THE GLOBAL HEALTH NETWORK
Enabling research by sharing knowledge

ALERRT
African coaLition for Epidemic Research, Response and Training

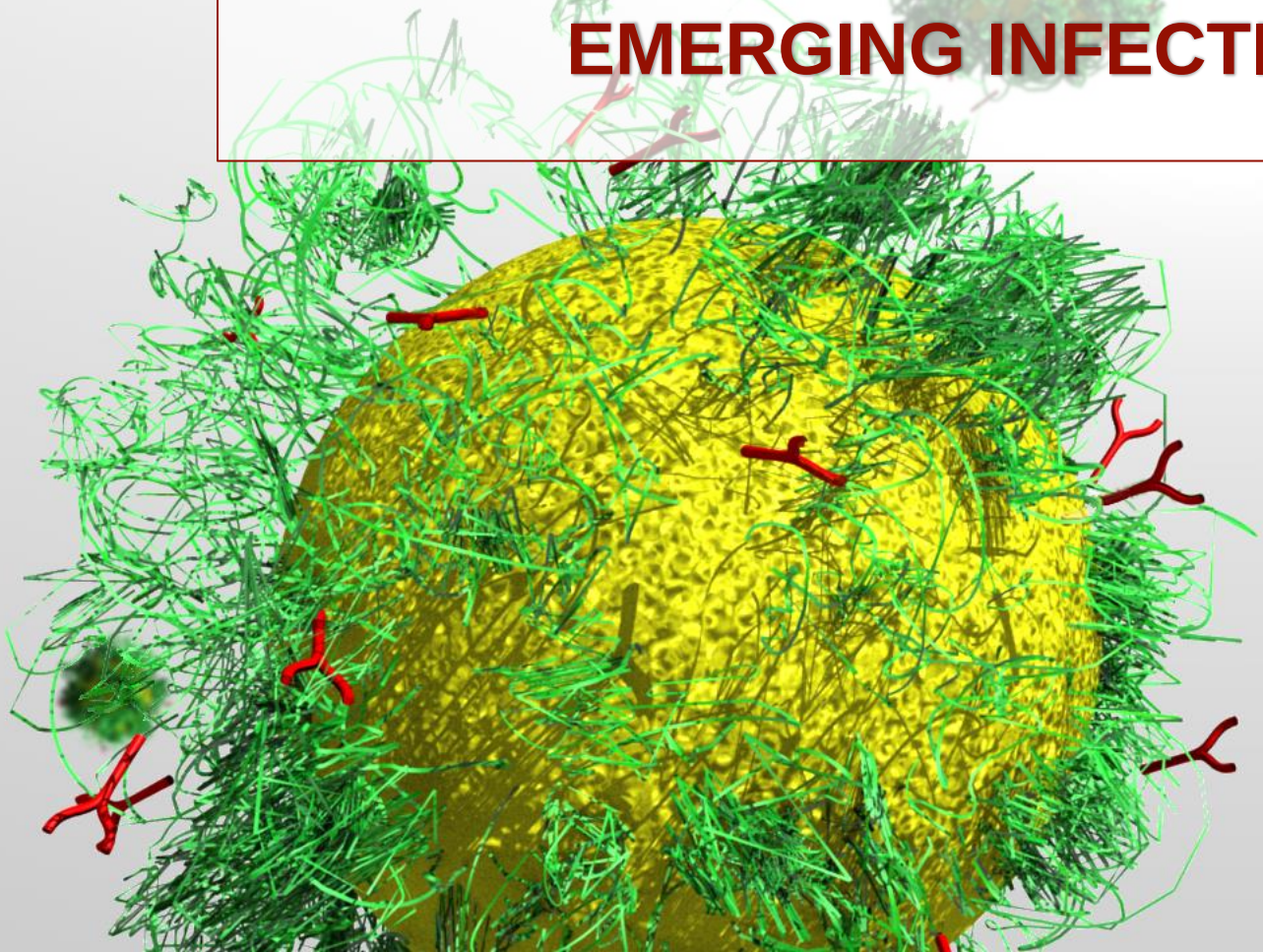
PANDORA
Pan-African Network For Rapid Research, Response and Preparedness for Infectious Diseases Epidemics

