



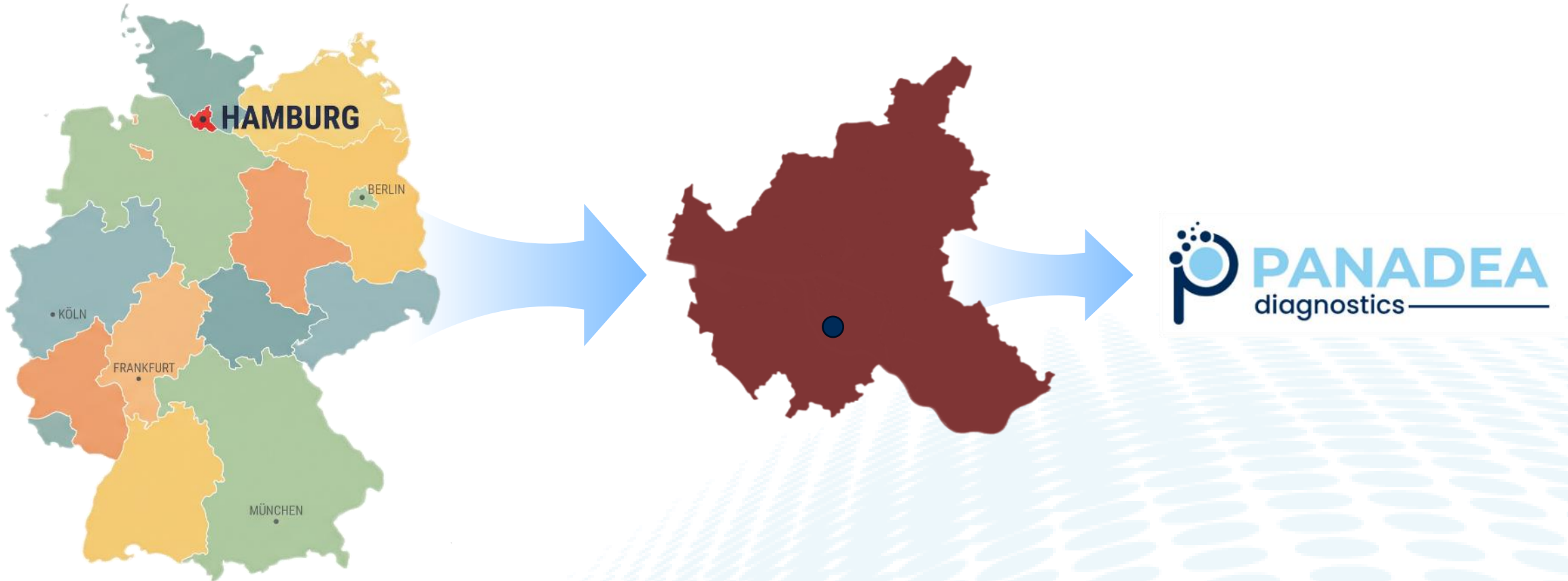
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INTRO

Dr. Yang Liu, CEO

On the map



Disease targets



Pathogen prioritization by International research organizations paves our pipeline

Climate change and globalization lead to:

- More frequent outbreaks with higher case numbers
- Geographic spread of vectors and pathogens

Priority diseases according to the WHO R&D Blueprint:

- Crimean-Congo hemorrhagic fever
- Lassa fever
- Zika fever
- COVID19/SARS/MERS
- Ebola, Sudan and Marburg virus diseases
- Rift valley fever
- Nipah and henipaviral diseases

ELISA portfolio (IgG/IgM)

Current portfolio

Crimean-Congo Hemorrhagic Fever Virus (CCHFV)
Dengue Virus (DENV)
Ebola Virus (EBOV)
Lassa Virus (LASV)
Marburg Virus (MARV)
Rift Valley Fever Virus (RVFV)
Sudan Virus (SUDV)
West Nile Fever Virus (WNV)
Zika Virus (ZIKV)

Development pipeline

Chikungunya Virus (CHIKV)
Hantaviruses
Japanese Encephalitis Virus (JEV)
Monkeypox Virus (MPXV)
Nipah Virus (NiV)
Oropouche Virus (OROV)
Tick-borne Encephalitis Virus (TBEV)
Yellow Fever Virus (YFV)
and more...



Trusted by

	Centers for Disease Control and Prevention, USA		Pasteur Institute of Dakar, Senegal		WHO, Uganda
	Friedrich-Loeffler-Institute, Germany		Tübingen University, Germany		The Pennsylvania State University, USA
	Charite Universitätsmedizin, Germany		University of Glasgow, Scotland		CEPI, Norway
	University Leipzig, Germany		Helmholtz Institute for One Health, Germany		UK Health Security Agency, UK
	CIBERESP, Spain		Nigeria Centre of Disease Control		Kumasi Centre for Collaborative Research in Tropical Medicine, Ghana
	Njala University, Sierra Leone		Institute of Tropical Medicine in Antwerp, Belgium		Federal University of Health Science Otukpo, Nigeria
	Bernhard-Nocht-Institute for Tropical Medicine, Germany		FIOCRUZ, Brazil		Tropical Infectious Diseases Research Center, UAC, Benin

