J. D., Gaddis MPH, B., Hunter DrPH, J. C., Monroe MPH, B., Pillai MD, S. K., Reed MD, C., Schafer DVM, I. J., Massaquoi MD, M., Dahn MD, B., & De Cock MD, K. M.

(2014). Ebola Virus Disease Cases Among Health Care Workers Not Working in Ebola Treatment Units - Liberia, June-August, 2014. In *MMWR. Morbidity and Mortality Weekly Report* (Vol. 63, Issue 46, pp. 1077–1081). http://search.proquest.com/docview/1628558631?accountid=15292%5Cnhttp://sfx.cbuc.cat/uab?url_ver=Z39.88-2004&rft_val_fmt=info:ofi/fmt:kev:mtx:book&genre=unknown&sid=ProQ:ProQuest+Health+%26+Medical+Complete&atitle=&title=Ebola+Virus+Disease+Cases+Among+He

McElroy, A. K., Akondy, R. S., Davis, C. W., Ellebedy, A. H., Mehta, A. K., Kraft, C. S., Lyon, G. M., Ribner, B. S., Varkey, J., Sidney, J., Sette, A., Campbell, S., Ströher, U., Damon, I., Nichol, S. T., Spiropoulou, C. F., & Ahmed, R. (2015). Human Ebola virus infection results in substantial immune activation. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(15), 4719–4724. https://doi.org/10.1073/pnas.1502619112

Mclean, C., Barry, H., Kieh, M., Anywaine, Z., Rogers, T., Doumbia, S., & Sirima, S. B. (2023). Articles Immune response of a two-dose heterologous Ebola vaccine regimen : summary of three African clinical trials using a single validated Filovirus Animal Nonclinical Group enzyme-linked immunosorbent assay in a single accredited laboratory. *EBioMedicine*, *91*, 104562. https://doi.org/10.1016/j.ebiom.2023.104562

McLean, C., Dijkman, K., Gaddah, A., Keshinro, B., Katwere, M., Douoguih, M., Robinson, C., Solforosi, L., Czapska-Casey, D., Dekking, L., Wollmann, Y., Volkmann, A., Pau, M. G., Callendret, B., Sadoff, J., Schuitemaker, H., Zahn, R., Luhn, K., Hendriks, J., & Roozendaal, R. (2023). Persistence of immunological memory as a potential correlate of long-term, vaccine-induced protection against Ebola virus disease in humans. *Frontiers in Immunology*, *14*(September), 1–14. https://doi.org/10.3389/fimmu.2023.1215302

Mehedi, M., Falzarano, D., Seebach, J., Hu, X., Carpenter, M. S., Schnittler, H.-J., & Feldmann, H. (2011). A New Ebola Virus Nonstructural Glycoprotein Expressed through RNA Editing. *Journal of Virology*, *85*(11), 5406–5414. https://doi.org/10.1128/jvi.02190-10

Meyers, L., Frawley, T., Goss, S., & Kang, C. (2015). Ebola virus outbreak 2014: Clinical review for emergency physicians. *Annals of Emergency Medicine*, *65*(1), 101–108. https://doi.org/10.1016/j.annemergmed.2014.10.009

Moghadam, S. R. J., Omidi, N., Bayrami, S., Moghadam, S. J., & SeyedAlinaghi, S. A. (2015). Ebola viral disease: A review literature. *Asian Pacific Journal of Tropical Biomedicine*, *5*(4), 260–267. https://doi.org/10.1016/S2221-1691(15)30341-5

Mutua, G., Anzala, O., Luhn, K., Robinson, C., Bockstal, V., Anumendem, D., & Douoguih, M. (2019). Safety and Immunogenicity of a 2-Dose Heterologous Vaccine Regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola Vaccines: 12-Month Data from a Phase 1 Randomized Clinical Trial in Nairobi, Kenya. *Journal of Infectious Diseases*, *220*(1), 57–67. https://doi.org/10.1093/infdis/jiz071

Orthoebolavirus ~ ViralZone. (n.d.).

Paladino, L., Sharpe, R. P., Galwankar, S. C., Sholevar, F., Marchionni, C., Papadimos, T. J., Paul, E., Hansoti, B., Firstenberg, M., Garg, M., Watson, M., Baxter, R. A., & Stawicki, S. P. (2017). Reflections on the ebola public health emergency of international concern, part 2: The unseen epidemic of posttraumatic stress among health-care personnel and

survivors of the 2014-2016 Ebola outbreak. *Journal of Global Infectious Diseases*, *9*(2), 45–50. https://doi.org/10.4103/jgid.jgid_24_17

Petti, S., Protano, C., Messano, G. A., & Scully, C. (2016). Ebola virus infection among western healthcare workers unable to recall the transmission route. *BioMed Research International*, *2016*(November 1976). https://doi.org/10.1155/2016/8054709

Pinski, A. N., & Messaoudi, I. (2020). To B or Not to B : Mechanisms of Protection Conferred by rVSV-EBOV-GP and the Roles of Innate and Adaptive Immunity. *Microorganisms*, *8*(1473), 1–24.

Prescott, J. B., Marzi, A., Safronetz, D., Robertson, S. J., Feldmann, H., & Best, S. M. (2017). Immunobiology of Ebola and Lassa virus infections. *Nature Reviews Immunology*, *17*(3), 195–207. https://doi.org/10.1038/nri.2016.138

Quattrochi, J. P., Malemo, L., & Niehuus, R. (2023). Impact of the 2018-2020 Democratic Republic of Congo Ebola epidemic on health system utilization and health outcomes. *Journal of Global Health Reports*, *7*, 1–14. https://doi.org/10.29392/001c.88108

Quick, J., Loman, N. J., Duraffour, S., Simpson, J. T., Severi, E., Cowley, L., Bore, J. A., Koundouno, R., Dudas, G., Mikhail, A., Ouédraogo, N., Afrough, B., Bah, A., & Carrol, M. W. (2016). Europe PMC Funders Group Real-time , portable genome sequencing for Ebola surveillance. *Nature*, *530*(7589), 228–232. https://doi.org/10.1038/nature16996.Real-time

Receptor, M. G. (n.d.). *Structural Rearrangement and*.

