Ebola & Marburg Virus Vaccine ELISA Kits, Recombinant Proteins, and Antibodies

Alpha Diagnostic Intl Inc. (ADI), a biotechnology company located in San Antonio, Texas, USA, has been researching and developing many prototype vaccines and diagnostic tests to determine the efficacy of Ebola candidate vaccines in animals and humans. We have cloned and expressed most Ebola viral proteins (GP, NP, and VP40) from Ebola/Marburg viruses, generated antibodies, and developed ELISA kits for the detection and measurement of Ebola related antigens and antibodies. Given the urgency of Ebola virus disease (EVD), the company is releasing many critical recombinant proteins, antibodies, and ELISA kits to further research into the development of Ebola vaccines and testing their efficacy. ADI's Ebola kits contain all animal derived antibodies made to purified recombinant proteins. ADI antibodies and kits have no Ebola virus or viral derived proteins and are completely safe to use and transport. The kits have been tested and validated with therapeutic antibodies, Zmapp. Additional ELISA kits and antibodies are available for Ebola vaccine vectors (Adenovirus, VSV, and Rabies virus proteins) to determine efficacy of Ebola vaccines.

Zaire-Ebola vaccine Related ELISA kits

(See Details at the website) http://4adi.com/commerce/catalog/spcategory.jsp?category_id=2762

Virus	ELISA Kit Description	Species	IgG Specific Cat#	IgM Specific Cat#
Zaire Ebola	Zaire-Ebola Virus Nucleoprotein (**EBOV NP**) antibody ELISA Kits**	Mouse	AE-320500-1	AE-320510-1
		Human	AE-320520-1	AE-320530-1
		Monkey/Chimp	AE-320550-1	AE-320560-1
	Zaire-Ebola Virus Glycoprotein (**EBOV GP**) antibody ELISA Kits**	Mouse	AE-320600-1	AE-320610-1
		Human	AE-320620-1	AE-320630-1
		Monkey/Chimp	AE-320650-1	AE-320660-1
		Dog	AE-322670-1	
	Zaire-Ebola Virus Glycoprotein (**EBOV VP40**) antibody ELISA Kits**	Mouse	AE-320700-1	AE-320710-1
		Human	AE-320720-1	AE-320730-1
		Monkey/Chimp	AE-320750-1	AE-320760-1
	Zaire-Ebola Virus Antigen ELISA kit, Qualitative (detect virus in human samples)	AE-320800-48 (48 tests) AE-320800-48 (96 tests)		
Sudan Ebola	Sudan-Ebola Virus Glycoprotein (**EBOV GP**) antibody ELISA Kits**	Mouse	AE-321600-1	AE-321610-1
		Human	AE-321620-1	AE-321630-1
		Monkey/Chimp	AE-321650-1	AE-321660-1
Marburg	Marburg Virus Glycoprotein (**MARV GP**) antibody ELISA Kits**	Mouse	AE-322600-1	AE-322610-1
		Human	AE-322620-1	AE-322630-1
		Monkey/Chimp	AE-322650-1	AE-322660-1
Zmapp	**ZMAPP ELISA** (Humanized Anti-Ebola GP IgGs, produced in tobacco plants) Activity ELISA kit (for mouse, human, monkey etc) (for determining the activity of Zmapp and its measurement in serum or plasma) # AE-320850-1, 96 tests			
	Anti-ZMAPP antibody ELISA (Anti-drug antibody/ADA ELISA for mouse, human, monkey etc), 96 tests, Quantitative # AE-320860-1			

****Notes:** The above ELISA kits contain recombinant protein made and purified from E. coli or sf9 host cell. There is no Ebola virus or antibodies in the kit. The kit transport or usage does not pose any safety tissue. However, if Ebola positive samples are tested using the kit then they must be used in appropriate BSL4 labs

Adenovirus, Rabies and VSV are being used to express Ebola genes (vaccines). ADI has many antibodies, recombinant proteins and ELISA kits for these vectors.

http://4adi.com/commerce/ccc2744-adenovirus-based-vaccines-and-elisa-kits-**adenovirus**-vaccines--elisa-kits0d0a.htm

http://4adi.com/commerce/ccc2726-rabies-vaccine-elisa-and-reagents-**rabies**-vaccine--elisa-reagents.htm

http://4adi.com/commerce/ccc2745-vaccinia-virus-based-vaccines-and-elisa-kits-**vaccinia**-virus--vaccines--elisa-kits0d0a.htm