Partner



Scientific advisors



Prof. Jonas Schmidt-Chanasit



Prof. Stephan Günther



Dr. Petra Emmerich

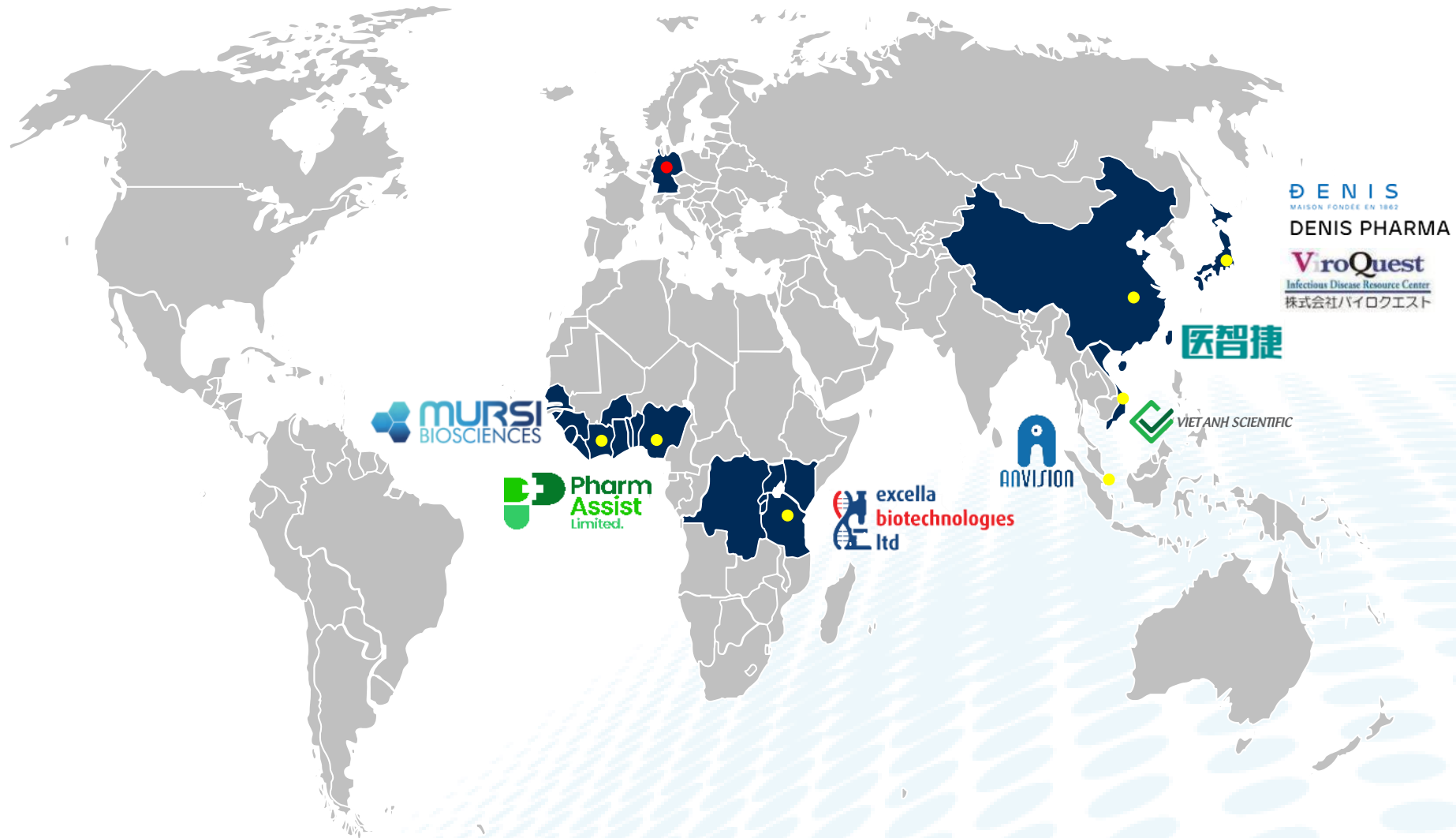


Dr. Markus Hess



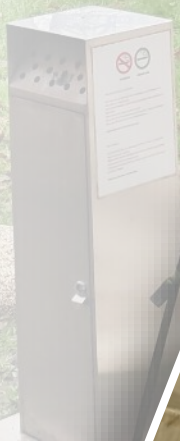
Prof. Rolf Horstmann

Distributors



Meet us

Looking forward to
meeting you at the
next event



Polling question 1 & 2

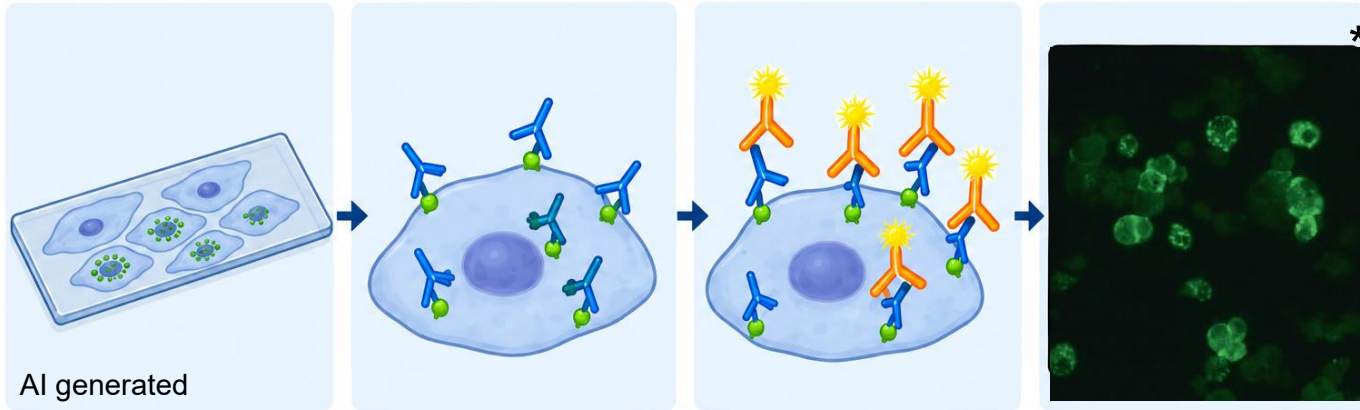


TECHNOLOGY

Mr. Ben Rushton, COO

IgG detection: classical methods

Indirect Immunofluorescence Testing (IIFT)



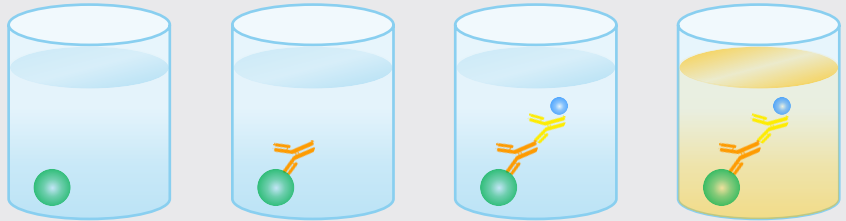
- Cells infected with native virus
- Sample serum
- Fluorescence-labeled species-specific secondary antibody

Enzyme-Linked Immunosorbent Assay (ELISA)



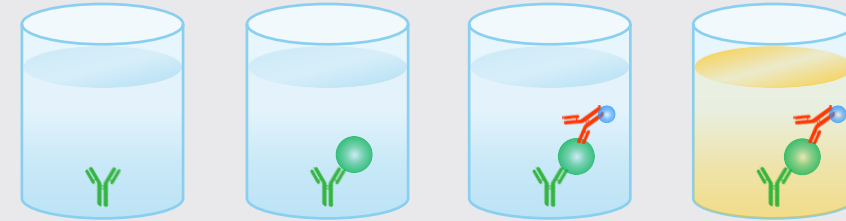
- Plates coated with antigen
- Sample serum
- Labeled species-specific secondary antibody

Traditional ELISA technologies



Indirect ELISA

- Antigen on surface “captures” analyte antibody
- Labelled secondary antibody for signal

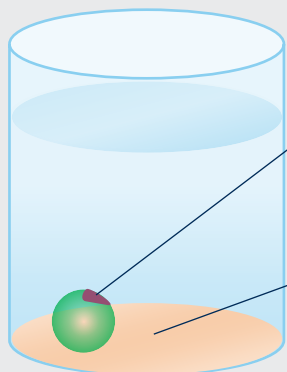


Capture (sandwich) ELISA

- Antibodies on surface capture analytes
- Labelled paired secondary antibody for signal

Parameter determining components

Indirect ELISA

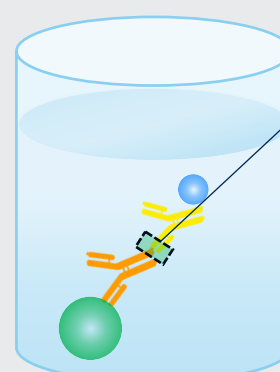


Target antigen epitopes

- Number & accessibility
- Surface variation/conservation

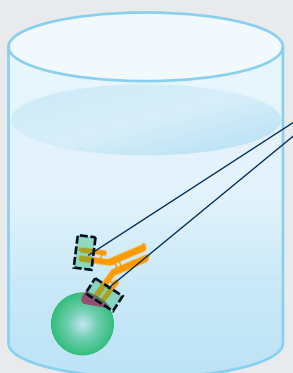
Surface blocking

- Blocking molecule size
- Completeness of coverage



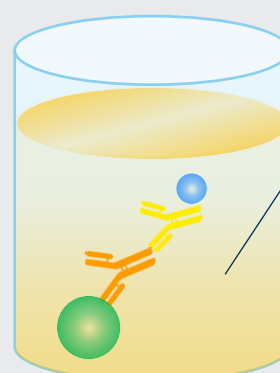
Detection antibody paratopes

- Affinity determines retention during wash
- Binding of both complexed and uncomplexed antibodies
- Species specific (anti-human/anti-mouse etc.)



Analyte antibody paratopes

- Strength of interaction (affinity)
- High conc. / low affinity may read as low conc. / high affinity



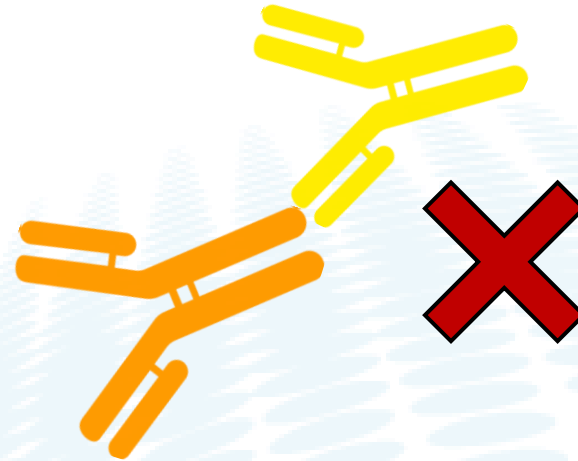
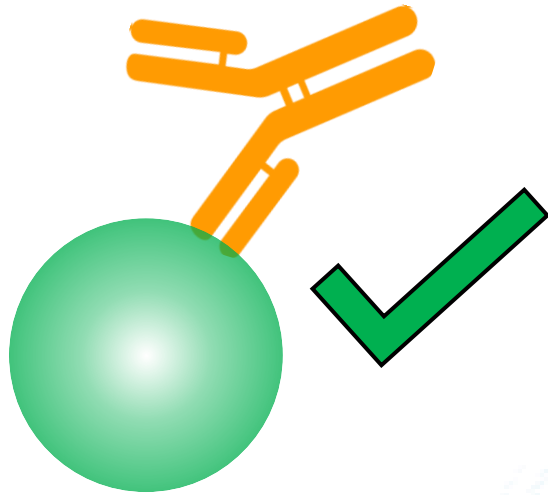
Detection physics/chemistry

- Chromogenic vs fluorescence
 - Assay operating ranges
 - Read-out

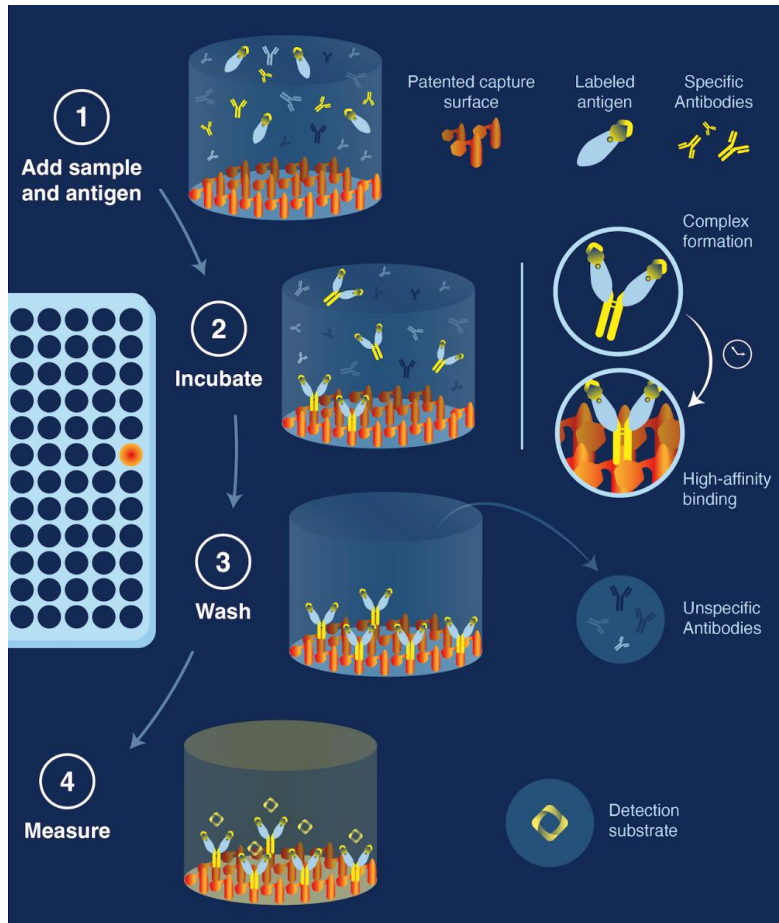
So what's new?