Reichart, P. A., Gelderblom, H. R., Khongkhunthian, P., & Schmidt-Westhausen, A. (2016). Ebola virus disease: any risk for oral and maxillo-facial surgery? An overview. *Oral and Maxillofacial Surgery*, *20*(2), 111–114. https://doi.org/10.1007/s10006-015-0542-1

Rewar, S., & Mirdha, D. (2014). Transmission of Ebola virus disease: An overview. *Annals of Global Health*, *80*(6), 444–451. https://doi.org/10.1016/j.aogh.2015.02.005

Richards et al. (2018). A recombinant. *Physiology & Behavior*, *176*(5), 139–148. https://doi.org/10.1056/NEJMoa1414216.A

Rimoin, A. W., Lu, K., Bramble, M. S., Steffen, I., Doshi, R. H., Hoff, N. A., Mukadi, P., Nicholson, B. P., Alfonso, V. H., Olinger, G., Sinai, C., Yamamoto, L. K., Ramirez, C. M., Wemakoy, E. O., Illunga, B. K., Pettitt, J., Logue, J., Bennett, R. S., Jahrling, P., … Simmons, G. (2018). *Ebola Virus Neutralizing Antibodies Detectable in Survivors of the Yambuku , Zaire Outbreak 40 Years after Infection*. *217*, 223–231. https://doi.org/10.1093/infdis/jix584

Rivera, A., & Messaoudi, I. (2020). *HHS Public Access*. *1*(5), 186–197. https://doi.org/10.1021/id5000426.Pathophysiology

Rudge, T. L., Sankovich, K. A., Niemuth, N. A., Anderson, M. S., Badorrek, C. S., Skomrock, N. D., Cirimotich, C. M., & Sabourin, C. L. (2019). Development, qualification, and validation of the Filovirus Animal Nonclinical Group anti-Ebola virus glycoprotein immunoglobulin G enzyme-linked immunosorbent assay for human serum samples. *PLoS ONE*, *14*(4), 1–28. https://doi.org/10.1371/journal.pone.0215457

Rugarabamu, S., Mboera, L., Rweyemamu, M., Mwanyika, G., Lutwama, J., Paweska, J., & Misinzo, G. (2020). *two years of responding to Ebola virus outbreaks in Sub- - Saharan Africa : a review*. 1–10. https://doi.org/10.1136/bmjgh-2019-001955

Samaranayake, L., Scully, C., Nair, R. G., & Petti, S. (2015). Viral haemorrhagic fevers with emphasis on Ebola virus disease and oro-dental healthcare. *Oral Diseases*, *21*(1), 1–6. https://doi.org/10.1111/odi.12298

Satterly, N. G., Voorhees, M. A., Ames, A. D., & Schoepp, R. J. (2017). Comparison of MagPix assays and enzyme-linked immunosorbent assay for detection of hemorrhagic fever viruses. *Journal of Clinical Microbiology*, *55*(1), 68–78. https://doi.org/10.1128/JCM.01693-16

Schindell, B. G., Webb, A. L., & Kindrachuk, J. (2018). Persistence and sexual transmission of filoviruses. *Viruses*, *10*(12). https://doi.org/10.3390/v10120683

Schudt, G., Dolnik, O., Kolesnikova, L., Biedenkopf, N., Herwig, A., & Becker, S. (2015). Transport of Ebolavirus Nucleocapsids Is Dependent on Actin Polymerization: Live-Cell Imaging Analysis of Ebolavirus-Infected Cells. *Journal of Infectious Diseases*, *212*, S160–S166. https://doi.org/10.1093/infdis/jiv083

Shaffer, K. C. L., Hui, S., Id, A. B., Id, L. B. K., Mutombe, R., Kavira, N., Kompany, J. P., Tambu, M., Musene, K., Id, P. M., Mbala, P., Id, A. G., West, B. R., Ilunga, K., Kaba, D., Muyembe-tanfum, J. J., Hoff, N. A., Id, W. R., Ollmann, E., & Id, S. (2022). *PLOS NEGLECTED TROPICAL DISEASES Pan-ebolavirus serology study of healthcare workers in the Mbandaka Health Region , Democratic Republic of the Congo*. 1–15. https://doi.org/10.1371/journal.pntd.0010167

Shorten, R. J., Brown, C. S., Jacobs, M., Rattenbury, S., Simpson, A. J., & Mepham, S. (2016). Diagnostics in Ebola Virus Disease in Resource-Rich and Resource-Limited Settings. *PLoS Neglected Tropical Diseases*, *10*(10), 1–16. https://doi.org/10.1371/journal.pntd.0004948

Singh, R. K., Dhama, K., Malik, Y. S., Ramakrishnan, M. A., Karthik, K., Khandia, R., Tiwari, R., Munjal, A., Saminathan, M., Sachan, S., Desingu, P. A., Kattoor, J. J., Iqbal, H. M. N., & Joshi, S. K. (2017). Ebola virus - Epidemiology, diagnosis, and control: Threat to humans, lessons learnt, and preparedness plans - An update on its 40 year's journey. *Veterinary Quarterly*, *37*(1), 98–135. https://doi.org/10.1080/01652176.2017.1309474

Ste, I., Lu, K., Ho, N. A., Mulembakani, P., & Okitolonda, E. (2020). *Seroreactivity against Marburg or related fi loviruses in West and Central Africa*. *9*, 0–4.

Sun, Y., Guo, Y., & Lou, Z. (2012). A versatile building block: the structures and functions of negative-sense single-stranded RNA virus nucleocapsid proteins. *Protein & Cell*, *3*(12), 893–902. https://doi.org/10.1007/s13238-012-2087-5

Takamatsu, Y., Kolesnikova, L., & Becker, S. (2018). Ebola virus proteins NP, VP35, and VP24 are essential and sufficient to mediate nucleocapsid transport. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(5), 1075–1080. https://doi.org/10.1073/pnas.1712263115

Taylor, K. L., Lanning, L., Wolfraim, L., Shrivastava Gales, S., Sico, C., Dowling, W. E., Ward, L. A., Florence, W. C., Nuzum, E., & Bryant, P. R. (2022). A U.S. Government-Coordinated Effort to Leverage Non-Human Primate Data to Facilitate Ebolavirus Vaccine Development. *Vaccines*, *10*(8), 4–9. https://doi.org/10.3390/vaccines10081201

Towner, J. S., Sealy, T. K., Khristova, M. L., Albariño, C. G., Conlan, S., Reeder, S. A., Quan, P. L., Lipkin, W. I., Downing, R., Tappero, J. W., Okware, S., Lutwama, J.,

Bakamutumaho, B., Kayiwa, J., Comer, J. A., Rollin, P. E., Ksiazek, T. G., & Nichol, S. T. (2008). Newly discovered Ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathogens*, *4*(11), 3–8. https://doi.org/10.1371/journal.ppat.1000212

V, V., V, V., S, W., K, H.-D., & F, H. (1998). Release of Viral Glycoproteins during Ebola virus Infection. In *Virology* (Vol. 245, pp. 110–119).