Zaire-Ebola vaccine Related Antibodies, Proteins and other Reagents

	Virus Type#	Catalog#	Product Description	Product Type
Bundibugyo	**Bundibugyo Ebola (BEBOV)**	BVGP41-A	Rabbit Anti-Bundibugyo Ebola virus glycoprotein (BDBV GP) IgG	Antibodies
		BVGP41-C	Recombinant (sf9) Bundibugyo Ebola virus glycoprotein (BEBOV GP ΔTM his-tag,>95%) control for Western Blot	Protein control
		BVGP45-R-10	Recombinant (sf9) Bundibugyo Ebola virus glycoprotein (BEBOV GP ΔTM his-tag,>95%), low endotoxin	Antigen protein
		BVRB46-R-10	Recombinant (HEK) Bundibugyo Ebola virus glycoprotein RBD domain (Uganda/2007/1-308aa, hIgG1-Fc-tag at CT, low endotoxin)	Rec. protein
		BVRB46-BTN	Biotin-Recombinant (HEK) Bundibugyo Ebola virus glycoprotein RBD domain (Uganda/2007/1-308aa, hIgG1-Fc-tag at CT, low endotoxin)	Rec. protein
		BVRB11-R-10	Recombinant (HEK) Bundibugyo Ebola virus glycoprotein RBD domain (Uganda 2007, 54-201aa, Fc-tag at CT, >95%, low endotoxin)	Rec. protein
		BVRB11-BTN	Biotin-Recombinant (HEK) Bundibugyo Ebola virus glycoprotein RBD domain (Uganda 2007, 54-201aa, Fc-tag at CT, >95%, low endotoxin)	Rec. protein
Zaire Ebola ZEBOV	**Zaire Ebola (ZEBOV) Glycoprotein**	EVGP11-A	Rabbit Anti-Zaire Ebola virus glycoprotein peptide (mayinga, EBOV GP) IgG, purified	Antibodies
		EVGP11-C	Recombinant (sf9) Zaire Ebola virus glycoprotein (Mayinga, 1-637aa, His-tag, >95%) control for Western Blot	Protein control
		EVGP15-A	Rabbit Anti-Zaire Ebola virus glycoprotein (GP, 1-676aa/DNA vaccine) IgG, purified	Antibodies
		EVGP16-A	Rabbit Anti-Zaire Ebola virus glycoprotein (GP 1-652aa/DNA vaccine) IgG, purified	Antibodies
		EVGP17-R-10	Recombinant (sf9) Zaire Ebola virus glycoprotein (Mayinga, 1-637aa, His-tag, >95%), Low endotoxin	Antigen protein
		EVGP17-BTN	Biotin-Recombinant (sf9) Zaire Ebola virus glycoprotein (Mayinga, 1-637aa, his-tag, >95%), low endotoxin	Antigen protein
		EVGP18-R-10	Recombinant (sf9) Zaire Ebola virus glycoprotein 1 (/GIN/2014/Kissidougou-C15, **GP1**, 1-501aa, his-ta at CT, >95%), Low endotoxin	Antigen protein
		EVGP20-R-10	Recombinant (sf9) Zaire Ebola virus glycoprotein (GIN/2014/Kissidougou-C15, 1-650aa, his-tag at CT, >95%), Low endotoxin	Antigen protein
		EVGP21-R-10	Recombinant (HEK) Zaire Ebola virus glycoprotein (GIN/2014/Kissidougou-C15, 1-650aa, his-tag at CT, >95%), Low endotoxin	Antigen protein
		EVGP18-M	Mouse monoclonal Anti-**Zaire** Ebola virus glycoprotein (EBOV GP) IgG	Antibodies
		EVRB11-R-10	Recombinant (HEK) **Zaire** Ebola virus glycoprotein **RBD domain** (1-308aa, **his-tag at CT**, low endotoxin)	Antigen protein
		EVRB11-BTN	Biotin-Recombinant (HEK) Zaire Ebola virus glycoprotein **RBD domain** (1-308aa, his-tag, >95%, low endotoxin)	Antigen protein
		EVRB12-R-10	Recombinant (HEK) **Zaire** Ebola virus glycoprotein **RBD domain** (1-308aa, **hIgG1-Fc-tag at CT**, >95% low endotoxin)	Antigen protein
		EVRB13-R-10	Recombinant (HEK) **Zaire** Ebola virus glycoprotein **RBD domain** (1-308 aa, **Fc tag at CT**, >95%, low endotoxin)	Antigen protein
		EVRB14-R-10	Recombinant (HEK) **Zaire** Ebola virus glycoprotein **RBD** domain (1-308 aa, **his tag at CT** >95%, low endotoxin)	Antigen protein
		EVRB14-BTN	Biotin-Recombinant (HEK) Zaire Ebola virus glycoprotein **RBD** domain (Mayinga 1976, 1-308 aa, his tag, >95%, low endotoxin)	Antigen protein
	VLPs	EVLP14-S	Rabbit Anti-Zaire Ebola virus-like particles (Mayinga, VLPs containing NP, GP, and VP40) antiserum	Antiserum
	NP	EVNP11-C	Recombinant (E. coli) Zaire-Ebola virus nucleoprotein (Mayinga EBOV NP) control for Western	Protein control
		EVNP11-S	Rabbit Anti-Zaire-Ebola virus nucleoprotein (Mayinga EBOV NP) protein antiserum	Antiserum
		EVNP13-A	Rabbit Anti-Zaire Ebola virus nucleoprotein (EBOV NP, 1-739/DNA vaccine) IgG	Antibodies
		EVNP13-C	Recombinant (E. coli) Zaire-Ebola virus nucleoprotein (Mayinga EBOV NP) control for Western	Protein control
		EVNP15-R-10	Recombinant (E.coli) Zaire Ebola virus nucleoprotein (EBOV NP) (full length, his-tag, 82 kda), purified	Antigen protein
		EVNP15-BTN	Biotin-Recombinant (E.coli) Zaire Ebola virus nucleoprotein (EBOV NP) (full length, his-tag, 82 kda), purified	Antigen protein
	VP35	EVP351-A	Rabbit Anti-Zaire Ebola virus **VP35** peptide (ZEBOV VP35) IgG, aff pure	Antibodies
	VP40	EVP401-A	Rabbit Anti-Zaire-Ebola virus **VP40** peptide (ZEBOV VP40) IgG, aff pure	Antiserum
		EVP401-C	Recombinant Zaire-Ebola virus VP40 protein control for Western blot	Protein control
		EVP405-R-10	Recombinant (E.coli) Zaire Ebola virus **VP40** (no-tag, ~40 kda, purified	Antigen protein
		EVP405-BTN	Biotin-Recombinant (E.coli) Zaire Ebola virus VP40 (no-tag, ~40 kda),	Antigen protein
	L-Polymerase	EVPO11-A	Rabbit Anti-Zaire Ebola virus (Mayinga) **L-polymerase** peptide IgG	Antibodies
	Ebola Virus Whole	EVZ12-M	Mouse Monoclonal Anti-Zaire Ebola virus (killed) IgG, aff pure	Antibodies
		EVZ13-M	Mouse Monoclonal Anti-Zaire Ebola virus (Killed) IgG, aff pure	Antibodies
		EVZ14-M	Mouse Monoclonal Anti-Zaire Ebola virus IgG (mixture of EVZ12-M and EVZ13-M), aff pure	Antibodies
	Ebola Peptides	SP-89925-1	Zaire Ebola virus Glycoprotein (GP), T cell epitope (577-584) (MW: 966.1)	Pure peptide
		SP-89926-1	Zaire Ebola virus negative control peptide for SP-89925-1 (MW: 1102.2)	Pure peptide