Consider:
How would the body do it?

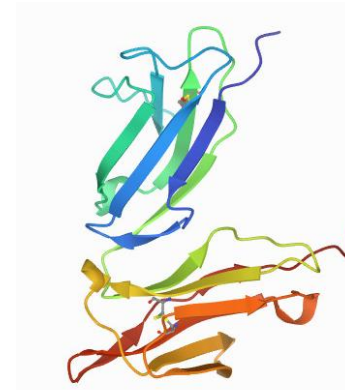
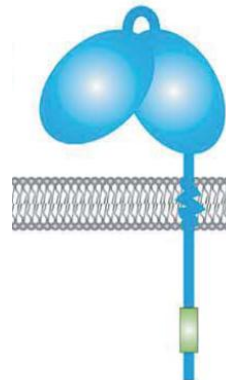


Fc receptors as capture molecules in immunoassays



Example: IgG Fc γ RIIA ELISA

- Patented technology
- Recombinant Fc-receptors for capture
- Platform tech flexible for different species / pathogens
- Preserves conformational epitopes
- Cross-reactivities are suppressible with competitor molecules (“Specificity Enhancer”)



Thank you for your attention

For further questions, please feel free to contact me:
B.Rushton@Panadea-Diagnostics.com

Polling question 3

PERFORMANCE

Dr. Christina Deschermeier, CSO



Intended use and choice of antigen/assay

Intended use e.g.:

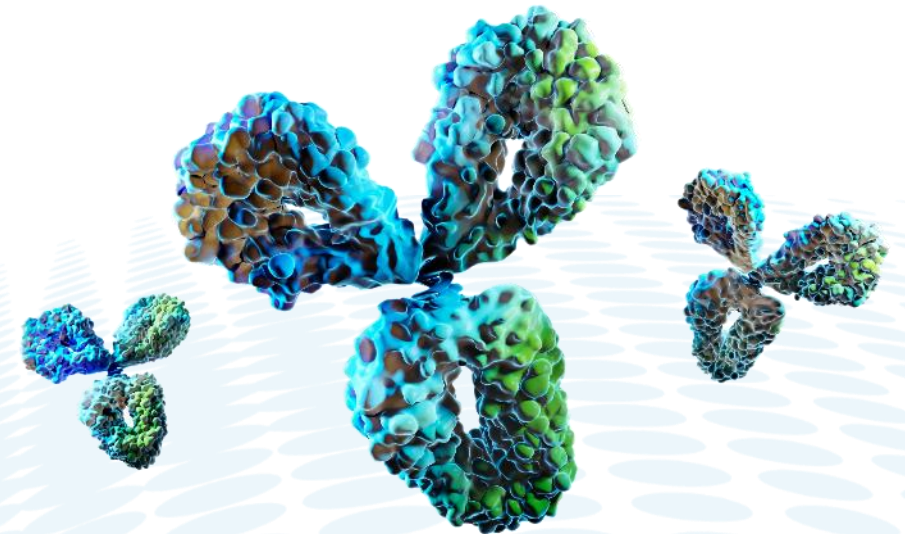
- Acute diagnostics or seroprevalence studies?
- Screening or differentiation?
- Evaluation of vaccine responses or response to infection?
- Differentiation between vaccination/infection?

Choice of antigen:

- Antigenicity?
- Pathogen specificity?
- Conservation between pathogen strains/lineages?
- Antigen used in vaccines?

Choice of assay:

- Antigen/assay principle matching intended use?
- Relevant validation data available for target population?



Human-pathogenic flaviviruses

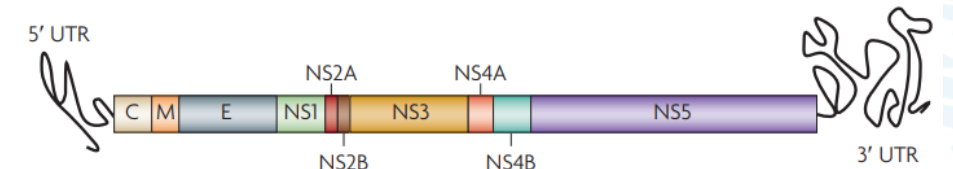
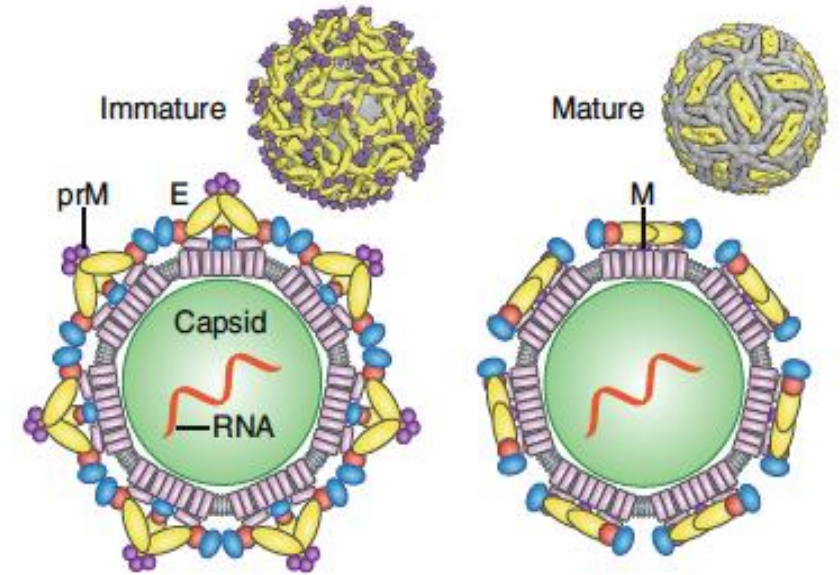
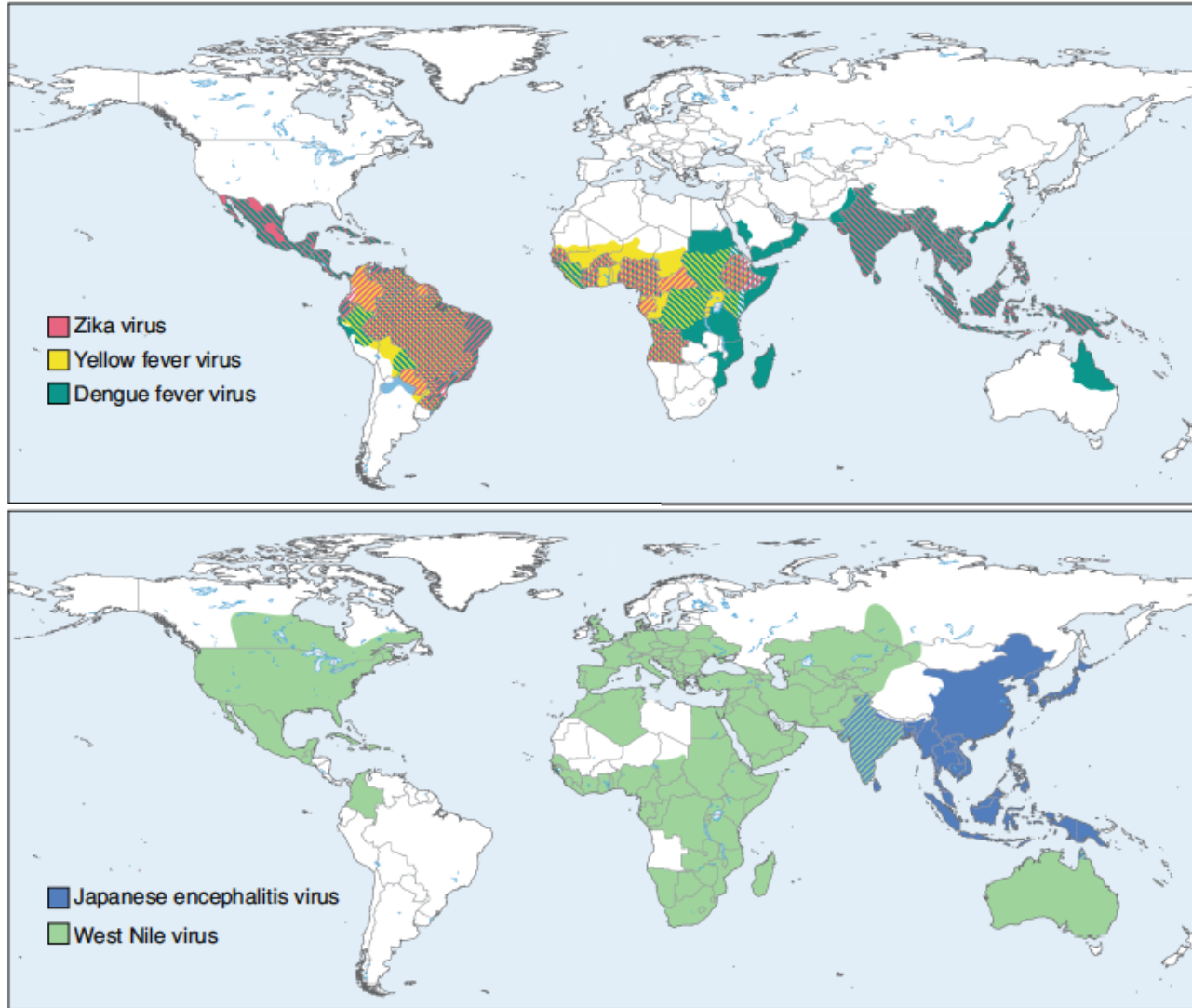
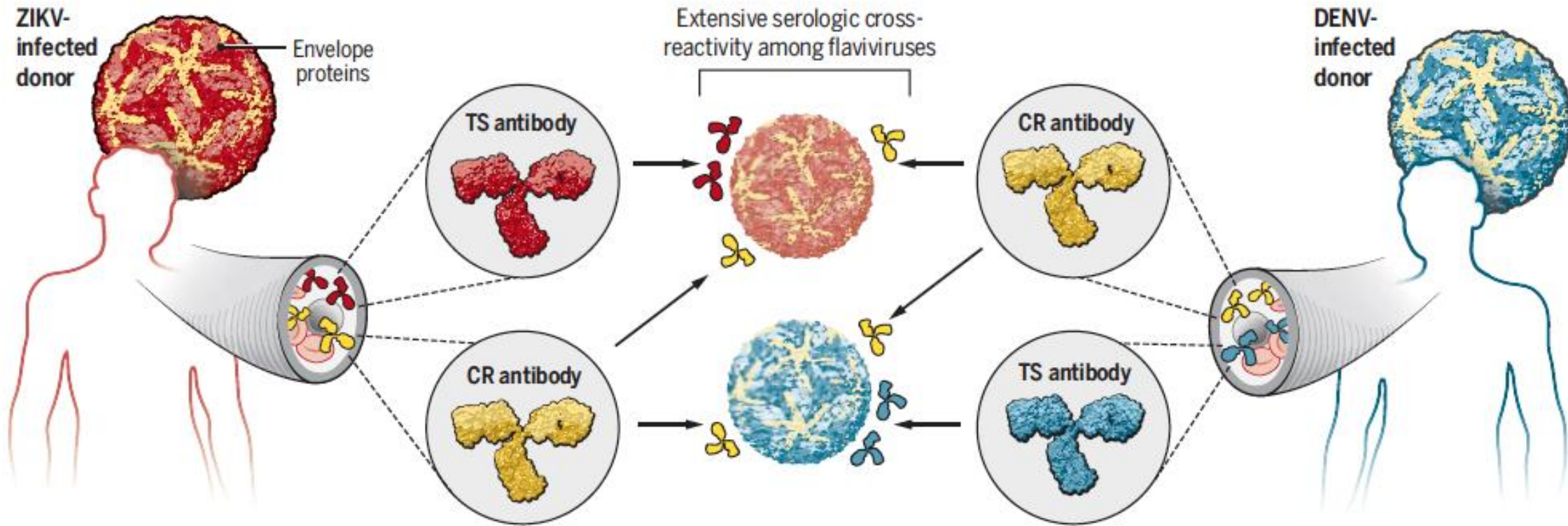