Valmas, C., Grosch, M. N., Schümann, M., Olejnik, J., Martinez, O., Best, S. M., Krähling, V., Basler, C. F., & Mühlberger, E. (2010). Marburg virus evades interferon responses by a mechanism distinct from Ebola virus. *PLoS Pathogens*, *6*(1). https://doi.org/10.1371/journal.ppat.1000721

Wauquier, N., Becquart, P., Padilla, C., Baize, S., & Leroy, E. M. (2010). Human fatal zaire ebola virus infection is associated with an aberrant innate immunity and with massive lymphocyte apoptosis. *PLoS Neglected Tropical Diseases*, *4*(10). https://doi.org/10.1371/journal.pntd.0000837

Wikipedia: Western African Ebola virus epidemic. (n.d.). https://en.wikipedia.org/wiki/Western_African_Ebola_virus_epidemic

Wilkinson, A., Parker, M., Martineau, F., & Leach, M. (2017). Engaging 'communities': Anthropological insights from the west African ebola epidemic. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1721). https://doi.org/10.1098/rstb.2016.0305

Wirsiy, F. S., Ako-Arrey, D. E., Nkfusai, C. N., Yeika, E. V., & Bain, L. E. (2021). Marburg virus disease outbreak in guinea: A spin framework of its transmission and control measures for an exemplary response pattern in West Africa. *Pan African Medical Journal*, *40*. https://doi.org/10.11604/pamj.2021.40.143.31709

World Health Organization. (2020). Ebola outbreak 2018-2020- North Kivu-Ituri. In *Situation*. https://www.who.int/emergencies/situations/Ebola-2019-drc-

Xu, D., Powell, A. E., Utz, A., Sanyal, M., Do, J., Patten, J. J., Moliva, J. I., Sullivan, N. J., Davey, R. A., & Kim, P. S. (2024). Design of universal Ebola virus vaccine candidates via immunofocusing. *Proceedings of the National Academy of Sciences of the United States of America*, *121*(7). https://doi.org/10.1073/pnas.2316960121

Yang, X., Wang, X., Song, Y., Zhou, P., Li, D., Zhang, C., Jin, X., Huang, Z., & Zhou, D. (2019). Chimpanzee adenoviral vector prime-boost regimen elicits potent immune responses against Ebola virus in mice and rhesus macaques. *Emerging Microbes and Infections*, *8*(1), 1086–1097. https://doi.org/10.1080/22221751.2019.1644968

APPENDICES

APPENDIX 1: ETHICAL CLEARANCE

ECOLE DE SANTE PUBLIQUE
Université de Kinshasa
Ministère de l'Enseignement Supérieur et Universitaire
République Démocratique du Congo
COMITE D'ETHIQUE

No d'Approbation: ESP/CE/022..2017

Kinshasa, le 14 avril 2017

A Monsieur le Prof. Dr Muyembe Tamfum
Investigateur Principal
INRB
République Démocratique du Congo

Concerne : Décision du Comité d'éthique portant sur l'étude intitulée : « *Epidémiologie, Immuno pathologie et Immunogénétique des séquelles des infections à virus Ebola et autres fièvres hémorragiques virales* ».

Monsieur l'Investigateur,

Le Comité d'Ethique de l'Ecole de Santé Publique de l'Université de Kinshasa a bien reçu le protocole dont le titre est repris en marge.

Après examen du protocole selon les normes d'éthique nationales sur les études impliquant les êtres humains, le Comité a donné un avis favorable à cette recherche et autorise sa mise en œuvre pour la période allant du 14 avril 2017 au 13 avril 2018.

Veuillez agréer, Monsieur l'Investigateur, l'expression de nos sentiments les meilleurs.

Prof. BONGOPASI MOKE SANGOL

Vice Président du Comité Ethique

ESP UNIKIN COMITE D'ETHIQUE

Université de Kinshasa, Ecole de Santé Publique : B.P 11850 Kin I.

APPENDIX 2: CONSENT TO PARTICIPANTS

Consent to Participate in a Research Study

Adult Participants (18 years and older)

Co-Investigators: Emile Okitolonda-Wemakoy, MD, PhD, Kinshasa School of Public Health; Jean Jacques Muyembe-Tamfum, MD, PhD, National Institute of Biomedical Research; Benoit Kebela Ilunga, MD, Division of Disease Control, Anne W. Rimoin, PhD, UCLA School of Public Health;

Sponsors: Bill and Melinda Gates Foundation, Seattle, Washington, USA
U.S. Food and Drug Administration, Washington DC, USA
Stanford University, Palo Alto, California, USA
Faucett Catalyst Fund, Los Angeles, California, USA

Study Title: Epidemiology, Immunopathology, Immunogenetics and Sequelae of Ebola Virus and other Viral Hemorrhagic Fever Infections

Version: 5

Version Date: August 3, 2018

Instructions: This Consent Form will be available in French, and other local languages. It should be read to the adults who are 18 years or older and are eligible to join the study. This form should be read in the language that he/she understands best.

Introduction

Hello, I am _____, working with a team of people from the Democratic Republic of Congo and the United States. We are doing research on Ebola virus and other viral hemorrhagic fevers, which has caused several epidemics in this country. I am going to give you information about our study and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research. If you do choose to participate in our

study, you can expect your participation to last no more than two hours today and we may ask to meet with you again over the next two years with up to 5 visits for follow-up which could include additional questions and sample collection.
There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask me, the study doctor, nurses or staff.

I am now going to tell you about the study and what will happen if you decide to participate. This will take about 40 minutes. Once you understand the study, if you decide to take part, you will be asked to sign this consent form.

Why is this study being done?