Sudan SUDV	**Sudan Ebola (SUDV) GP**	SVGP21-A	Rabbit Anti-Sudan Ebola virus glycoprotein (sf9, SUDV GP) IgG	Antibodies
		SVGP21-C	Recombinant (sf9) Sudan-Ebola virus glycoprotein (his-tag control for Western	protein control
		SVGP22-M	Mouse Monoclonal Anti-Sudan Ebola virus glycoprotein (sf9, SUDV GP) IgG, purified	Antigen protein
		SVGP25-R-10	Recombinant (sf9) Sudan-Ebola virus glycoprotein (1-637aa, his-tag at CT, >95%), low endotoxin	Antigen protein
		SVGP24-R-10	Recombinant (HEK) Sudan-Ebola virus glycoprotein (Gulu, 1-637aa, his-tag at CT, >95% low endotoxin),	Antigen protein
		SVGP24-BTN	Biotin-Recombinant (HEK) Sudan-Ebola virus glycoprotein (Gulu, 1-637aa, his-tag at CT, >95% low endotoxin)	Antigen protein
		SVRB11-R-10	Recombinant (**HEK**) Sudan-Ebola virus **RBD domain** (Uganda-00/1-320aa, his-tag, low endotoxin), purified	Antigen protein
		SVRB12-R-10	Recombinant (**sf9**) Sudan-Ebola virus **RBD domain** (Uganda-00/1-320aa, his-tag, low endotoxin), purified	Antigen protein
		SVRB12-BTN	Biotin-Recombinant (sf9) Sudan-Ebola virus RBD domain (Gulu, 1-320aa, his-tag, low endotoxin), purified	Antigen protein
		SVRB13-R-10	Recombinant (**HEK**) Sudan-Ebola virus **RBD domain** (Gulu, 1-320aa, Fc-tag at CT, >95%, low endotoxin),	Antigen protein
	Sudan NP	SVNP23-A	Rabbit Anti-Sudan Ebola virus Nucleoprotein (SUDV NP) peptide IgG, aff pure	Antibodies
	Sudan VP40	SVP402-A	Rabbit Anti-Sudan Ebola virus VP40 (SUDV VP40) IgG, aff pure	Antibodies
		SVP403-M	Mouse Monoclonal Anti-Sudan Ebola virus VP40 (SUDV VP40) IgG	Antibodies
Reston RESTV	**Reston Ebola (RESTV)**	RVGP31-A	Rabbit Anti-Reston Ebola virus Glycoprotein (RESTV GP) peptide IgG	Antibodies
		RVGP31-C	Purified Reston Ebola virus Glycoprotein (RESTV GP) control for western blot	protein control
		RVGP35-R-10	Recombinant (sf9) Reston Ebola virus glycoprotein (his-tag~72 kda), purified	Rec. protein
Marburg MARV	**Marburg Virus (MARV) GP**	MVGP12-A	Rabbit Anti-Marburg virus glycoprotein peptide (MARV GP) IgG	Antibodies
		MVGP13-M	Mouse Monoclonal Anti-Marburg virus glycoprotein (MARV GP) IgG	Antigen protein
		MVGP15-R-10	Recombinant (sf9) Marburg virus glycoprotein (Angola his-tag >95%)	Rec. protein
		MVGP16-R-10	Recombinant (sf9) Marburg virus glycoprotein (Musoke HA-tag, >95%	Rec. protein
		MVGP16-BTN	Biotin-Recombinant (sf9) Marburg virus glycoprotein (Musoke, HA-tag, >95%),	Rec. protein
		MVLP12-A	Rabbit Anti-Marburg virus-like Particles (VLPs containing NP, GP, and VP40) IgG	Antibodies
	Marburg NP	MVNP13-A	Rabbit Anti-Marburg virus nucleoprotein (MARV NP) peptide IgG, aff pure	Antibodies
	Marburg VP40	MVP401-M	Mouse Monoclonal Anti-Marburg virus VP40 (Muskoe MARV VP40) IgG purified	Antibodies
		MVP402-A	Rabbit Anti-Marburg virus VP40 peptide (Muskoe MARV VP40) IgG	Antibodies