CANTAM
Central Africa Network on Tuberculosis, HIV/AIDS and Malaria

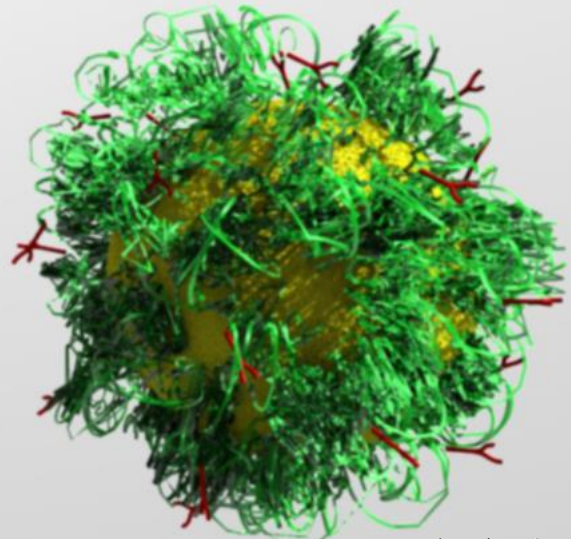
FIOCRUZ
Fundação Oswaldo Cruz

REDe
Research Capacity Network

DEVELOPMENT OF DIAGNOSTIC TOOLS FOR EMERGING INFECTIOUS DISEASES



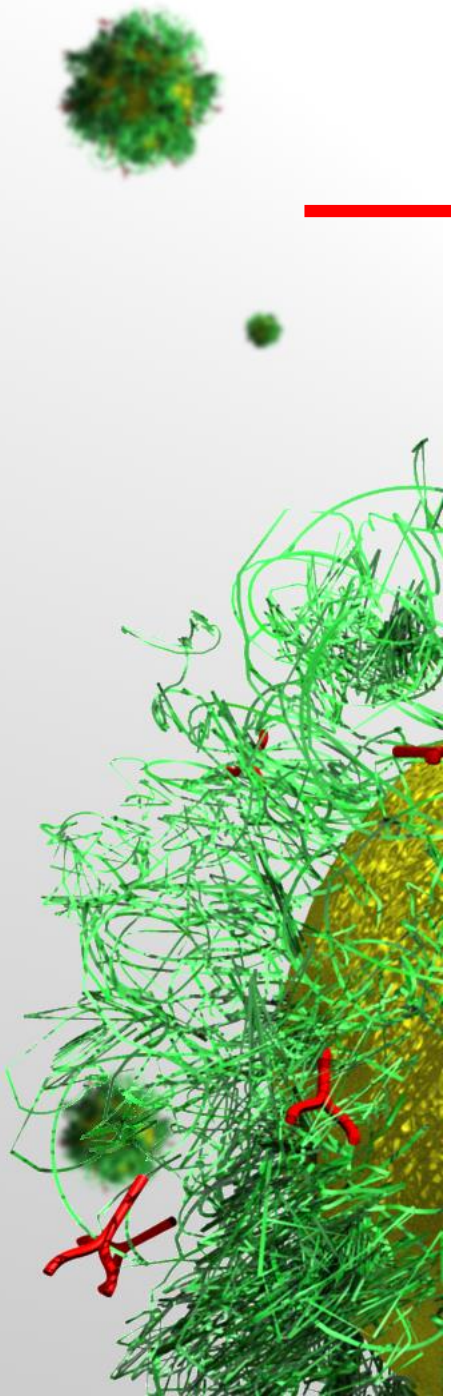
Alice Versiani
DVM, Ph.D.
School of Medicine of
Sao Jose do Rio Preto
FAMERP/Brazil



LPV
FAMERP

Massachusetts Institute of Technology

NanoBioMedical
Research Group



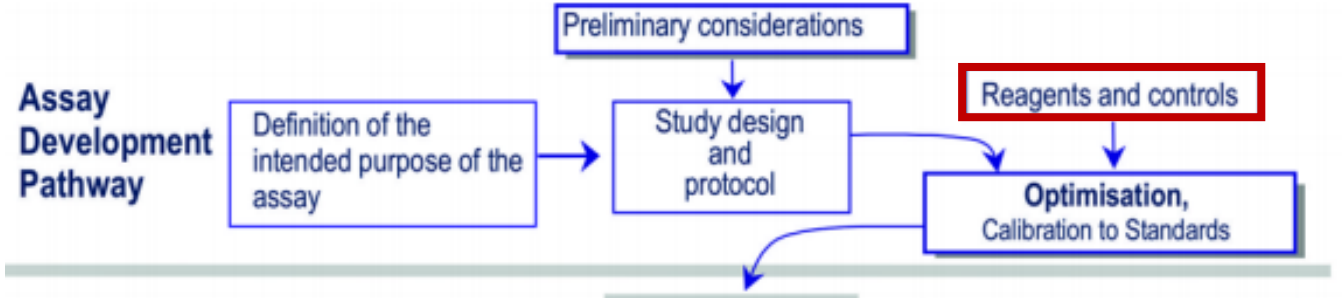
Overview

1. Assay development pathway
   - Definition of intended purpose
   - Controls and samples
   - Validation
   - Types of diagnostic tools (Classic x Novelty)
   - Point-of-care x Laboratory-based tests

2. Brazilian experience
   - Nanotechnology
   - Classic methodologies x new biomolecules
   - New methodologies x classic biomolecules
   - Technologies improvements

3. How to deal with emerging or re-emerging pathogens
   - Scientific networks

Assay Development Pathway

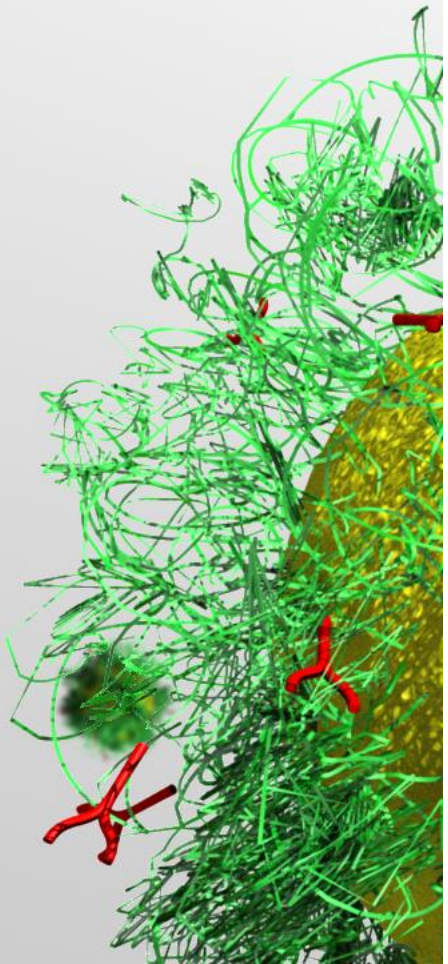


- Definition of the intended purpose of the assay:
  - Quantitative x Qualitative assay
  - Biological sample
    - Individual or pooled, matrix composition, host/organism interactions affecting the target analyte…
  - Assay system
    - Physical, chemical, biological, operator-related factors affecting the capacity of the assay to detect a specific analyte in the sample.
  - Teste results interpretation
    - The capacity of a test result, derived from the assay system, to predict accurately the status of the individual or population relative to the purpose for which the assay is applied.

- Selection, collection, preparation, preservation and management of samples are critical variables in design and development of an assay to ensure valid test results.