Ebola virus is a very dangerous disease. It causes a hemorrhagic fever called Ebola virus disease. "Hemorrhagic" means that the victim will bleed a lot, inside and outside their body. Out of every 10 people who get Ebola, on average five to nine die. There is no cure for Ebola, but if people get care quickly from doctors and nurses at a hospital, more of them live. Scientists have not yet found a cure for Ebola, but efforts are underway. There are also other diseases that can cause the same symptoms as Ebola such as Marburg Virus or Bas-Congo Virus that are called “Viral Hemorrhagic Fevers (VHF). These viruses also circulate here in the Democratic Republic of the Congo.
We are interested in answering several questions about Ebola and other VHFs including: 1) How many people get these infections without showing any symptoms)? 2) What happens to a person’s health following infection with one or more viral hemorrhagic fevers (with or without symptoms)? 3) How does the health of people infected with these viruses compare to the health of people who have never been exposed to them?

We are also interested in learning more about what your body may do after infection with Ebola virus or another VHF. If you had Ebola or another VHF or had close contact with somebody infected with Ebola or another VHF while they were sick, you may have antibodies to this disease. Antibodies are made in your body when it thinks something is trying to hurt it. These antibodies can also stop future infection with Ebola virus or another VHF. In this research study, the researchers will collect blood samples for the purposes of finding and characterizing antibodies to try to find out why antibodies are sometimes helpful to the body, sometimes do nothing, and sometimes are harmful. We will study those who were sick because of Ebola or another VHF, those who had contact (family, friends, health care workers) with someone who

was sick, and those who never had Ebola or another VHF or had contact with anyone who had Ebola or another VHF. We will also examine whether the cells in your blood can be used to help other people with Ebola or other VHFs. If they can, we may try to change these cells so that they can live indefinitely for this purpose.

This information will help us find better ways to prevent and treat these diseases in the future.

Participant selection

We are inviting all adults (18 years and older) to participate in the research study. We are interested in people who have become sick because of Ebolavirus or other VHFs, those who were in close contact or provided care to people with Ebolavirus or other VHFs, and also those who were not sick and did not come in contact with anyone who was sick with Ebolavirus or other VHFs in the Democratic Republic of Congo.

The research team is asking you to be in this study because you are a healthy person over the age of 18 who may have knowingly or unknowingly been exposed to the diseases we would like to study, even if you have never been sick.

Question to determine if the participant understands: *Do you know why we are asking you to take part in this study? Do you know what the study is about?*

Question to determine if the participant understands: *Do you know that you do not have to take part in this research study, if you do not wish to? Do you have any questions?*

What will happen if I agree to participate in this study?

If you agree to be part of this study, your first visit will take about two (2) hours. Depending on your test results, we may ask you for additional information and blood samples. To identify you, we will use numbers instead of your name. These numbers will be placed on all of your forms and blood samples so that no one other than our study staff will know which forms and blood samples are yours.

During the first visit:

- You will be given a questionnaire that will ask you to provide detailed information about your history, daily life and activities, your family, and your medical history. You might get bored or tired answering these questions. You have the right to refuse to answer any question that you may not wish to answer.

- You will also be asked to have a physical examination, which includes taking information such as how tall you are, how much you weigh, your blood pressure, your general health status including ocular and neurologic examinations.
- We will then collect a small amount of blood (approximately 10 mL) from your arm.
- We may also take a thumbprint so we can identify you if we need to contact you for a follow-up visit.

We will send these blood samples to a laboratory in Kinshasa or in the United States that will test the samples to see if you have ever had or been potentially exposed to Ebola or other VHFs. We are not providing any treatment or other type of intervention in this study. You will not receive the results of these tests because they are for research purposes only and are not appropriate for use in health care decisions.

If you are contacted for follow-up visits (3 days to two years after the first visit):

- We will ask you to provide us with an additional blood sample (approximately 10mL to 50 mL of blood from the arm). You may also be asked some additional questions.
- We may test the genetic information of your blood for the types of antibodies being produced. The results of this analysis will be used for research purposes only and will not be shared with you, your representatives, or your health care providers. The results from your blood samples will not be used to make decisions about your health care.

How long will I be in this study?

The research will take place over one (1) day for the initial visit and will require about three (3) hours of your time. If you are contacted for a follow-up visit, it will take place over the course of up to 5 additional visits between 3 days and two years after the initial visit. The follow-up visits will take about 30 minutes each time,

Question to determine if the participant understands: *Can you tell me how much blood will be taken from your veins, using a syringe and needle during the first visit? Do you have any other questions? Do you want me to go through the procedures again?*

What kind of risks or discomforts could I expect?

By participating in this research, it is possible that you will be at greater risk than you would otherwise be. If you take part in this study, you will be asked to give blood. Drawing blood

may cause pain, bleeding, swelling, and rarely, infection at the place where the blood is taken. Sometimes, drawing blood causes people to feel lightheaded or faint.

While the possibility of this happening is very low, you should still be aware of the possibility. We will try to decrease the chances of this event occurring, but if something unexpected happens, we will provide you with treatment at no cost.

Are there any benefits if I participate?

There is no direct benefit to you, but your participation is likely to help us find the answer to the research question. In addition, you and others may benefit in the future from the information that will be learned from the study. You will be helping us find out more about long-term effects of Ebolavirus and other VHFs. This information may help to find better methods of preventing and treating Ebola and other diseases in the future.

Will I be paid for my participation?

We will provide a small gift plus the cost of travel if necessary. You will not be given any other money or gifts to take part in this research. If we need to take additional blood samples during follow-up visits, we will provide you with a small gift plus the cost of travel if necessary. If you are contacted to take part in participation which includes in depth analysis, you may receive up to $10 reimbursement for your time plus the cost of travel if necessary and provide you with drinks and a snack after you have given your sample.

It will not cost you anything to take part in this study. The program will pay for the cost of any testing that is done for this study.

Question to determine if the participant understands: *Can you tell me if you have understood correctly the benefits that you will have if you take part in the study? Can you tell me if you will receive this gift if you chose not to participate in this study? Do you have any other questions?*

How will information about me and my participation be kept confidential?

With this research, something out of the ordinary is being done in your community. It is possible that if others in the community are aware that you are participating, they may ask you questions. We will not be sharing the identity of those participating in the research.

The researchers will make every attempt to protect your confidentiality and to make sure that your personal identity does not become known. This signed consent form will be stored in a locked file that will be accessible only to a very small number of authorized people involved in

this project. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key or using encrypted software on the computer. It will not be shared with or given to anyone except the research sponsors and collaborating institutes who will be testing the blood samples. Your name will only be used to contact you for future studies if you choose to participate through your local health zone medical staff. All your records related to this study will be stored apart from your general health records. In the future, data collected for this study may be shared with other researchers for other studies that are unknown at this time. Any data shared with other researchers, will not include your name or other personal identifying information.