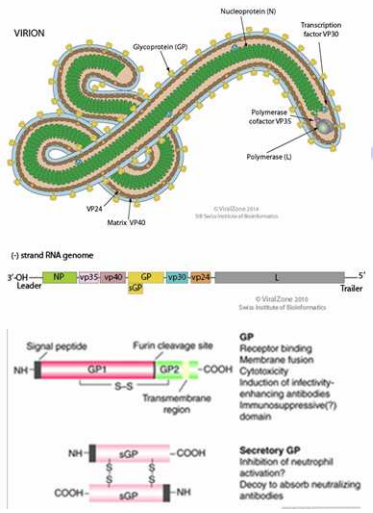
Adenovirus, Rabies and VSV are being used to express Ebola genes (vaccines). ADI has many antibodies, recombinant proteins and ELISA kits for these vectors.

Ebola Virus –General Information, Therapeutics and Vaccines

Ebola virus (**EBOV**) causes severe disease in humans and in nonhuman primates in the form of viral hemorrhagic fever. The name Ebola virus is derived from the Ebola River (a river that was at first thought to be in close proximity to the area in Zaire where the first recorded Ebola virus disease outbreak occurred) and the taxonomic suffix virus. Zaire Ebolavirus is a virological taxon included in the genus Ebolavirus, family Filoviridae, order Mononegavirales. The family Filoviridae (members are called Filovirus or filovirids; filum is derived from latin meaning filamentous) is a group of several related viruses that form filamentous infectious viral particles (virions) and encode their genome in the form of single-stranded negative-sense RNA. The family currently includes the three virus genera Cuevavirus, Ebolavirus, and Marburgvirus. The family members are:

Genus name	*Species name*	*Virus name (Abbreviation)*
Cuevavirus	*Lloviu cuevavirus**	Lloviu virus (LLOV)
Ebolavirus	*Bundibugyo ebolavirus*	Bundibugyo virus (BDBV; previously BEBOV)
	Reston ebolavirus	Reston virus (RESTV; previously REBOV)
	Sudan ebolavirus	Sudan virus (SUDV; previously SEBOV)
	Taï Forest ebolavirus	Taï Forest virus (TAFV; previously CIEBOV)
	*Zaire ebolavirus**	Ebola virus (EBOV; previously ZEBOV)
Marburgvirus	*Marburg marburgvirus**	Marburg virus (MARV)

The two members of the family that are commonly known are Ebola virus and Marburg virus. Both viruses, and some of their lesser known relatives, cause severe disease in humans and nonhuman primates (NHP) in the form of viral hemorrhagic fevers. All Ebola viruses and Marburg viruses are Select Agents Group 4 Pathogens. Filoviruses have a history that dates back several tens of millions of years. The most recent common ancestor of both the Reston and Zaire species has been estimated to be ~1960. The most recent common ancestor of the Marburg and Sudan species appears to have evolved 700 and 850 years before present respectively. The family Filoviridae represents significant health risks as emerging infectious diseases as well as potentially engineered biothreats. Ebolavirus species Zaire (ZEBOV) causes a highly lethal hemorrhagic fever, resulting in the death of 90% of patients within days. Ebola Zaire attacks every organ and tissue in the human body except skeletal muscle and bone. Ebola is classified as a Level 4 pathogen (higher than AIDS) with a 2 to 21 day (7 to 14 days average) incubation period. There are currently four known strains of Ebola: Zaire, Sudan, Reston and Tai. All of them cause illness in sub-human primates. Only Ebola Reston does not cause illness in humans. The mortality rate of Ebola victims is between 60% and 90%; with Ebola Sudan at 60% and Ebola Zaire at 90%.