Figure 2 | **The dengue virus genome.** The single open reading frame encodes three structural proteins (the capsid (C), membrane (M) and envelope (E) glycoproteins) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5).

Cross-reactivity of anti-flavivirus IgG



Why specificity of flavivirus serological tests is important?

- Complications (congenital, hemorrhagic, neurological)
- Serostatus prior to vaccination (DENV)
- Informative value of seroprevalence studies

Quiz question 1

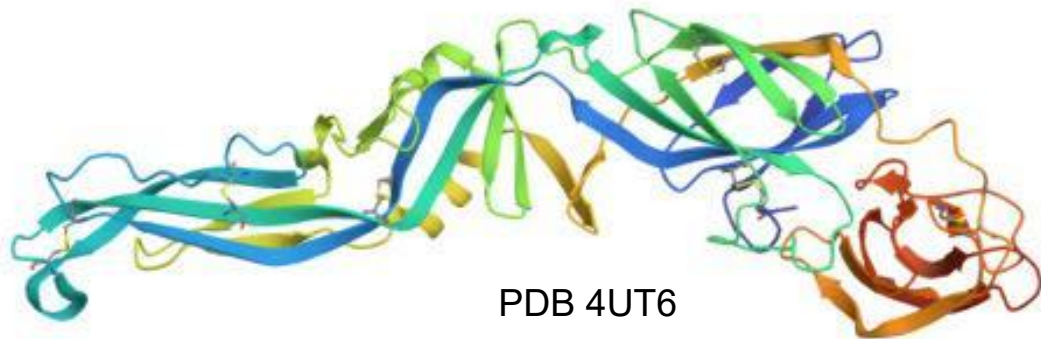
Use of flavivirus antigens in serology

Extent of observed cross-reactivity is influenced by:

- Serum panel (e.g. primary vs. secondary infections, previous flavivirus vaccinations)
- Assay principle (e.g. IIFT, ELISA)
- Antigen or antigen fragment (e.g. full virus, EP, EDIII, NS1)

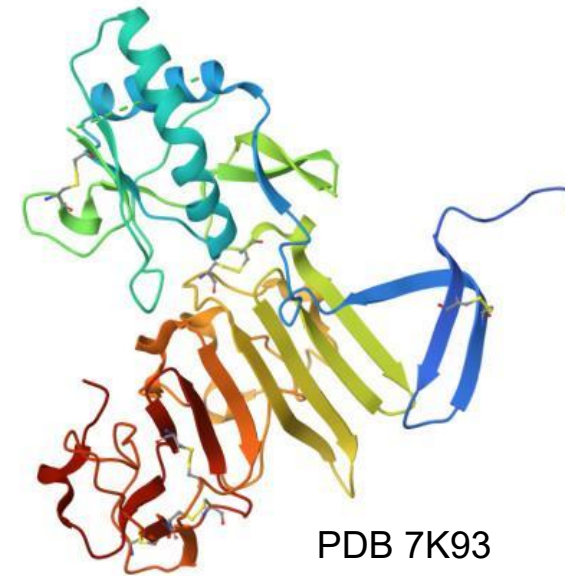
Envelope Protein EP (DI/II/III)

Envelope Protein Domain III (EDIII)



PDB 4UT6

Non-Structural Protein 1 (NS1)



PDB 7K93

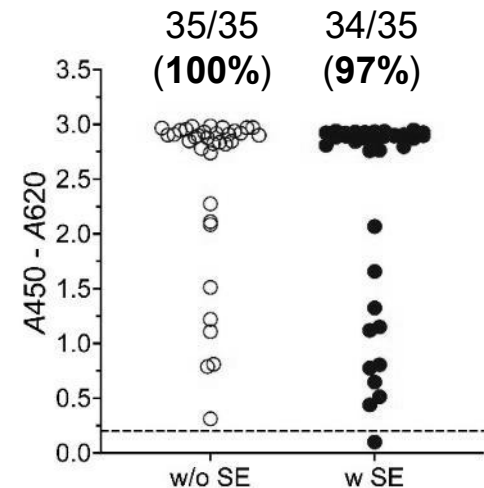
Suppression of cross-reactivity (CR) in IgG ELISAs

Assay	Antigen	Optional: Specificity Enhancer (SE)
pan-DENV IgG FcγR ELISA	DENV1-4 NS1 ^{HRP}	Suppression of ZIKV, WNV, USUV, JEV, YFV IgG CR
YFV IgG FcγR ELISA	YFV NS1 ^{HRP}	Suppression of DENV, ZIKV, WNV, USUV, JEV IgG CR
ZIKV IgG FcγR ELISA	ZIKV NS1 ^{HRP}	Suppression of DENV, WNV, USUV, JEV, YFV IgG CR
WNV IgG FcγR ELISA	WNV NS1 ^{HRP}	Suppression of DENV, ZIKV, USUV, JEV, YFV IgG CR

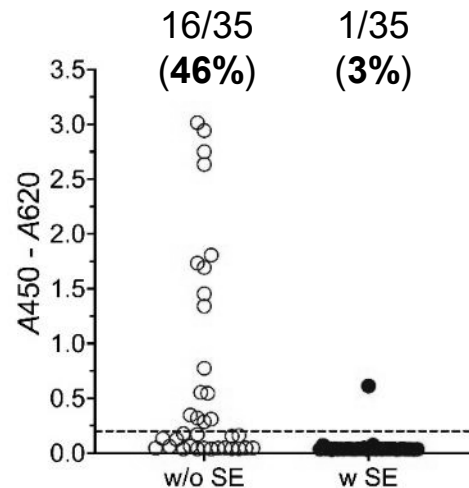
Samples:

- 35 samples from patients with PCR-confirmed DENV infection
- 2013 Savannakhet Provincial Hospital (Lao PDR)
- median 20 days post onset of symptoms (range 14 – 42)

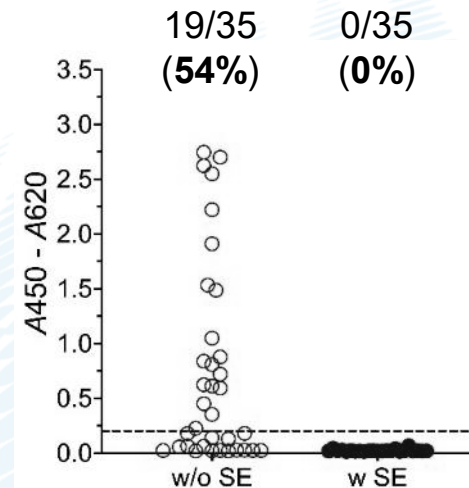
pan-DENV IgG FcγR ELISA



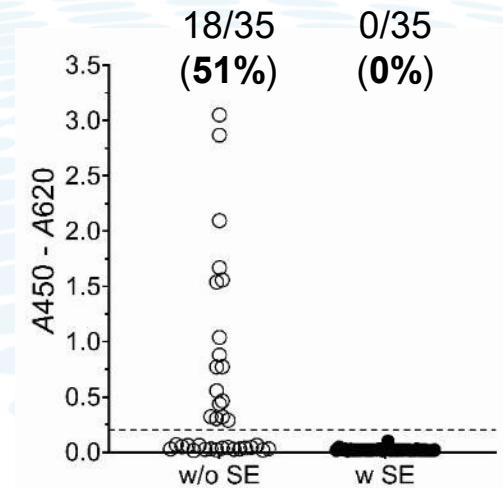
YFV IgG FcγR ELISA



ZIKV IgG FcγR ELISA



WNV IgG FcγR ELISA



Panadea DENV (NS1) IgG SE ELISA reagents

Assay reagents	
Plate	Capture molecule <i>HsFcγRIIa</i>
Recombinant antigens	Conjugate (HRP-labeled DENV NS1)
	Specificity Enhancer (<i>SE</i>)
Controls	Positive Control (ready-to-use)
	Crossreactive Control (ready-to-use)
	Negative Control (ready-to-use)
Other reagents	Serum Dilution Buffer
	Conjugate Dilution buffer
	10X Wash Buffer
	TMB Substrate
	Stop Solution



Panadea DENV (NS1) IgG SE ELISA protocol

Assay protocol

Step 1	Pipette 25 µl Conjugate/SE working solution per well
Step 2	Pipette 25 µl serum dilution or ready-to-use control per well
Step 3	Seal plate and incubate for 24h at 4°C in wet chamber
Step 4	Wash plate: 3 x 300 µl with 30 sec soak between washes
Step 5	Pipette 100 µl TMB Substrate per well
Step 6	Incubate for 20 min at room temperature in the dark
Step 7	Stop reaction by adding 100 µl Stop Solution per well
Step 8	Measure OD at 450 nm and 620 nm

- Check assay validity
- Calculate index values for tested samples

Analysis of results

Results can be interpreted as valid if the following criteria are met:

- $OD_{450} - OD_{620}$ of Positive Control $> PC_{min}$ value as shown on the batch-specific Certificate of Analysis
- $OD_{450} - OD_{620}$ of Specificity Control < 0.100
- $OD_{450} - OD_{620}$ of Negative Controls < 0.100

Calculate $OD_{neg,av}$ from the absolute OD values of the two Negative Control wells by using the following formula:

$$OD_{neg,av} = \frac{(OD_{450_{neg1}} - OD_{620_{neg1}}) + (OD_{450_{neg2}} - OD_{620_{neg2}})}{2}$$

Use $OD_{neg,av}$ to calculate the assay cut-off OD_{CO} according to the following formula:

$$OD_{CO} = OD_{neg,av} + 0.150$$

The Index Values (IV) for the tested samples can then be calculated according to:

$$IV_{Sample} = \frac{OD_{450_{Sample}} - OD_{620_{Sample}}}{OD_{CO}}$$

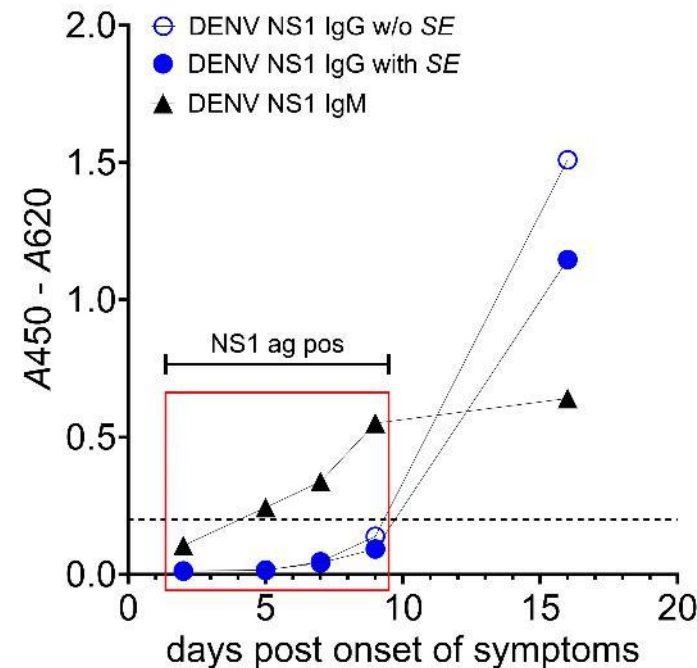
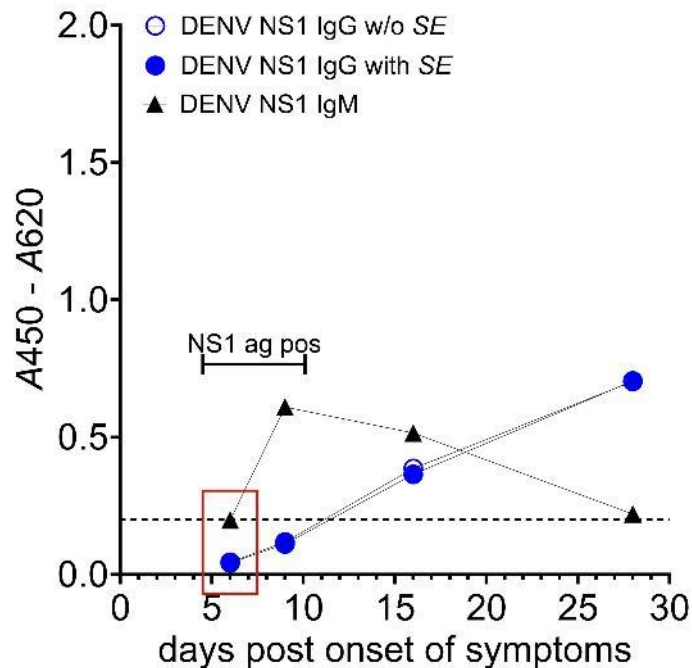
Classify the obtained results according to the table below.

Index Value (IV)	Result
$IV_{Sample} \geq 1.100$	positive
$0.900 < IV_{Sample} < 1.100$	equivocal
$IV_{Sample} \leq 0.900$	negative