Sharing the Results

Confidential information, or information which could help in identifying you as a participant, will not be shared. Results of this study are likely to be published in medical journals. Your name will never be used in these reports, and the program will not release you name to the public.

Right to Refuse or Withdraw

Your participation in this study is your choice. You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. It is your choice and all of your rights will still be respected.

What other things should I consider before participation?

Use of Specimens

Any blood specimen obtained for the purposes of this study may be provided to DRC Ministry of Health, the Institut National de Recherche Biomedicale (INRB), University of California, Los Angeles and Stanford University. These specimens will not include information that identifies you directly. Once you provide the specimens you will not have access to them. The specimens will be used for research and such use may result in discoveries that could become the basis for new products or therapeutic agents. In some instances, these discoveries may be of potential commercial value. You will not receive any money or other benefits derived from such a product.

On the checklist at the end of this consent form, you will be asked to indicate if you would permit part of this sample to be shared with other researchers. If you agree to have your sample shared with other researchers and later decide to withdraw, we may not be able to retrieve any

or all of your sample from other researchers. The researcher is not required to store your sample(s) indefinitely.

Who can I contact if I have questions about this study?

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following:

- **Dr. Jean Paul Kompany,** the Laboratory Director for the UCLA-DRC program. He lives in Kinshasa and can be reached by cell phone at **(+243) 815121187**.
- You can also contact the co-Principal Investigators of this study in Kinshasa by cell phone. They are: **Professor Emile Okitolonda** (cellphone: **(+243) 999945183** or **Professor Jean Jacques Muyembe** (cellphone: +**243 898949249**).

What are my rights if I take part in this study?

Taking part in this study is your choice. You can choose whether or not you want to participate. Whatever decision you make, there will be no penalty to you and you will not lose any of your regular benefits.

- You have a right to have all of your questions answered before deciding whether to take part.
- Your decision will not affect the medical care you receive.
- If you decide to take part, you can leave the study at any time.

This proposal has been reviewed and approved by the UCLA Office for Protection of Research Subjects in the United States and The Kinshasa School of Public Health Committee for Bioethics, which are a committee whose task it is to make sure that research participants are protected from harm.

Question to determine if the participant understands: *Do you know that you do not have to take part in this study if you do not wish to? You can say No if you wish to? Do you know that you can ask me questions later, if you wish to? Do you know that I have given the contact details of the person who can give you more information about the study?*

You can ask me any more questions about any part of the research study, if you wish to. Do you have any questions?

PART II: Certificate of Consent

How do I indicate my agreement to participate?

Participant: I have listened to the information about the study. I have asked any questions I had, and all my questions were answered. I know that joining the study is my choice. I understand that I may stop participating in this study at any time. I freely agree to participate in the study.

Person taking consent: I have read the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Check one of the following boxes and have the researcher initial:

I agree to participate in this study (questionnaire and sample collection):

□Yes

□No

Do you understand that you do NOT have to participate?

□Yes

□No

Do you understand that you will still receive a small gift even if you choose not to participate?

□ Yes

□ No

I agree to have my blood sample shared with other researchers.

☐ Yes

☐ No

I agree that my data and/or specimens may be kept for use in future research to learn about, prevent or treat other health related problems

☐ Yes

☐ No

Should the need arise, I agree to be re-contacted for additional sample and data collection, as described in the "What Will Happen If I Agree to participate in this study" section above.

☐ Yes

☐ No

Should the need arise, I agree to be contacted for future research purposes.

☐ Yes

☐ No

Name of participant _____

Thumb print or Signature of participant

Date _____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands the study.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily. A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent _____

Signature of Researcher /person taking the consent

_____

Date _____

Day/month/year

APPENDIX 3 : PROCEDURE FOR PERFORMING SEROLOGICAL TESTS

NAME OF OPERATOR : _____

DATE : _____

PLATE # : ____

INTEGRATED RESEARCH FACILITY-FREDERICK

FANG Human Anti-Zaire Ebola Virus Glycoprotein IgG Assay: PREPARE/PREVAC WA-0056 v03

FANG

Reagents	Suppliers	Lot #	Expiration Date
Reference Standard (RS)	BMI	BMIZAIRE 108 (G3)	N/A
Negative Control (NC)	BMI	BMI 530	N/A
QC High (QCH)	BMI	BMIZAIRE 110 (G3)	N/A
QC Low (QCL)	BMI	BMIZAIRE 109 (G3)	N/A
Wash Buffer ELISA (FW)	LIBR	FW	
Diluent ELISA (DE)	LIBR	DE	
Secondary Antibody Conjugated to HRP	Jackson Labs	122214	
TMB Substrate	Thermo Scientific		
Stop Solution	Thermo Scientific		

Equipment	Numéro de Série
12-channel pipette P200	
12-channel pipette P1200	
P1000 Pipette	
P200 Pipette	
P20 Pipette	
Réfrigerator	1
Freezer at -80°C	S1
Biosafety Cabinet (BSC)	N/A
Microplate Washer	
Microplate reader	C138487
Incubator	0211V1120

1.0 **Reagent Preparation: Initials and Date _____**

1.1 Remove reagents

All reagents, including ELISA microplates, must be brought to room temperature at least 30 minutes before starting the test.

1.2 Prepare samples

- ☐ Thaw serum samples previously inactivated by heating.
- ☐ Record sample number and microplate number on the sample identification sheet.
- ☐ Number of pages of sample identification document : _____

1.3 Prepare Standard Curve with Reference Standard (RS). Prepare the Negative Control (NC). Use a dilution box (ELISA microplate format tube rack with micro-titration tubes).

- ☐ Depending on the RS batch, dilute the RS as follows:
- ☐ RS lot BMIZAIRE108: Prepare a 1/87.6 dilution by pipetting 11.4µL of RS into 988.6µL of ELISA diluent in row A, column 1 of a dilution box.
- ☐ Dispense 300µL of ELISA diluent into columns 2-11 of row A
- ☐ Serially dilute RS by transferring 600µL from column 1 to column 2, then 600µL from column 2 to column 3 and so on up to column 11
- ☐ Mix each dilution at least 6 times before transferring.