The virions are tubular in general form but variable in overall shape and may appear as the classic shepherd's crook or eyebolt, as a U or a 6, or coiled, circular, or branched. Ebolavirions consist of seven structural proteins. At the center is the helical ribonucleocapsid, which consists of the genomic RNA wrapped around a polymer of **nucleoproteins (NP).** Associated with the ribonucleoprotein is the RNA-dependent RNA polymerase (L) with the polymerase cofactor (VP35) and a transcription activator (VP30). The ribonucleoprotein is embedded in a matrix, formed by the major (**VP40**) and minor (VP24) matrix proteins. These particles are surrounded by a lipid membrane derived from the host cell membrane. The membrane anchors **a glycoprotein (GP1,2**) that projects 7 to 10 nm spikes away from its surface. While nearly identical to marburgvirions in structure, ebolavirions are antigenically distinct. Being acellular, viruses do not grow through cell division; instead, they use the machinery and metabolism of a host cell to produce multiple copies of themselves, then assembling in the cell.

Ebola virus disease (EVD) is clinically indistinguishable from **Marburg virus disease (MVD)** and can be easily be confused with many other diseases prevalent in Equatorial Africa, such as other viral hemorrhagic fevers, falciparum malaria, typhoid fever, shigellosis, and rickettsial diseases such as typhus, cholera, gram-negative septicemia, borreliosis such as relapsing fever or EHEC enteritis. The most common **diagnostic methods** are therefore RT-PCR in conjunction with antigen-capture ELISA which can be performed in field or mobile hospitals and laboratories. **Vaccines** have successfully protected nonhuman *primates*; however, the six months needed to complete immunization made it impractical in an epidemic. In 2003, a vaccine using an adenoviral (ADV) vector carrying the Ebola spike protein was tested on crab-eating macaques. The monkeys were challenged with the virus 28 days later, and remained resistant. In 2005, a vaccine based on attenuated recombinant vesicular stomatitis virus (VSV) vector carrying either the Ebola glycoprotein or Marburg glycoprotein successfully protected nonhuman primates, opening clinical trials in humans. There are currently **no Food and Drug Administration-approved vaccines** for the prevention of EVD. The most promising ones are DNA vaccines or are based on adenoviruses, vesicular stomatitis Indiana virus (VSIV) or filovirus-like particles (VLPs) as all of these candidates could protect nonhuman primates from Ebola virus-induced disease.

Experimental Drugs and Vaccines (ZMapp, Favipravir, TKM-Ebola etc)

From 1976 (when it was first identified) through 2013, the WHO reported a total of 1,716 cases. The largest outbreak to date is the ongoing 2014 West Africa Ebola outbreak, which is affecting Guinea, Sierra Leone, Liberia and Nigeria. As of 26 August 2014, 3,069 suspected cases resulting in the deaths of 1,552 have been reported. Currently, neither a specific treatment nor a vaccine licensed for use in humans is available. However, a number of vaccine candidates have been developed in the last decades that are highly protective in non-human primates. Among these vaccines are recombinant Adenoviruses (Ad5/chAd3), recombinant Vesicular Stomatitis viruses (VSV), recombinant Human Parainfluenza viruses and virus-like particles. There is sufficient evidence from studies in animal studies and NHP (non-human primates) that a vaccine protective against ebolaviruses is possible.

Ebola Therapeutics

The FDA has allowed two drugs, **ZMapp** and an RNA interference drug called **TKM-Ebola**, to be used in people infected with Ebola under these programs during the 2014 outbreak. **ZMapp**, the top-secret magic serum, is an experimental biopharmaceutical drug comprising three humanized monoclonal antibodies (anti-Zaire Ebola GP) under development as a treatment for Ebola virus disease. The ZMapp drug is being developed by Mapp Biopharmaceutical Inc., a result of the collaboration between Mapp Biopharmaceutical (San Diego), LeafBio (the commercial arm of Mapp Biopharmaceutical), Defyrus Inc. (Toronto), the U.S. government and the Public Health Agency of Canada. ZMapp is composed of three monoclonal antibodies (mAbs) that have been humanized by genetic engineering and combine "the best components of MB-003 (Mapp) and ZMAb (Defyrus/PHAC)", each of which were combinations of mAbs. Zmapp components are humanized monoclonal antibody c13C6 from MB-003 and two humanized mAbs from ZMab, c2G4 and c4G7. Like intravenous immunoglobulin therapy, ZMapp contains neutralizing antibodies that provide passive immunity to the virus by directly and specifically reacting with virus GP in a "lock and key" fashion. ZMapp is manufactured in the tobacco plant Nicotiana benthamiana in the bioproduction process known as "pharming" by Kentucky BioProcessing, a subsidiary of Reynolds American. **ADI has developed the first rapid ELISA kit to measure the activity or potency of the drug during its manufacturing. The kit also allows the measurement of active drug in serum or plasma of animals or humans**.