OIE. 2019. Principles and methods of validation of diagnostic assays for infectious diseases (chapter 1.1.2)

Validation



- Choosing samples:
  - Gold-standard assay (reference method)
  - Controls
  - Open sera bank
  - Disease epidemiology
  - Cross-reaction

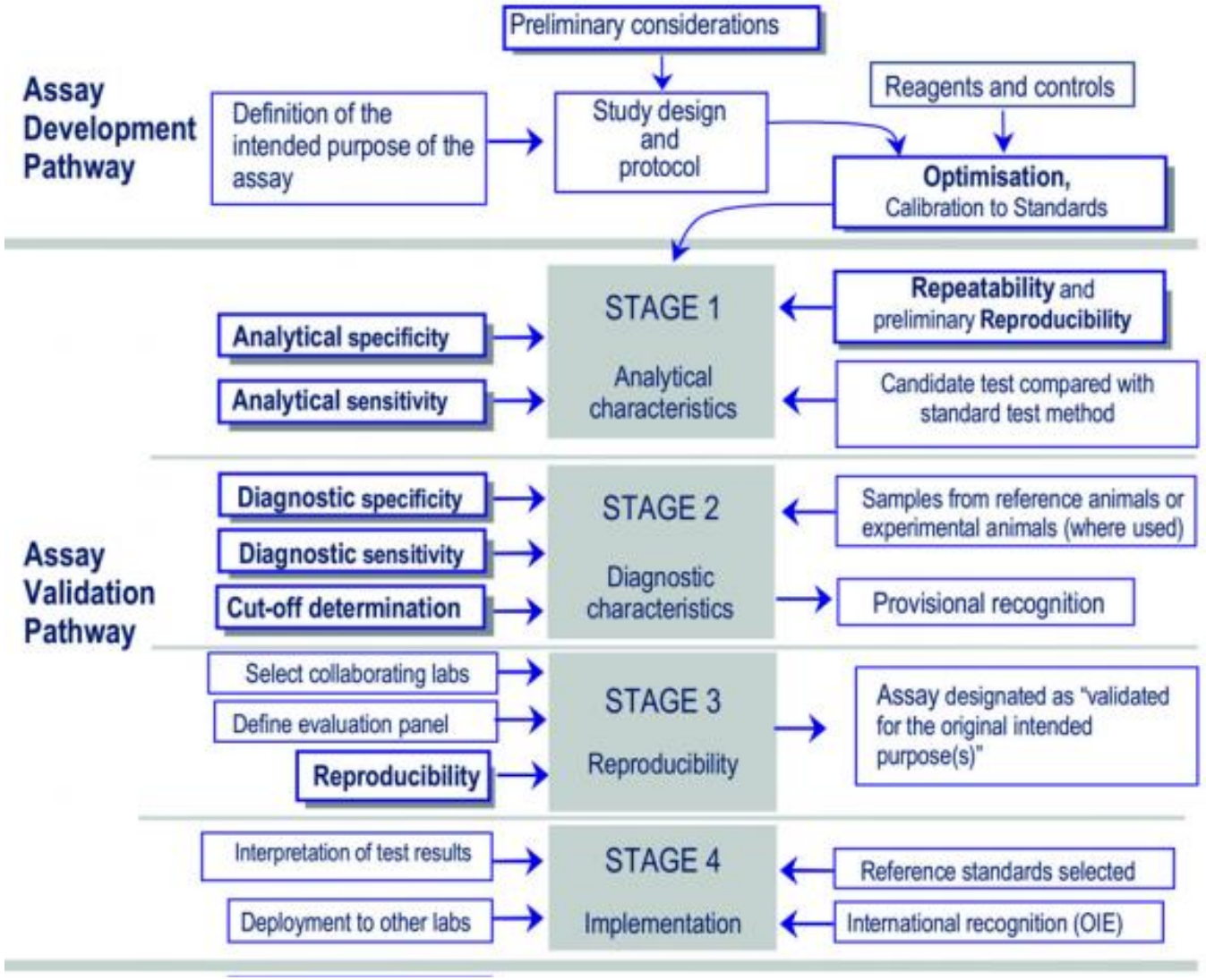
Reference Samples	SARS-CoV-2 Spike Protein Serological IgG ELISA Results					
	Positive	Negative	Inconclusive	Total	PPA	NPA
Positive	28	0	0	28	100%	—
Presumed Negative	2	59	1	62	—	95.2%

SPECIFICATIONS

- Serological Sensitivity - Secondary infection: 97.9% (92/94)
- Serological Sensitivity - Primary infection: 33.3% (28/84)
- Serological Specificity - Negatives: 100% (108/108)

DENV IgG ELISA

Assay Development Pathway

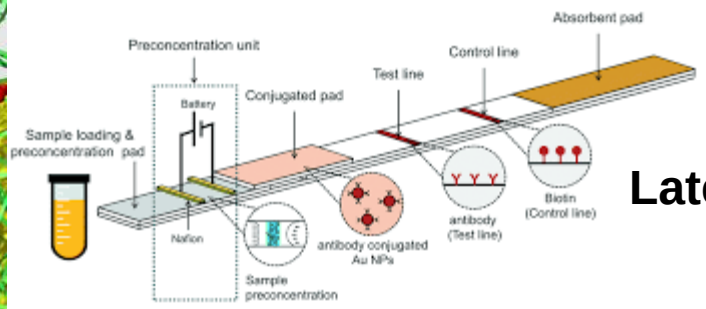


OIE. 2019. Principles and methods of validation of diagnostic assays for infectious diseases (chapter 1.1.2)