- ☐ Take care not to introduce bubbles.
- ☐ Add 5 µL of NC to 495 µL of ED to rows A and B, column 12 and mix.
- ☐ Repeat the above steps to prepare sufficient reference standard and negative control for all plates.
- ☐ Each row is sufficient for one plate.

1.4 Serial dilution of Samples, QCH and QCL. Using a dilution box

- ☐ Create a serial dilution of 1:50, 1:1,000 and 1:4,000 for all samples, QCH and QCL following steps 5.2 to 5.5.
- ☐ Add 294 µL of ED to rows C and F, columns 1-12 of the dilution block to generate a 1:50 dilution.
- ☐ Add 285 µL of ED to rows D and G, columns 1-12 of the dilution block to generate a 1:1,000 dilution.
- ☐ Add 225 µL of ED to row E and H, columns 1-12 of the dilution block to generate a 1:4,000 dilution.
- ☐ Prepare the dilution as follows and as shown in the diagram:

1.4.1 Prepare a 1:50 dilution of samples, QCH and QCL:

- ☐ Add 6 µL of sample; according to the sample layout; to the 294 µL of ED in rows C and F, columns 1-12 of the dilution block.
- ☐ Mix each dilution at least 10 times before transferring.
- ☐ Change tip between each sample addition.
- ☐ Be careful not to produce bubbles or introduce contamination.

1.4.2 Prepare a 1:1000 dilution:

- ☐ Transfer 15 µL of the initial sample diluted 1:50 into the 285 µL of ED from rows D and G, columns 1-12 of the dilution block.
- ☐ Mix each dilution at least 10 times before transferring.
- ☐ Change tip between each sample addition.

1.4.3 Prepare a 1:4000 dilution:

- ☐ Transfer 75 µL of sample diluted 1:1,000 into the 294 µL of ED in rows E and H, columns 1-12 of the dilution block.

- ☐ Mix each dilution at least 10 times.
- ☐ Change tip between each sample addition.
- ☐ Be careful not to produce bubbles or introduce contamination.

2.0 Prepare the ELISA microplates: Initials and Date: _____

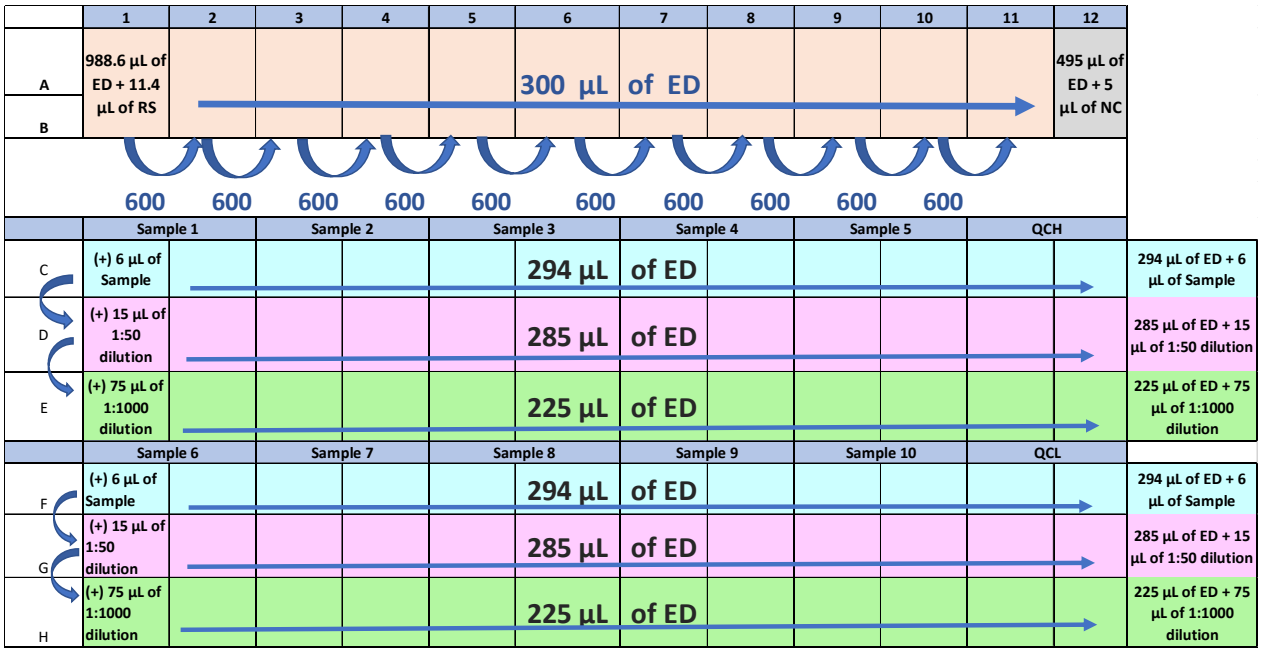
- ☐ Start the microplate washer. Prime the wash system (Prime)
- ☐ Wash the scored microplates 3 times with at least 300µL of wash buffer per wash
- ☐ After the last wash, remove any residual droplets still present in the wells by turning and gently tapping the microplates several times on dry absorbent paper.

3.0 Procedure: Initials and Date: _____

- ☐ Using a multichannel pipette, mix the solutions of the standard curve (RS) and the negative control (NC) 10 times
- ☐ Add 100µl of RS and NC to rows A and B of each plate
- ☐ Using a multichannel pipette, mix the diluted sample solutions 3-5 times
- ☐ Add 100µl of diluted sample solutions to rows C-H of each plate
- ☐ Incubate for 60 minutes at 37°C
- ☐ Record the time on the Incubation Time Document (Time Sheet)
- ☐ Number of pages of the Incubation Time document: _____
- ☐ Dilute the secondary antibody 1/10,000 (6.6µL in 66mL of ELISA diluent for 6 plates)
- ☐ Wash the plates 3 times with at least 300µL of washing buffer per wash
- ☐ Drain the plates by gently tapping them several times on absorbent paper
- ☐ Add 100µL of secondary antibody diluted 1/10,000 to each well
- ☐ Incubate for 60 minutes at 37°C
- ☐ Record the time on the Incubation Time Document (Time Sheet)
- ☐ Wash the plates 5 times with at least 300µL of washing buffer per wash
- ☐ Drain the plates by gently tapping them several times on absorbent paper