TKM-Ebola is being developed by Tekmira Pharmaceuticals Corp., a company located in Vancouver, Canada. The drug was formerly known as Ebola-SNALP. It is a combination of Small interfering RNAs (siRNAs) targeting three of the seven proteins in Ebola virus: Zaire Ebola L polymerase, Zaire Ebola membrane-associated protein (VP24), and Zaire Ebola polymerase complex protein (VP35), formulated with Tekmira's lipid nanoparticle technology. ADI has produced recombinant proteins, antibodies, and antibody ELISA kits to research the efficacy of TKM-Ebola therapy.

Current and Future Ebola Vaccines

A number of vaccines have been successfully tested in animals and NHP. Human safety studies of an experimental **Ebola vaccine developed by the National Institutes of Health (NIH) and GlaxoSmithKline will launch in September 2014**. NIH is also working with Crucell, Profectus Biosciences, Immunovaccine and researchers at Thomas Jefferson University to develop other candidate vaccines for Ebola. Human trials of the Crucell vaccine are planned for late 2015 or early 2016. Another experimental **Ebola vaccine, VSV-EBOV**, has been developed by the Public Health Agency of Canada and is licensed to NewLink Genetics. The clinical trials are expected to begin soon. NIAID also is funding Profectus Biosciences, a Baltimore, Maryland-based biotechnology company, to develop a candidate vaccine targeting **Ebola and Marburg infections**. The vaccines is based upon recombinant vesicular stomatitis Indiana virus (rVSV) vectored vaccines for EBOV and MARV glycoproteins (rVSV vector-GP construct (delta G1,2). This highly attenuated genetically modified rVSV vector is a replicating virus with good immunogenicity and low virulence. This strategy may mitigate the risk of poor of immunogenicity in vaccine recipients with immunologic memory to vector variants delivered in previous vaccinations. This vaccine is currently in preclinical testing.

Human trials of the candidate Ebola vaccine, co-developed by the US National Institutes of Health (NIH) and GlaxoSmithKline (GSK), are scheduled to start in September 2014 in the UK, The Gambia and Mali. The candidate vaccine is against the Zaire species of Ebola, which is the one circulating in West Africa, and uses a single **Zaire Ebola virus glycoprotein protein (GP)** to generate an immune response. NIAID is testing this same vaccine in the USA (**VRC 207 study**) in addition to a related vaccine that is designed to protect against two Ebola species (**Ebola Zaire and Ebola Sudan**). The NIAID/GSK Ebola vaccine candidate is based on an attenuated strain of chimpanzee cold virus, called chimp adenovirus type 3 (**ChAd3**). This approach uses ChAd vectors to obviate the issue of background immunity to human Ad5 vectors. The adenovirus is used as a carrier, or vector, to deliver benign genetic material derived from the Ebola virus Zaire species that has caused the current Ebola outbreak in West Africa. The genetic material contained in the investigational vaccine cannot cause a vaccinated individual to become infected with Ebola. The vaccine candidate delivers the Ebola genetic material to human cells but does not replicate further. Rather, the Ebola gene that it carries allows the cells of the vaccine recipient to express a single Ebola protein, and that protein prompts an immune response in the individual. The vaccine has shown promising protection in non-human primates (NHP) exposed to Ebola without significant adverse effects.

NIAID support is assisting Crucell (a Netherlands based biotechnology company) and Bavarian Nordic, based in Denmark. Crucell is developing a **multivalent Ebola/Marburg vaccine** using a recombinant adenovirus platform. Phase 1 clinical trial of this candidate vaccine is anticipated to begin by late 2015. The Multivalent **filovirus vaccine is based on recombinant adenovirus (Ad) vectors Ad26 and Ad35** that infect humans at low seroprevalence. Protective efficacy studies to date have all involved an Ad26 prime and an Ad35 boost with various viral GP antigens (EBOV, SUDV, MARV, and TAFV), followed by an exposure of four weeks after the boost immunization.

NIAID and Thomas Jefferson University in Philadelphia have developed an investigational **Ebola vaccine using the established rabies virus vaccine** platform. Ebola virus (EBOV) vaccine platform is based on: (a) replication competent rabies virus (RABV); (b) replication-deficient RABV;or (c) chemically inactivated RABV expressing EBOV glycoprotein (GP). The vaccines were found to be safe and produced potent immune responses against both rabies and Ebola viruses when tested in nonhuman primates. NIAID supported researchers are currently pursuing the development of multivalent vaccine candidates against Ebola, Marburg and rabies viruses for use in humans.