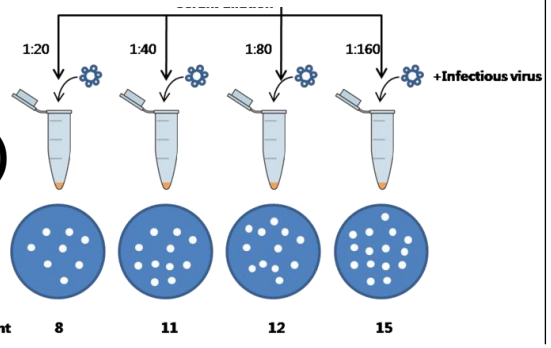
Types of diagnostic assays

SEROLOGICAL ASSAYS

ELISA

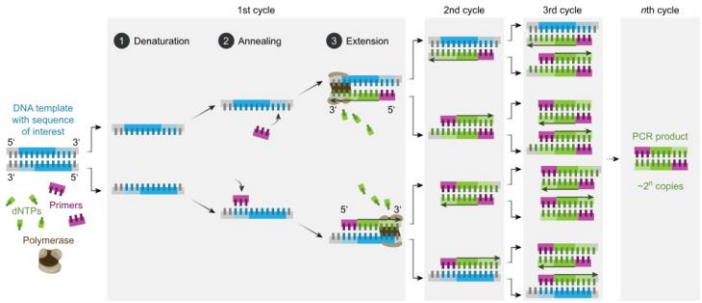


Lateral flow (LFA)

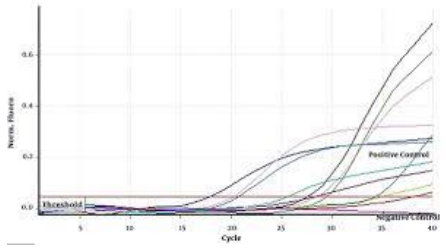


Neutralization (PRNT)

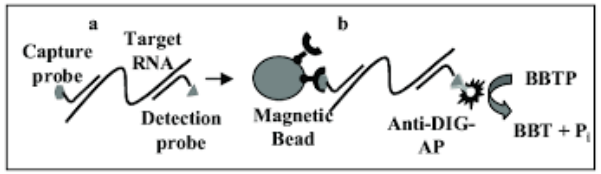
MOLECULAR ASSAYS



PCR

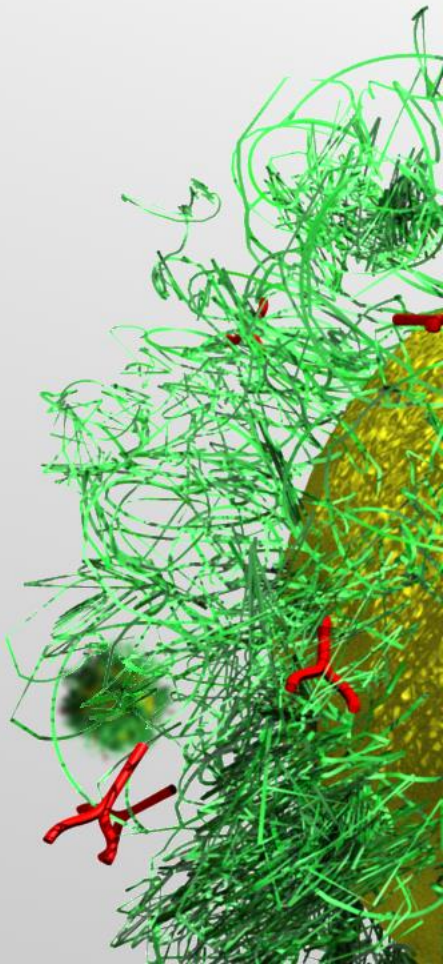


RT-PCR



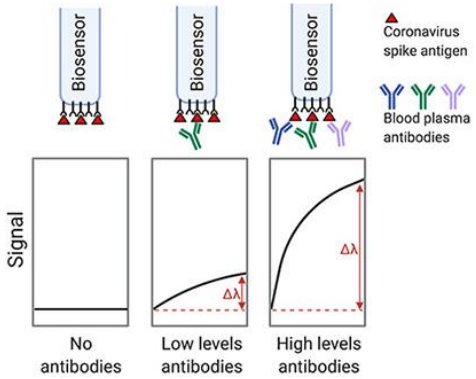
Hybridization

Types of diagnostic assays

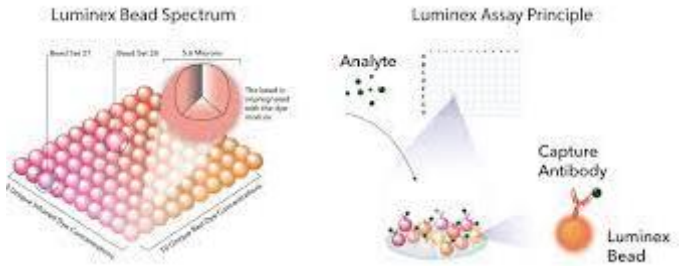


SEROLOGICAL ASSAYS

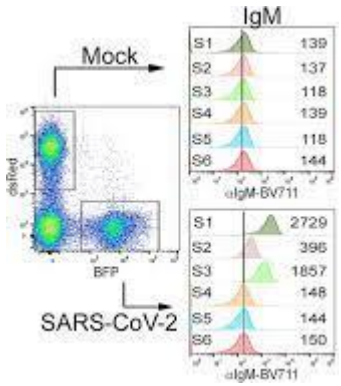
Biosensors



Luminex (bead array)