Panadea DENV (NS1) IgG SE ELISA validation

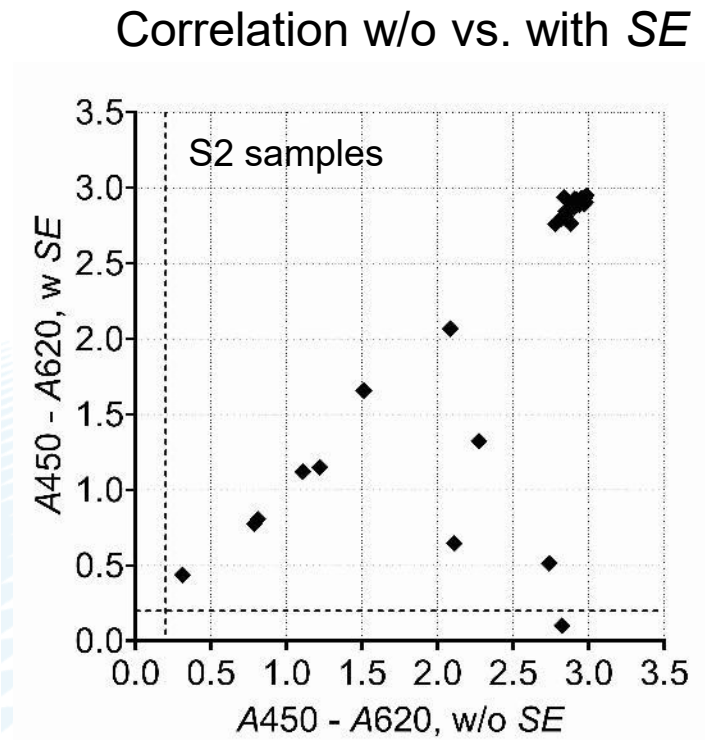
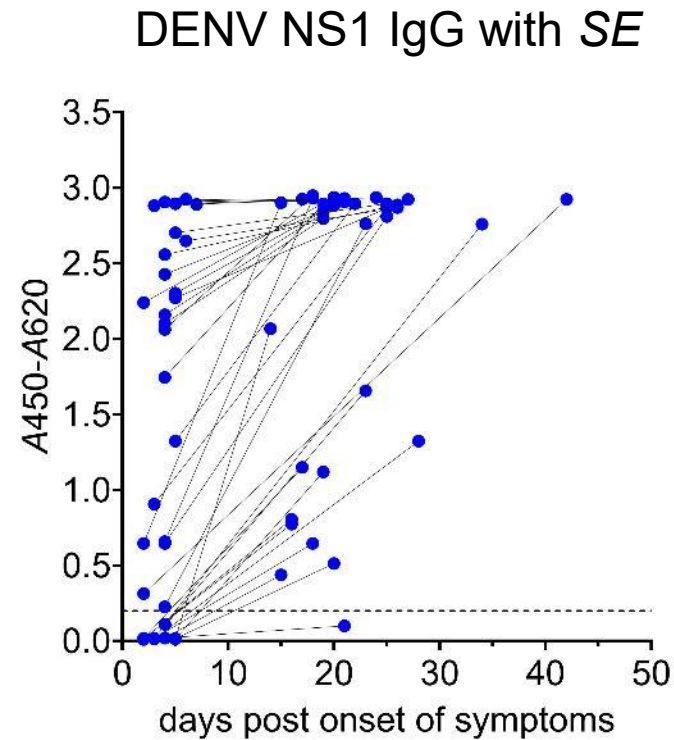
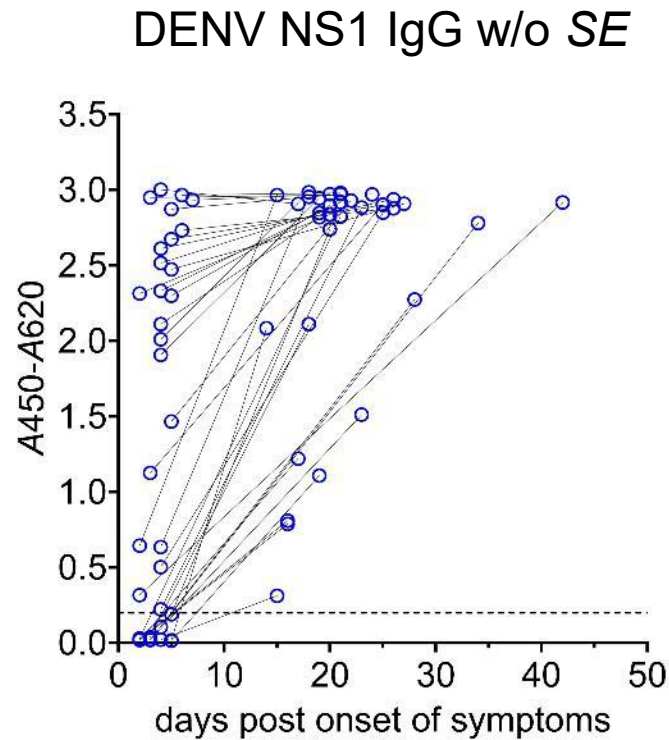
Specificity: healthy donors from non-endemic countries with and w/o YFV/TBEV/JEV vaccinations
patients with flavivirus infections other than DENV
patients with other infections (e.g. CHIKV, SARS-CoV-2, *Plasmodium*)
650/662 (98.2%) negative

Sensitivity: seroconversion in patients with PCR-confirmed DENV infection (travelers)



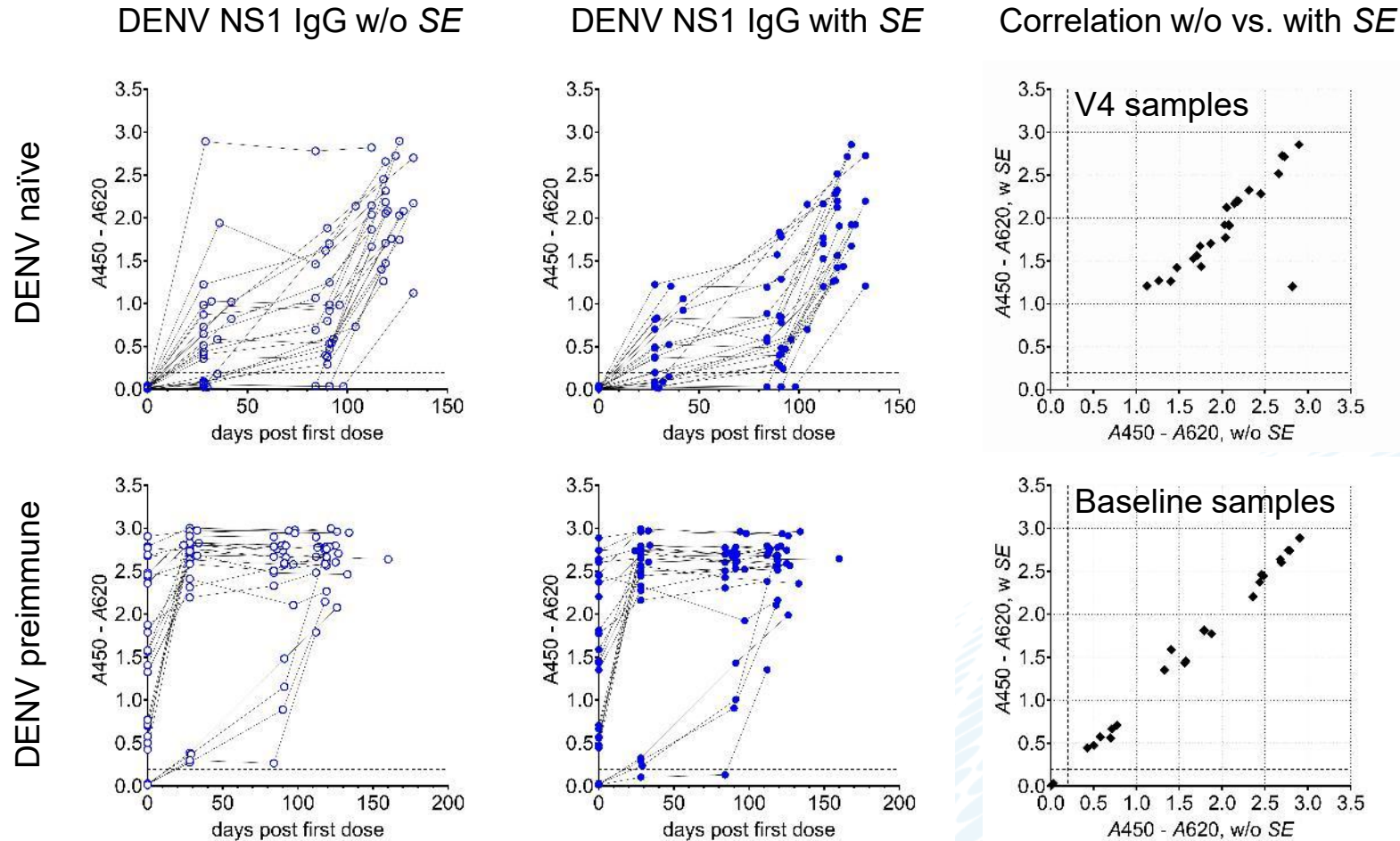
Panadea DENV (NS1) IgG SE ELISA validation

Sensitivity: seroconversion in patients with PCR-confirmed DENV infection (Lao PDR residents)



Panadea DENV (NS1) IgG SE ELISA validation

Sensitivity: seroconversion in individuals post DENV vaccination (Qdenga)



50 individuals receiving DENV vaccination (25 naïve, 25 preimmune)

Sampling:

- Baseline/administration of first dose
- 30 days after first dose
- 90 days after first dose/administration of second dose
- 120 days after first dose

in collaboration with

BNITM

Bernhard Nocht Institute for Tropical Medicine



Application: seroprevalence pilot study Gabon

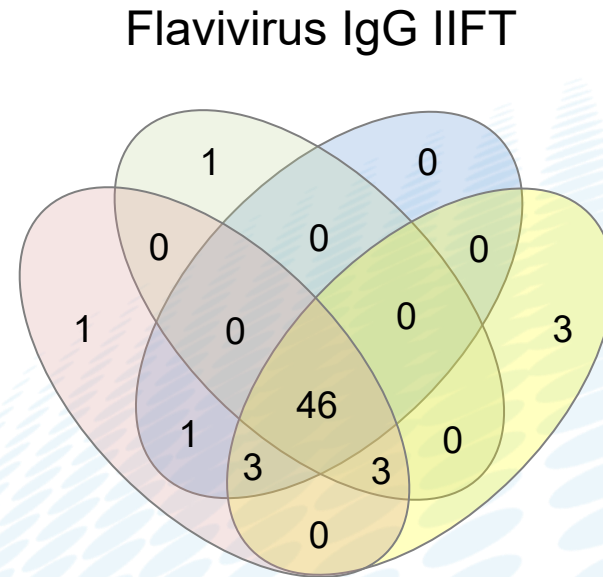
Sample ID	Matrix	Flavivirus IgG IIFT	pan-DENV (NS1) IgG		ZIKV (NS1) IgG		WNV (NS1) IgG		WNV (NS2) IgG		YFV (NS1) IgG	
			with SE	with SE	with SE	with SE	with SE	with SE	with SE	with SE	with SE	with SE
Eco-005	pos	pos	7.07	7.01	0.15	nt	0.31	nt	0.27	nt	0.31	nt
Eco-014	pos	pos	1.02	1.15	0.15	nt	0.23	nt	0.20	nt	0.21	nt
Eco-024	pos	pos	7.89	5.37	0.14	nt	0.45	nt	0.35	nt	0.41	nt
Eco-033	pos	pos	6.54	5.18	0.17	nt	0.30	nt	0.25	nt	0.43	nt
Eco-067	pos	pos	1.72	1.51	0.14	nt	0.34	nt	0.26	nt	0.44	nt
Eco-067	neg	pos	13.17	14.37	0.35	nt	0.43	nt	0.40	nt	0.70	nt
Eco-113	pos	pos	6.35	1.54	0.14	nt	0.25	nt	0.20	nt	0.20	nt
Eco-114	pos	pos	2.49	1.91	0.15	nt	0.33	nt	0.23	nt	0.27	nt
Eco-119	pos	pos	4.45	6.25	0.15	nt	0.19	nt	0.26	nt	0.25	nt
Eco-132	pos	pos	11.59	13.89	0.15	nt	0.71	nt	0.69	nt	0.49	nt
Eco-145	pos	pos	4.55	5.52	0.14	nt	0.21	nt	0.22	nt	0.85	0.99
Eco-163	neg	pos	6.76	6.63	0.49	nt	0.42	nt	0.31	nt	0.32	nt
Eco-168	neg	pos	13.74	14.52	0.49	nt	0.53	nt	0.45	nt	0.32	nt
Eco-197	pos	pos	1.72	1.63	0.15	nt	0.30	nt	0.26	nt	0.24	nt
Eco-217	neg	pos	2.40	1.94	0.27	nt	0.23	nt	0.22	nt	0.22	nt
Eco-262	neg	pos	8.51	6.13	0.20	nt	0.38	nt	0.27	nt	0.25	nt
Eco-269	neg	pos	10.23	14.88	0.19	nt	0.28	nt	0.28	nt	0.23	nt
Eco-347	neg	pos	2.56	2.44	0.15	nt	0.21	nt	0.24	nt	0.20	nt
Eco-434	pos	pos	12.19	15.14	0.20	nt	0.34	nt	0.34	nt	0.89	nt
Eco-438	neg	pos	10.25	11.51	0.18	nt	0.35	nt	0.35	nt	0.80	0.23
Eco-483	pos	pos	1.94	1.77	0.22	nt	0.47	nt	0.42	nt	1.04	0.30
Eco-495	neg	pos	6.94	7.24	0.29	nt	0.48	nt	0.39	nt	1.04	0.34
Eco-539	neg	pos	5.50	6.16	0.23	nt	0.65	nt	0.54	nt	0.97	0.89
Eco-564	neg	pos	16.24	16.94	2.27	0.16	0.59	nt	0.57	nt	0.93	nt
Eco-565	pos	pos	13.14	15.55	2.60	0.26	0.59	0.36	0.50	0.36	1.19	nt
Eco-564	neg	pos	7.13	6.47	0.13	nt	0.76	0.16	0.80	0.16	1.07	0.88
Eco-578	neg	pos	10.37	13.15	0.29	nt	1.16	0.31	1.36	0.36	1.40	nt
Eco-578	neg	pos	7.18	6.40	0.18	nt	0.21	nt	0.20	nt	1.26	0.85
Eco-580	pos	pos	7.94	6.53	0.19	nt	0.41	nt	0.30	nt	1.19	0.87
Eco-585	pos	pos	9.80	11.95	0.46	nt	0.79	nt	0.80	nt	2.75	0.88
Eco-587	pos	pos	1.96	1.36	0.45	nt	0.97	0.16	0.89	0.16	1.72	0.33
Eco-595	pos	pos	6.54	6.44	0.17	nt	0.21	nt	0.37	nt	1.30	0.59
Eco-598	neg	pos	6.94	6.70	0.30	nt	0.89	nt	0.71	nt	1.19	0.56
Eco-598	neg	pos	2.50	1.85	0.28	nt	0.75	nt	0.56	nt	1.86	0.34
Eco-598	neg	pos	6.95	6.95	0.16	nt	0.16	nt	0.16	nt	4.28	0.80
Eco-594	pos	pos	8.51	4.35	0.34	nt	1.34	0.36	1.32	0.40	2.11	1.08
Eco-603	neg	pos	14.12	16.50	2.27	0.16	1.39	0.16	1.19	0.16	1.89	0.34
Eco-130	neg	pos	16.22	17.85	2.45	0.16	1.39	0.16	1.19	0.16	1.78	0.34
Eco-413	pos	pos	2.60	1.62	1.53	0.16	0.21	0.16	2.35	0.16	0.80	1.05
Eco-591	neg	pos	2.32	1.77	0.15	nt	0.33	nt	0.24	nt	0.44	0.46
Eco-481	neg	pos	10.38	16.19	1.45	0.16	0.85	0.21	1.10	0.20	3.64	1.21
Eco-555	neg	pos	7.11	6.02	0.26	nt	0.70	0.24	0.65	0.24	2.11	nt
Eco-560	pos	neg	6.57	7.39	1.54	0.24	0.73	0.25	4.31	0.24	13.23	1.71
Eco-121	neg	pos	0.15	nt	0.21	nt	0.59	nt	0.49	nt	1.67	1.68
Eco-261	neg	pos	0.25	nt	0.21	nt	0.45	nt	0.36	nt	0.33	7.28
Eco-448	neg	pos	0.44	nt	0.19	nt	0.33	nt	0.30	nt	1.30	1.34
Eco-070	pos	pos	0.76	nt	2.67	1.65	0.32	nt	0.31	nt	0.22	nt
Eco-077	neg	pos	1.89	1.07	0.15	nt	0.29	nt	0.19	nt	0.21	nt
Eco-186	neg	pos	1.25	1.00	0.16	nt	0.21	nt	0.18	nt	0.35	nt
Eco-195	pos	pos	2.46	1.07	0.16	nt	0.21	nt	0.27	nt	0.35	nt
Eco-032	pos	pos	1.62	0.65	0.13	nt	0.29	nt	0.23	nt	0.27	nt
Eco-365	neg	pos	1.49	0.52	0.60	nt	1.66	0.18	1.19	0.19	1.32	0.27
Eco-145	neg	pos	4.28	0.51	0.24	nt	1.59	0.22	2.75	0.47	0.22	nt
Eco-162	neg	pos	6.91	0.15	0.16	nt	2.09	1.06	2.46	0.63	0.63	nt
Eco-139	pos	pos	1.06	0.80	0.19	nt	0.39	nt	0.31	nt	0.43	nt
Eco-122	pos	pos	0.15	nt	0.15	nt	0.24	nt	0.26	nt	0.39	nt
Eco-164	pos	pos	0.36	nt	0.15	nt	0.29	nt	0.24	nt	0.26	nt
Eco-584	neg	pos	0.21	nt	0.14	nt	0.19	nt	0.21	nt	0.17	nt
Eco-437	neg	pos	0.13	nt	0.17	nt	0.25	nt	0.26	nt	0.26	nt
Eco-542	pos	pos	0.26	nt	0.16	nt	0.29	nt	0.26	nt	0.44	nt
Eco-179	neg	pos	0.79	nt	0.25	nt	0.37	nt	0.37	nt	0.60	nt
Eco-305	neg	neg	0.65	nt	0.27	nt	0.55	nt	0.41	nt	0.55	nt
Eco-019	neg	neg	0.15	nt	0.11	nt	0.24	nt	0.24	nt	0.26	nt
Eco-019	neg	neg	0.13	nt	0.13	nt	0.19	nt	0.19	nt	0.51	nt
Eco-019	neg	neg	0.13	nt	0.13	nt	0.19	nt	0.19	nt	0.19	nt
Eco-038	pos	neg	0.26	nt	0.17	nt	0.27	nt	0.35	nt	0.45	nt
Eco-047	pos	neg	0.16	nt	0.14	nt	0.14	nt	0.14	nt	0.26	nt
Eco-043	pos	neg	0.13	nt	0.13	nt	0.18	nt	0.17	nt	0.21	nt
Eco-049	pos	neg	0.17	nt	0.16	nt	0.21	nt	0.20	nt	0.29	nt
Eco-050	neg	neg	0.16	nt	0.12	nt	0.26	nt	0.21	nt	0.19	nt
Eco-050	neg	neg	0.20	nt	0.16	nt	0.20	nt	0.27	nt	0.38	nt
Eco-064	neg	neg	0.15	nt	0.12	nt	0.26	nt	0.19	nt	0.22	nt
Eco-068	neg	neg	0.15	nt	0.24	nt	0.56	nt	0.40	nt	0.34	nt
Eco-107	neg	neg	0.16	nt	0.16	nt	0.16	nt	0.16	nt	0.30	nt
Eco-108	neg	neg	0.19	nt	0.15	nt	0.21	nt	0.21	nt	0.26	nt
Eco-109	neg	neg	0.18	nt	0.16	nt	0.23	nt	0.21	nt	0.23	nt
Eco-118	neg	neg	0.13	nt	0.13	nt	0.13	nt	0.13	nt	0.31	nt
Eco-119	neg	neg	0.21	nt	0.15	nt	0.22	nt	0.23	nt	0.31	nt
Eco-120	pos	neg	0.13	nt	0.14	nt	0.22	nt	0.20	nt	0.26	nt
Eco-127	neg	neg	0.13	nt	0.16	nt	0.22	nt	0.20	nt	0.26	nt
Eco-174	neg	neg	0.13	nt	0.16	nt	0.22	nt	0.20	nt	0.22	nt
Eco-195	neg	neg	0.13	nt	0.15	nt	0.24	nt	0.21	nt	0.21	nt
Eco-211	neg	neg	0.20	nt	0.15	nt	0.20	nt	0.24	nt	0.26	nt
Eco-221	neg	neg	0.14	nt	0.14	nt	0.14	nt	0.22	nt	0.22	nt
Eco-441	pos	neg	0.24	nt	0.18	nt	0.29	nt	0.32	nt	0.32	nt
Eco-305	pos	neg	0.20	nt	0.17	nt	0.24	nt	0.26	nt	0.33	nt
Eco-273	pos	neg	0.13	nt	0.15	nt	0.22	nt	0.21	nt	0.22	nt
Eco-348	pos	neg	0.17	nt	0.17	nt	0.42	nt	0.34	nt	0.30	nt
Eco-417	neg	neg	0.12	nt	0.15	nt	0.24	nt	0.25	nt	0.23	nt
Eco-450	pos	neg	0.16	nt	0.16	nt	0.30	nt	0.24	nt	0.31	nt