DoD-USAMRIID is working on a **VLP (virus like particles) vaccine for filoviruses**. VLPs are virus-sized particles formed by viral proteins (EBOV and MARV glycoproteins) which retain virus morphology but are noninfectious. VLPs have the advantages of rapid production in large quantities and generate robust innate, humoral and cellular immunity in rodents, NHPs and humans. There are no issues regarding vector immunity. A single vaccine may be effective against EBOV, SUDV, and MARV.

University of Texas at Austin researchers are evaluating the mucosal vaccine against **EBOV GP using an Ad5-based vaccine**. The goal is a vaccine that provides systemic and mucosal immunity with memory, low toxicity, and ease of administration and delivery.

Researchers at the University of Hawaii are exploring **recombinant filovirus antigens (GP1.2, VP24, and VP40) as vaccines**. Advantages of the subunit approach include the ability to precisely select antigen doses and the elimination of translation of protein antigens in the host.

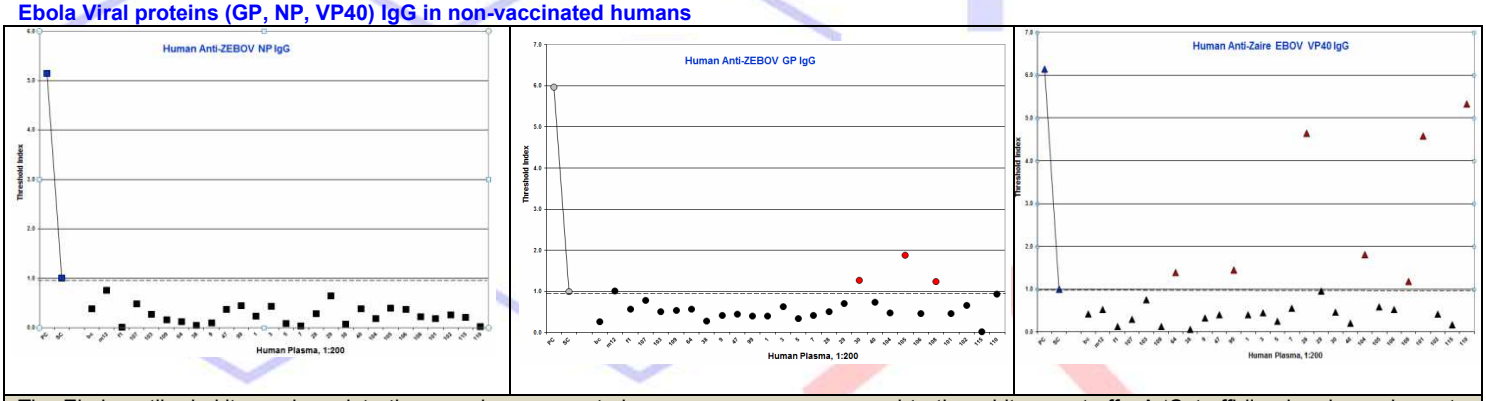
Are some people or animals immune to Ebola?

Although few medical experts realize and appreciate it, a small but significant population of in West Africa is immune to the Ebola virus. There are about 1,800 survivors of the current West African outbreak, all of whom are now immune, of course. Dr. Leroy, ICMR Gabon, tested 4,349 samples from the Gabon region that had four Ebola outbreaks from 1994 to 2002. Approximately 15% population showed antibodies to various Ebola proteins (Np, GP, and VP40). They study used viral antigens derived from the Ebola virus, so antibodies to individual Ebola proteins were not measured in all the samples, but the Western blot showed the presence of antibodies to various Ebola proteins. **ADI has developed specific antibody ELISA kits using highly purified recombinant Ebola proteins (GP, NP, and VP40) that can be used to screen the presence of Ebola antibodies in the general population that are exposed to the Ebola outbreak and the ones outside the 'Ebola Ground Zero"**.

ADI has developed Ebola viral antigens (GP, NP, and VP40) and antibody (IgG and IgM) ELISA kits for human, NHP (non-human primates), mouse, rabbit, and pig. We measured the antibody levels (IgG and IgM) in random samples that were presumably not exposed to Ebola. All the samples (human and animals) were collected in the USA.