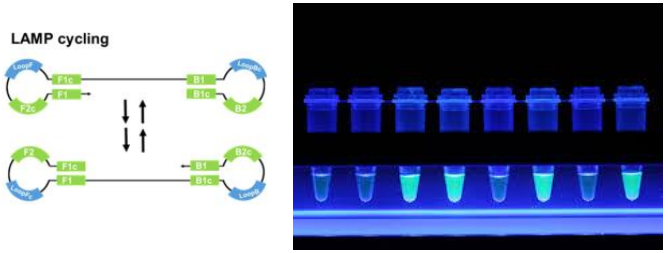


FACS

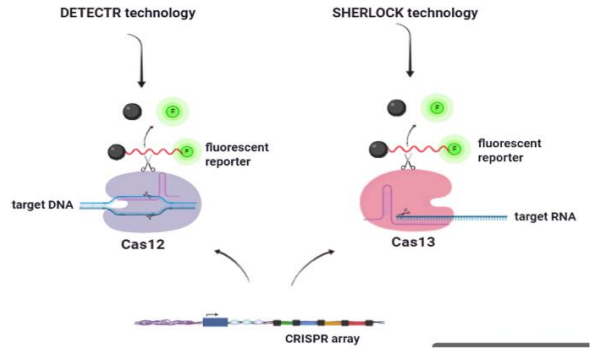


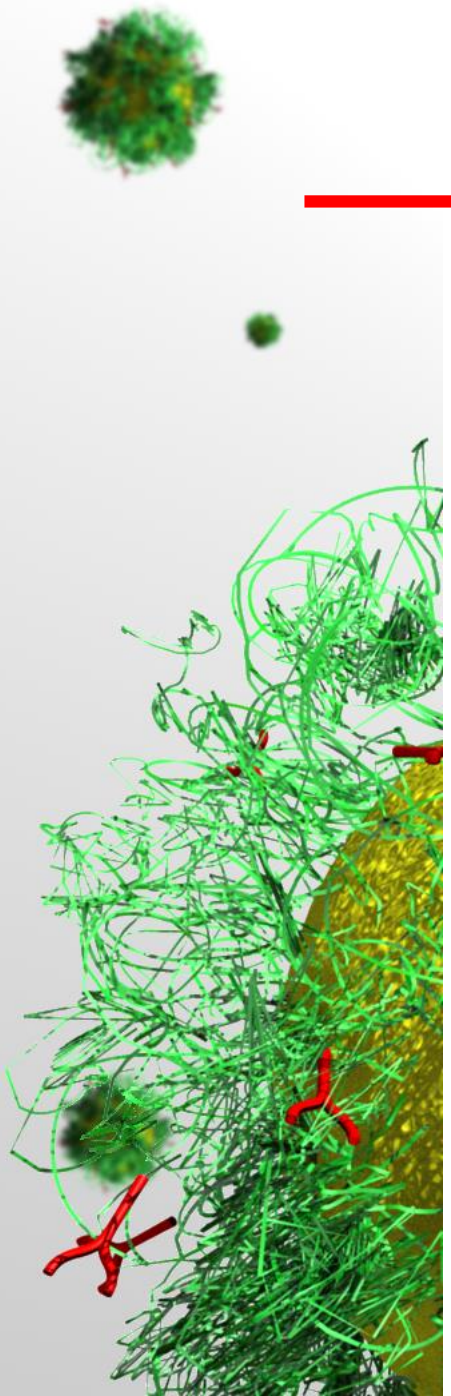
MOLECULAR ASSAYS

LAMP



CRISPR-Cas





Types of diagnostic assays

Point-of-care

- Can be performed near/at the point of patient care.

- Easy to use;
- Portability;
- Low cost;
- Do not require power or additional reagents;
- Results in minutes.

- Inaccuracy;
- Low sensitivity;
- Single-use device;
- Cross-reactivity.

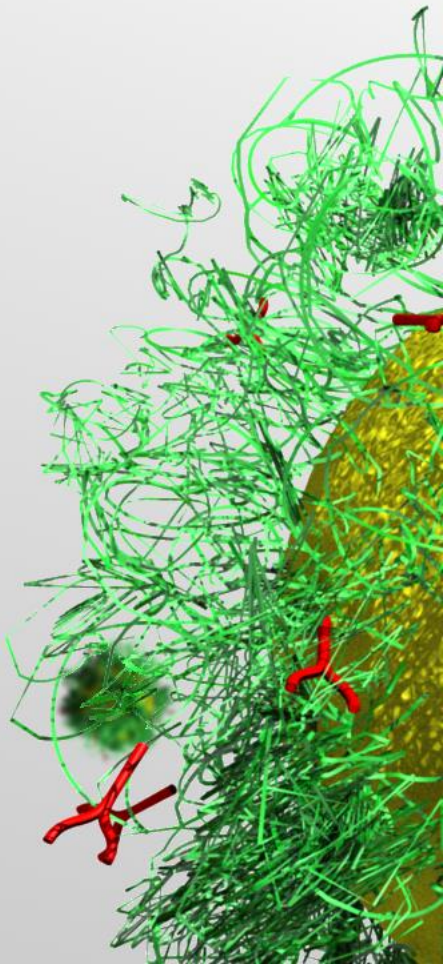
Lab-based tests

- Samples sent to a central laboratory for analysis.

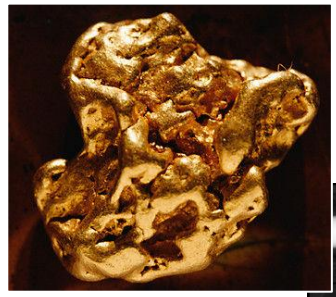
- Accuracy;
- Multiplex assays;
- High diagnostic performance;
- Reproducibility;
- High-end technology.

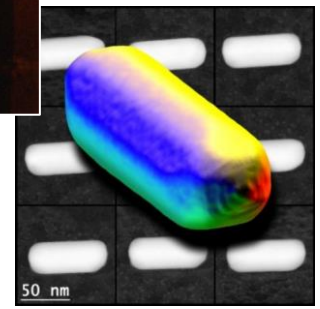
- Higher costs;
- Needs specialized personal and equipment;
- Requires infrastructure.

Nanomedicine



- **Nanotechnology** → creation, manipulation and exploration of materials on a nanoscale.
  - The physical and chemical properties of matter are, to a large extent, determined by the type of motion of its electrons.