- ☐ Add 100µL of TMB Substrate to each well
- ☐ Incubate the plates in the dark and at room temperature for 10 minutes
- ☐ Record the time on the Incubation Time Document (Time Sheet)
- ☐ Add 100µL of Stop Solution to each well
- ☐ Record the time on the Incubation Time Document (Time Sheet)
- ☐ Read the optical densities at 450 nm using the plate reader (also programmed at 650 nm in order to subtract the absorbances corresponding to the background of the wells) within 30 minutes following the addition of the Stop Solution
- ☐ Record the time on the Incubation Time Document (Time Sheet)

	1	2	3	4	5	6	7	8	9	10	11	12
A	**RS 1**	**RS 2**	**RS 3**	**RS 4**	**RS 5**	**RS 6**	**RS 7**	**RS 8**	**RS 9**	**RS 10**	**RS 11**	NC
B	**RS 1**	**RS 2**	**RS 3**	**RS 4**	**RS 5**	**RS 6**	**RS 7**	**RS 8**	**RS 9**	**RS 10**	**RS 11**	NC
C	S1 1:50	S1 1:50	S2 1:50	S2 1:50	S3 1:50	S3 1:50	S4 1:50	S4 1:50	S5 1:50	S5 1:50	QCH 1:50	QCH 1:50
D	S1 1:1,000	S1 1:1,000	S2 1:1,000	S2 1:1,000	S3 1:1,000	S3 1:1,000	S4 1:1,000	S4 1:1,000	S5 1:1,000	S5 1:1,000	QCH 1:1,000	QCH 1:1,000
E	S1 1:4,000	S1 1:4,000	S2 1:4,000	S2 1:4,000	S3 1:4,000	S3 1:4,000	S4 1:4,000	S4 1:4,000	S5 1:4,000	S5 1:4,000	QCH 1:4,000	QCH 1:4,000
F	S6 1:50	S6 1:50	S7 1:50	S7 1:50	S8 1:50	S8 1:50	S9 1:50	S9 1:50	S10 1:50	S10 1:50	QCL 1:50	QCL 1:50
G	S6 1:1,000	S6 1:1,000	S7 1:1,000	S7 1:1,000	S8 1:1,000	S8 1:1,000	S9 1:1,000	S9 1:1,000	S10 1:1,000	S10 1:1,000	QCL 1:1,000	QCL 1:1,000
H	S6 1:4,000	S6 1:4,000	S7 1:4,000	S7 1:4,000	S8 1:4,000	S8 1:4,000	S9 1:4,000	S9 1:4,000	S10 1:4,000	S10 1:4,000	QCL 1:4,000	QCL 1:4,000



Layout diagram of the standard curve, controls and samples: Initials and Date: _____

NOM: _____

DATE: _____

PLAQUE #: ____

MAGPIX HAWAII

1.0 **Preparation of PBS- 1% BSA+ 0.02% Tween-20 as Working Solution: Initials and Date** _____

- ☐ Prepare 1 X PBS by diluting 2 PBS tablets in 1 liter of distilled water;
- ☐ Filter the PBS using a syringe filter or a 1 liter filter;
- ☐ Add 10 g of BSA (fraction V) in 1 liter of 1X PBS;
- ☐ Dissolve by keeping it on the magnetic stirrer for 10 min until BSA dissolves completely;
- ☐ Or alternatively make PBS-BSA overnight and keep it in the refrigerator at +4°C until BSA dissolves in solution.
- ☐ Add 200 µL of Tween 20, then mix vigorously

Note: Prepare small quantities PBS -1%BSA Tween 20 to avoid bacterial contamination or if a large quantity it can be kept for several days and before using check the solution if it is cloudy, prepare another.

0.2. Beads coupled to Ebola virus filovirus antigens (EBOV, SUDV, BDBV, MARV)

0.3. Controls:

- ☐ Negative control sample
- ☐ EBOV, SUDV, BDBV, MARV IgG positive control

0.4. Goat anti-human IgG PE, Fcγ fragment specific, 0.5 mg/ml (use 1:200 dilution) (Jackson Immunoresearch West Grove, PA,)

0.5. Magnetic plate for washing the 96-well plate.

0.6. MAGPIX machine.

Procedure :

I. Calculate the volume of bead dilution you will need based on the number of wells you want to use for your plate:

Initials and Date _____

- ☐ Vortex the master mix of the beads (beads stored in PBS-1%BSA and 0.05% sodium azide away from light) for 10 seconds.
- ☐ Dilute the beads 1/30 using PBS-1%BSA (final dilution factor).

For example, for 100 wells: 100 × 50ul =5000 µL then make a 1/30 dilution of the beads: 166.6 µL mastermix + 5000 µL of PBST.

Note: Luminex beads are sensitive to light, so during incubations they should be covered with aluminum foil to prevent long exposure to light.

- ☐ Add 50 µL of bead dilution to each well of the plate using a multichannel pipette.
- ☐ All subsequent steps are performed in the Biosafety Cabinet (BSC) using appropriate biosafety practices.
- ☐ Keep all materials needed for analysis such as vortex/mixer, plate shaker, boxes of tips, pipettes, dilution tubes, plate with dilution beads, inside the BSC.

II. Dilution of samples and negative and positive controls 1:100

plates _____

- ☐ Vortex the samples, negative control and positive control for 10 minutes
- ☐ Samples and negative control IgG: 2 µL 198 µL PBS-BSA (1:100 dilution) (Total 200 µL)
- ☐ The positive control: 3 µL of each positive control (EBOV, SUDV, MARV, BDBV) 191 µL of PBS-BSA (1:100 dilution) (Total 200 µL)

Note: Vortex the samples at the lowest speed possible. Prepare 10% bleach and keep it inside the BSC. Discard the tips used for sample dilution after rinsing it with 10% bleach.)

- ☐ Turn off the BSC (safety station) or room lights before adding the diluted samples to the plate with bead dilutions.
- ☐ Add 50 µL of the diluted sample duplicates to the plate according to the plate set up.