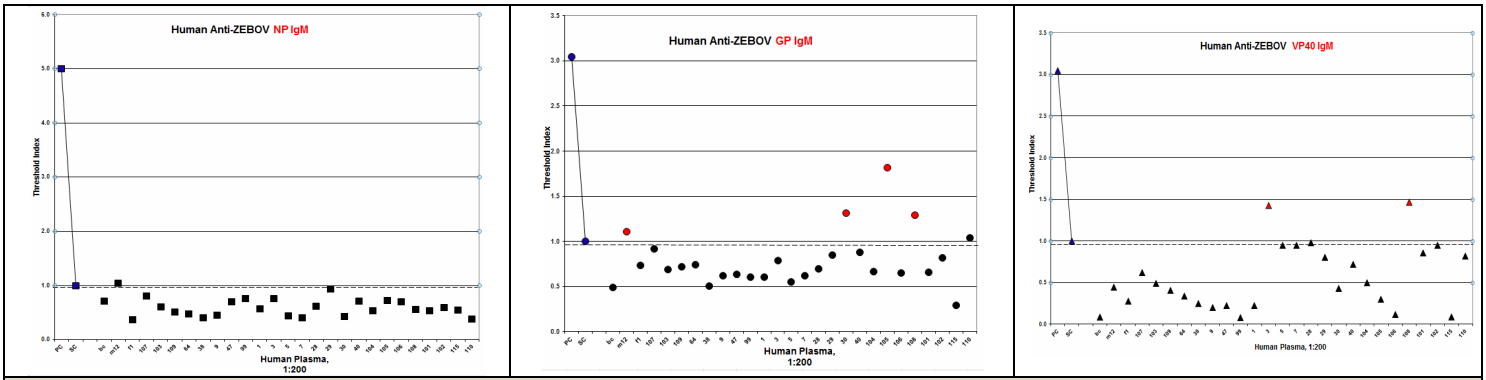
Human samples: A set of random humans samples (n=7) were tested at 1:500 for antibodies to EBOV GP, NP, and VP40 using ADI ELISA kits. We are presenting cumulative data for all 3 proteins in order to gain an understating of what to expect and how to make an interpretation of the data. **Several human samples gave higher basal levels of reactivity** (0.100-0.500). Samples that have elevated antibodies to all 3 EBOV antigens must be examined with other supporting data to determine if these samples are actually positive for EBOV antibodies.

Ebola Viral proteins (GP, NP, VP40) IgG in non-vaccinated humans



The Ebola antibody kits produce data than can be represented as –ve or +ve as compared to the arbitrary cut-off. A 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives. Vaccinated samples can be quantitated as compared to the standards provided in each kit. Human samples were tested at 1:200 dilutions for antibodies to Ebola NP (left panel), anti-GP (middle), and anti-VP40 IgG (right panel). Three samples were above the cut-off for GP IgG and 7 samples were +ve for VP40 IgG. No samples were elevated for antibodies to Ebola NP IgG. Due to the absence of internationally accepted Ebola antibody controls, it is not known if the above samples are truly positive. The presence of antibodies in the samples must not be seen as a sign of active disease. It may indicate exposure to the Ebola virus or related viruses that may have imparted immunity. These kits will be immensely useful to assess the presence of an "**Ebola Immunity**" in the general population and in samples after vaccination.

Ebola Viral proteins (GP, NP, VP40) IgM in non-vaccinated humans



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Monkey/Primate

Non-vaccinated rhesus monkey (R1-3), 2 cynomolgous (C1-2) and 2 baboon (B1-2) serum samples were tested for EBOV IgGs (NP, GP, VP40) at 1:500.

	Random Monkey Samples (non-vaccinated, non-Ebola region)						
	R1	**R2**	**R3**	**C1**	**C2**	**B1**	**B2**
GP IgG	0.01	0.06	0.25	0.22	0.04	0.03	0.03
VP40 IgG	0.01	0.02	0.21	0.04	0.00	0.01	0.02
NP IgG	0.01	0.02	0.02	0.04	0.01	0.01	0.01

Arbitrary A450 values Cut-offs for IgG : Average=<0.100 (Cut-off=0.150)

Unlike the human samples, primate's sera had lower basal antibody values. One rhesus (R3) and one Cyno (C1) had elevated antibody levels.

IgM antibodies to various Ebola proteins were not detectable in non-vaccinated monkey samples.

Pig:

Non-vaccinated pig sera (n=7)) were tested for EBOV IgGs (NP, GP, VP40) at 1:500.

	Random Pig Samples (non-vaccinated, non-Ebola region)					
	95	**96**	**97**	**98**	**99**	**80**
GP IgG	0.05	0.05	0.05	0.17	0.04	0.05
VP40 IgG	0.05	0.04	0.06	0.02	0.07	0.07
NP IgG	0.05	0.05	0.06	0.16	0.04	0.06

Arbitrary A450 values Cut-offs for IgG: Average=<0.100 (Cut-off=0.150)
Unlike the human samples, pig sera had lower basal antibody values.

IgM antibodies to various Ebola proteins were not detectable in non-vaccinated pig samples.

Summary of Human and Animal Testing for Ebola Virus Antibodies

Some non-vaccinated and presumably non-Ebola virus exposed human samples showed the presence of VP40 and GP IgG and IgM but not the NP antibodies. Out of the 3 Ebola virus antibodies, anti-VP40 IgG and IgM appear to be present at higher concentrations and therefore may appear to be more prevalent than GP and NP. Interestingly, other potential mammals (Monkey/primates and pig) have no detectable level or very low levels. Our preliminary but limited data in humans clearly suggests that there is a significant immunity to Ebola virus in non-vaccinated populations, even in areas that are outside the Ebola epidemic, ie. USA. Clearly, more work needs to be done to determine the source of Ebola virus antibodies and its significance.