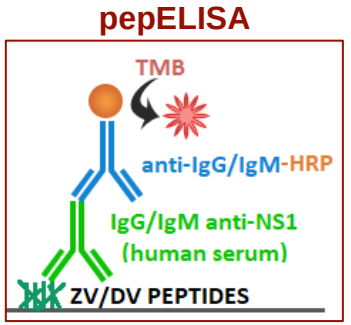


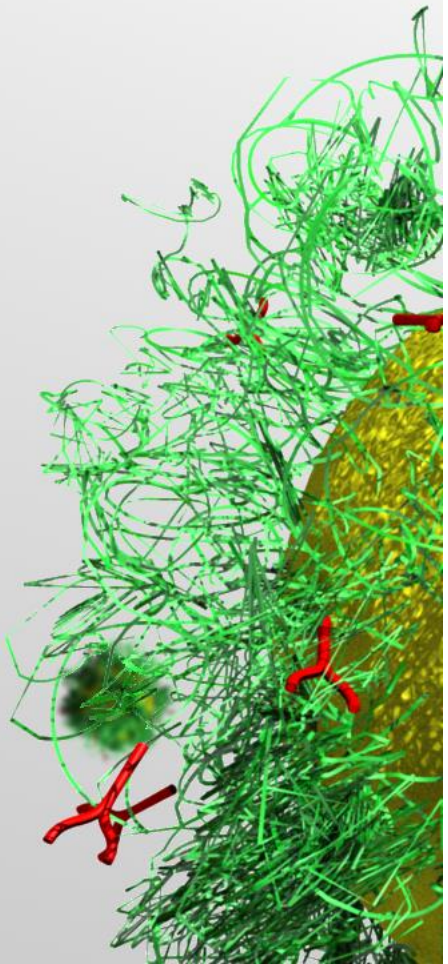
Nanomaterials special properties

- **Gold nanoparticles**
  - Strong **optical peak**, which is variable with the particle's morphology;
  - Electron-dense and radiopaque;
  - Its surface chemistry allows the bonding of organic molecules;
  - Low toxicity when introduced into biological systems.

Brazilian experience

- DENV Peptides to avoid ELISA cross-reaction





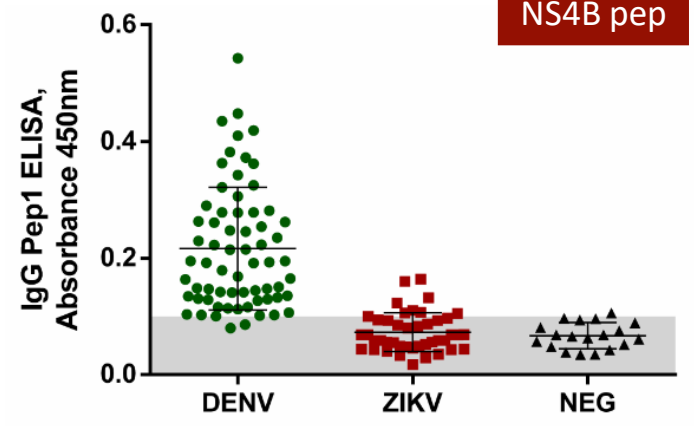
viruses MDPI

Article

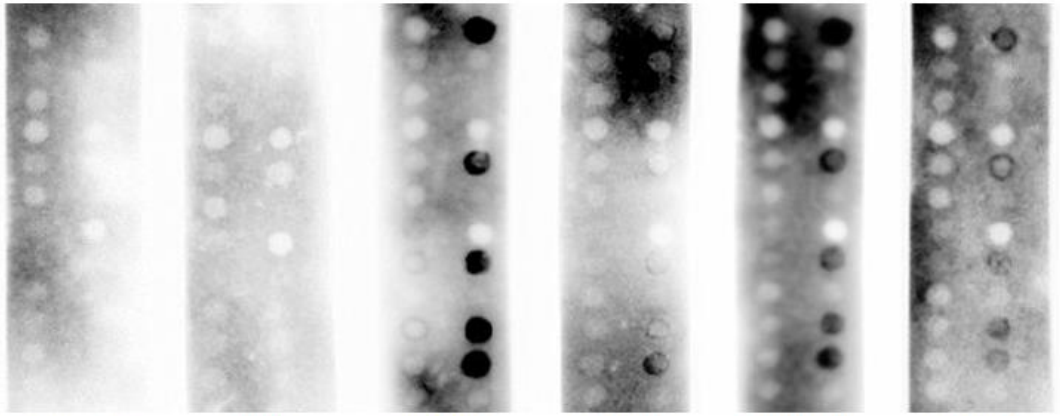
Identification of B-Cell Epitopes with Potential to Serologicaly Discrimnate Dengue from Zika Infections

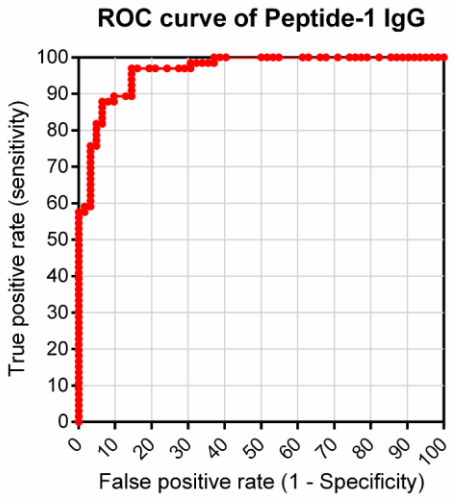
Alice F. Versiani [1,2], Raissa Prado Rocha [1], Tiago A. O. Mendes [3], Glauco C. Pereira [4], Jordana Graziella A. Coelho dos Reis [1], Daniella C. Bartholomeu [3] and Flávio G. da Fonseca [1,*]