- ☐ Always distribute the samples in the plate in this way (A1, B1, C1, D1, E1, F1, G1, H1, A2....) since this is the direction of the machine reading. This way the duplicates will be read consecutively.
- ☐ Cover the plate with aluminum foil,

III. Plate incubation

- ☐ Incubate at a temperature of 37°C on the shaker for 3 hours at 700 rpm in an incubator.
- ☐ Write the time on the Incubation Time Sheet
- ☐ Number of pages of the Incubation Time sheet: _____
- ☐ Remove other materials from the safety station or workroom after disinfecting it with 70% ethanol.
- ☐ Turn on the MAGPIX machine and log into the logbook.
- ☐ Check for drive fluid and waste container.
- ☐ Run magpix machine maintenance. (See SOP on magpix maintenance)
- ☐ Remove PBS-1% BSA tween 20 from the refrigerator for plate washing.
- ☐ Keep the biohazard receptacle with 10% bleach to neutralize waste generated by washing the plates.
- ☐ Just before the end of the 3 hour incubation, prepare the anti-human IgG phycoerythrin conjugate in PBS-1% BSA. For 100 wells: 5000 µL PBST: 25 µL of the secondary antibody (dilution 1:200).

Note: Do not vortex IgG and IgM PE stock solutions at high speed. Mix by pipetting up and down.

- ☐ After 3 hours of incubation, place the plates on the magnetic plate for 2 minutes on the magnetic plate,
- ☐ After 2 minutes throw the liquid into a tray containing the 10% bleach solution.,
- ☐ Wash the plate twice by adding 200ul of PBST to each well using a P200 multichannel pipette.
- ☐ Place the plate on the shaker for 10 to 15 seconds, always protected from light

- ☐ Remove the stirrer plate
- ☐ Place the plate on the magnetic plate for 2 minutes.
- ☐ After 2 minutes throw the liquid forcefully into a tray containing the 10% bleach solution.
- ☐ Dilute the secondary antibody 30 minutes before the end of the first incubation at 1/200 (25µL in 5mL of PBS-BSA –Tween 20 for 100 wells)
- ☐ Distribute 50 µL of the diluted secondary antibody into the respective wells according to the direction of the plate.
- ☐ Cover the plate with aluminum foil,
- ☐ Incubate in an incubator at 37°C for 1 hour at 700 rpm on the plate shaker.
- ☐ After 1 hour of incubation, Place the plates on the magnetic plate for 2 minutes on the magnetic plate,
- ☐ After 2 minutes throw the liquid into a tray containing the 10% bleach solution.,
- ☐ Wash the plate twice by adding 200 µL of PBST to each well,
- ☐ Place the plate on the plate shaker for 10 to 15 seconds
- ☐ Place the plate on the magnetic plate for 2 minutes.
- ☐ After 2 minutes throw the liquid forcefully into a tray containing the 10% bleach solution.
- ☐ Add 120 µl of MAGPIX Training Fluid to each plate well.
- ☐ Cover the plate with the aluminum foil and keep it on the shaker at 700 rpm for 10 to 15 seconds at room temperature.
- ☐ Create a batch using the "Liberia Human Project" protocol on MAGPIX. Immediately after mixing the contents, read the plate on a Luminex instrument

Note :

- ➢ Keep the plate in the refrigerator after reading.
- ➢ Check the number of beads as well as the MFI for all the wells.

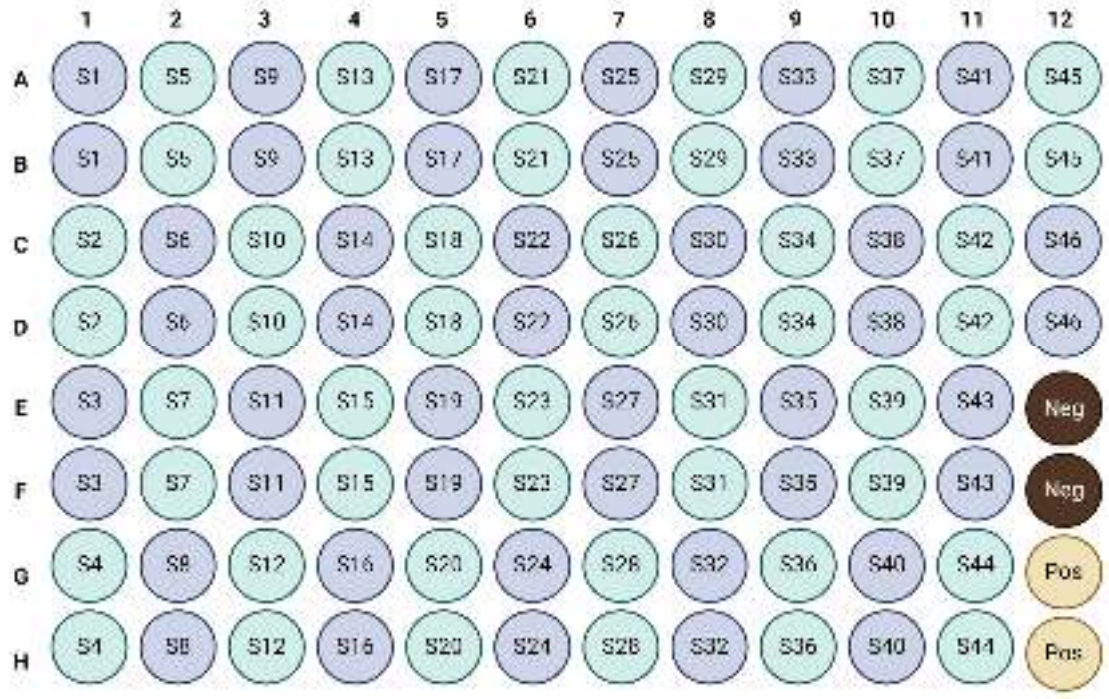
- You can re-run the plate the next day in case you have any problems with the MFI or the account.
- Simply add more drive fluid and shake the plate for 1 min on plate shaker before reading the plate.

- ☐ Be sure to add the reagents for the routine post-batch before starting the plate reading (Distilled water, 70% ethanol, 0.1 N NaOH in wells A, B and C so respective).
- ☐ Disinfect the MAGPIX instrument daily. Use 5% bleach and DI water. If bleach is not available then use 70% ethanol or 70% isopropanol. Turn off the instrument and log out of the logbook.
- ☐ Plate set up 1:100 dilution (S1-S45, 45 Samples can be tested on one plate or in duplicate)

Bead regions used for HFV IgG assay-human panel

Antigen	Bead region
EBOV VP40	**12**
BSA	**15**
SUDV GP	**20**
EBOV NP	**33**
MARV VP40	**42**
MARV GP	**51**
BDBV	**55**
EBOV GP	**72**

Note: The mastermix contains antigen-coupled beads (as indicated in the table above in bold)



MIA PLATE MAP