Table 1. ELISA results displayed as index values, nt: not tested; samples with an index value ≥ 1.1 are classified as positive.

90 serum samples from febrile patients from Gabon tested with:

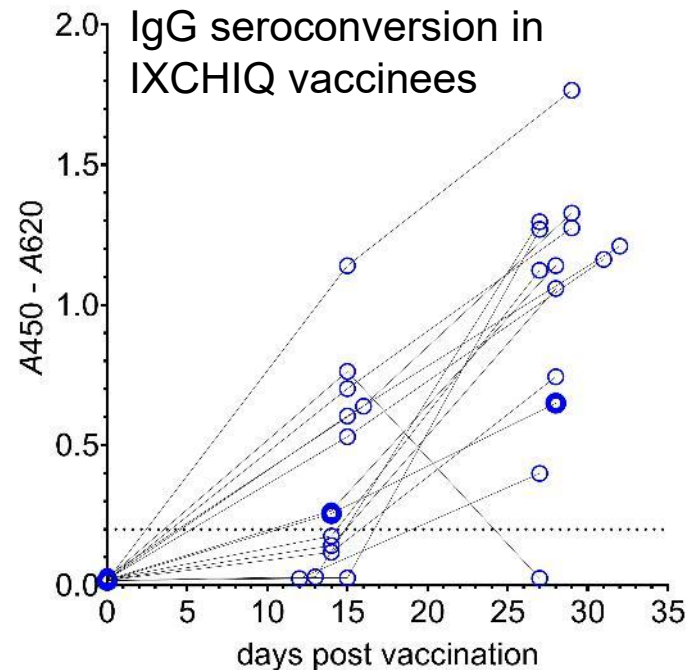
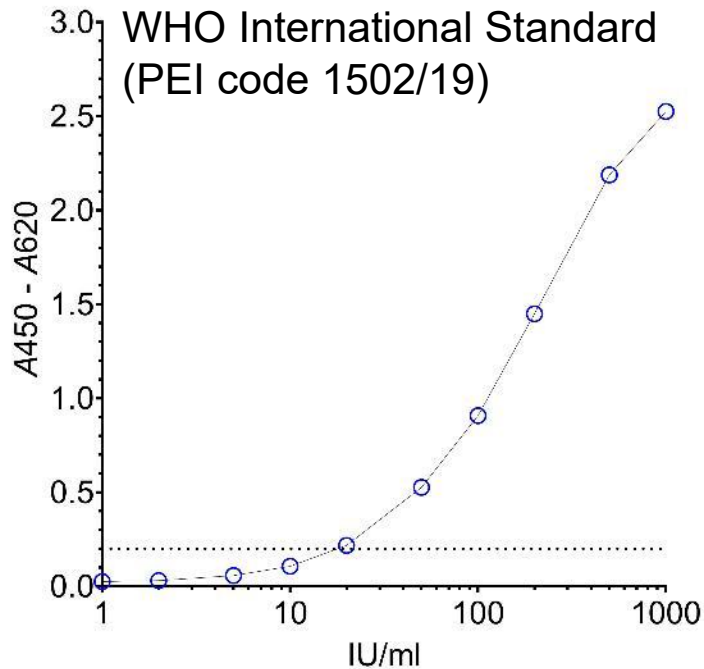
- pan-DENV (NS1) IgG +/- SE
- ZIKV DENV (NS1) IgG +/- SE
- WNV DENV (NS1) IgG +/- SE
- YFV DENV (NS1) IgG +/- SE

in collaboration with



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Panadea CHIKV (E2) IgG ELISA (prototype)



- Seroconversion Lao PDR patient with PCR-confirmed CHIKV infection
- Reactivity with *recomLine* Tropical Fever IgG CHIKV pos serum
- Negative panel HD Germany: 40/40 negative, mean A450 – A620: 0.037, standard deviation: 0.019

in collaboration with

BNITM

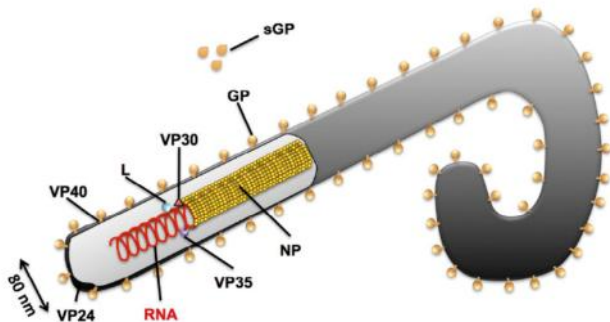
Bernhard Nocht Institute for Tropical Medicine



Panadea filovirus IgG ELISAs

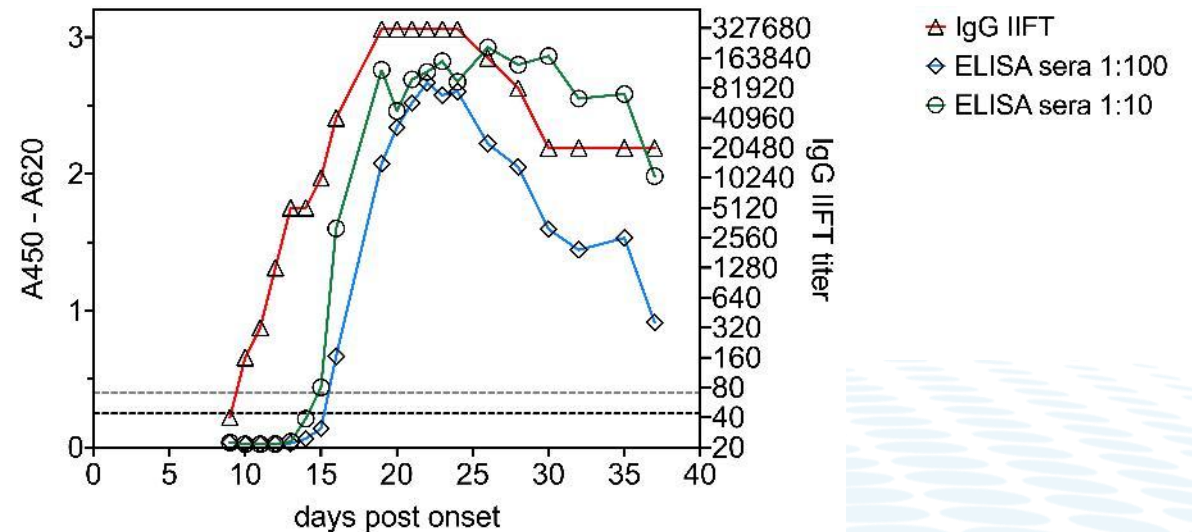
EBOV (NP) IgG ELISA
SUDV (NP) IgG ELISA
MARV (NP) IgG ELISA
Prototype: BDBV (NP) IgG ELISA

Ongoing:
Validation of assays in collaboration with
UVRI (Uganda) and RBC (Rwanda)



Emperor *et al.* (2019), *BMJ Glob Health* 4 (Suppl 2):e001112

Application: seroconversion in acute EBOV infection



in collaboration with

BNITM

Bernhard Nocht Institute for Tropical Medicine



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Product booklet