NS4B pep



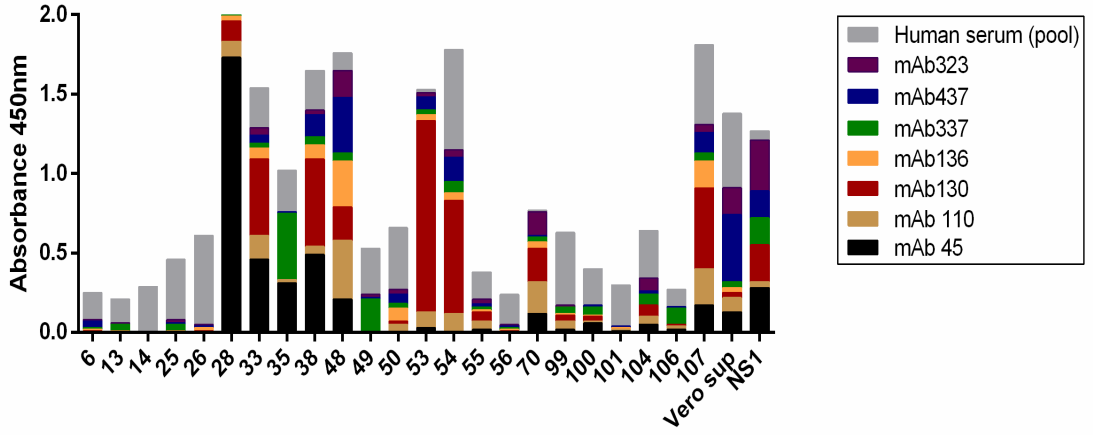
CPRITETE	SGDPLKND
SSQDEKGVT	SYDPKFEK
TAPGTFKTPEGE	YHGSYEAP
SSQSTTPET	KGGPGHEE
IATQQPES	AGWDTRIT
IPYDPKFE	QRGSGQVG
QRKKTGKP	VRKDIPQW
TEDGQGKA	PEPEKQRT
NAEPDGPT	PEREKSAA
MSKEPGVV	ATREAQKR
PETPNMDV	ISRKDQRG
AGATEVDS	WFKKGSSI

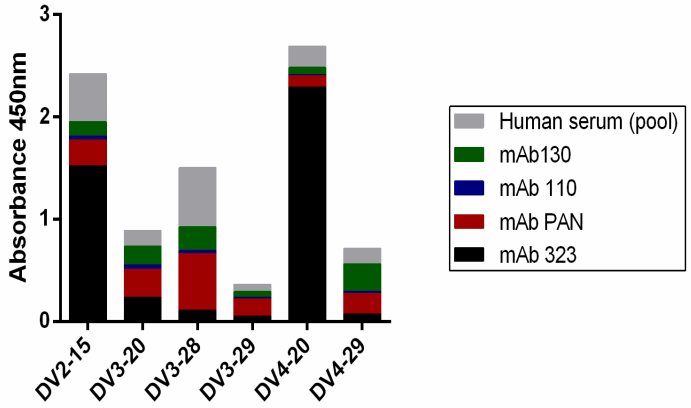


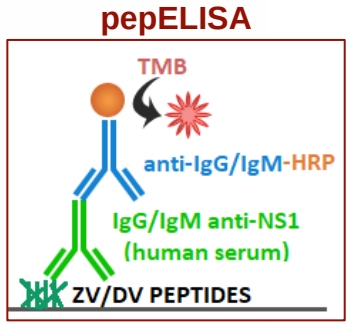


Brazilian experience

- DENV/ZIKV NS1 Peptides to avoid ELISA cross-reaction

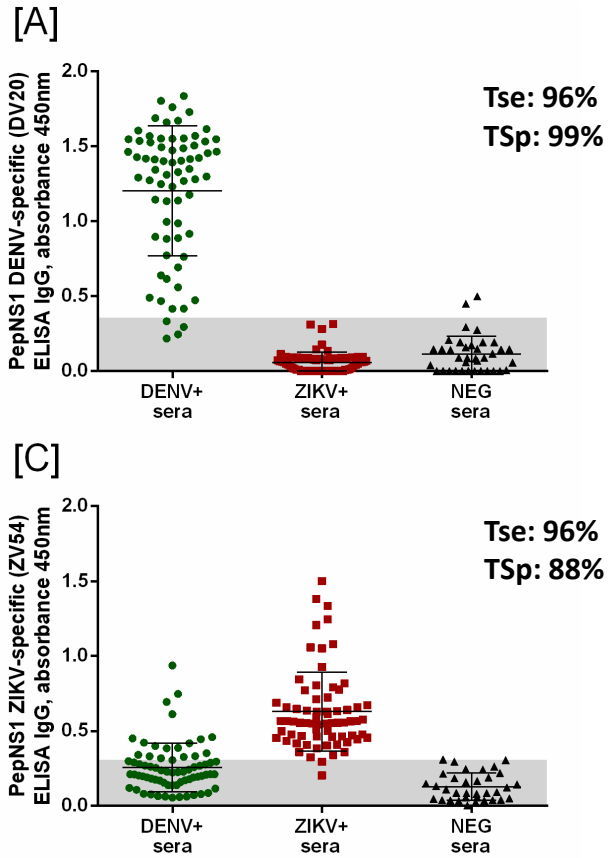






Screening with monoclonal ab's

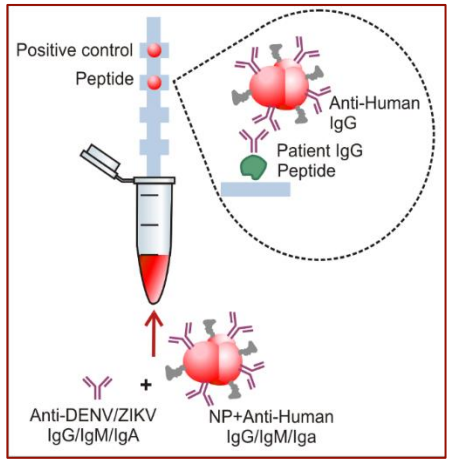
Endemic area samples for validation

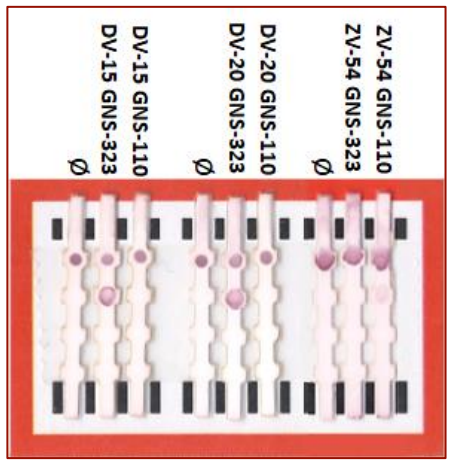


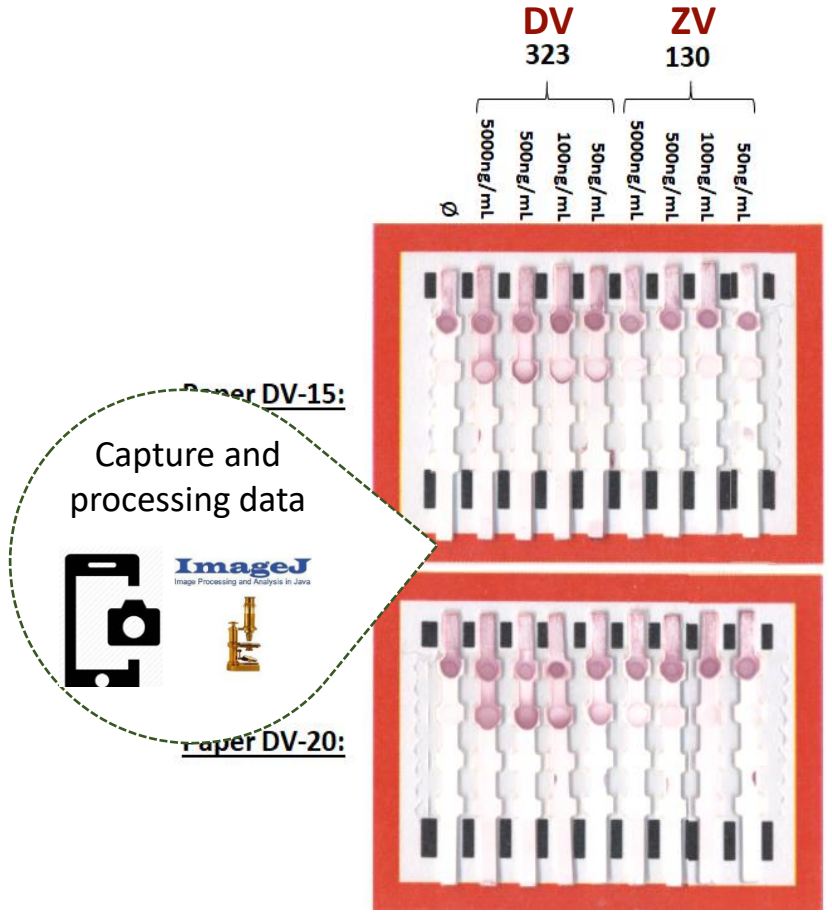
Versiani et al, 2021. Manuscript in preparation

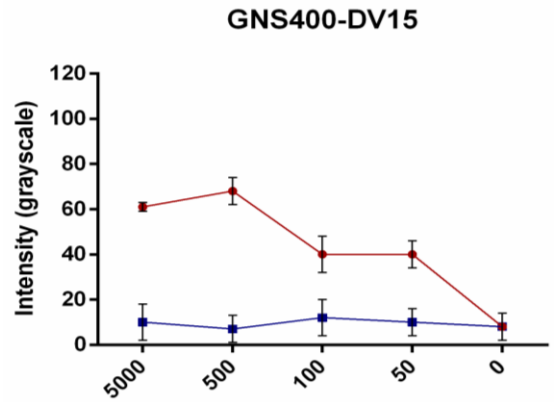
Brazilian experience

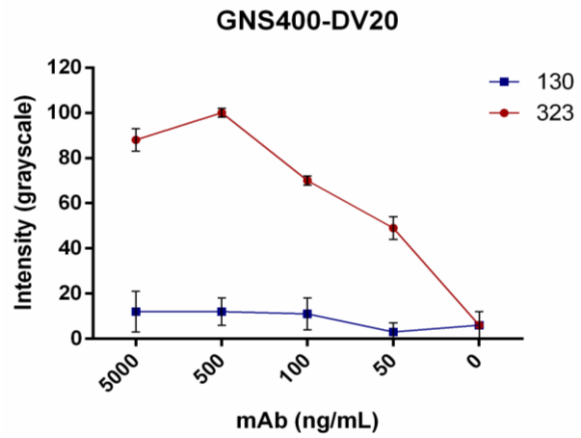
- DENV/ZIKV NS1 Peptides in a multiplex LFA + image processing







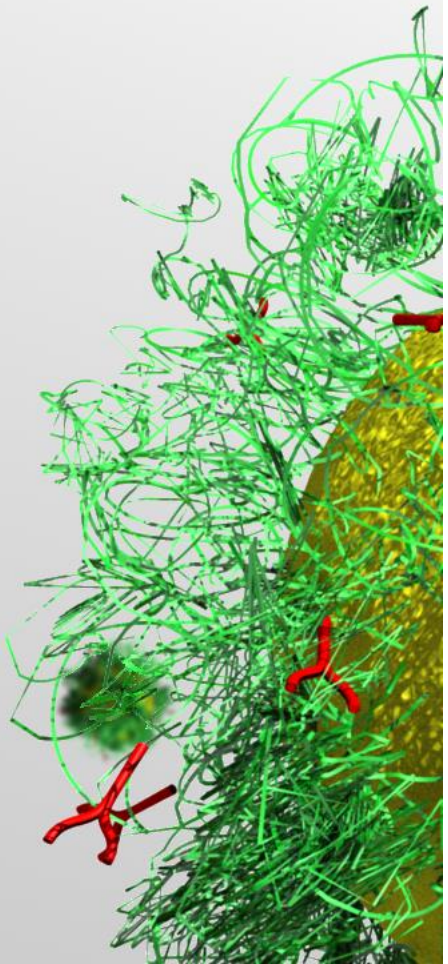




Versiani et al, 2021. Manuscript in preparation

Brazilian experience

- LFA signal improvement:



Molecular Systems Design & Engineering

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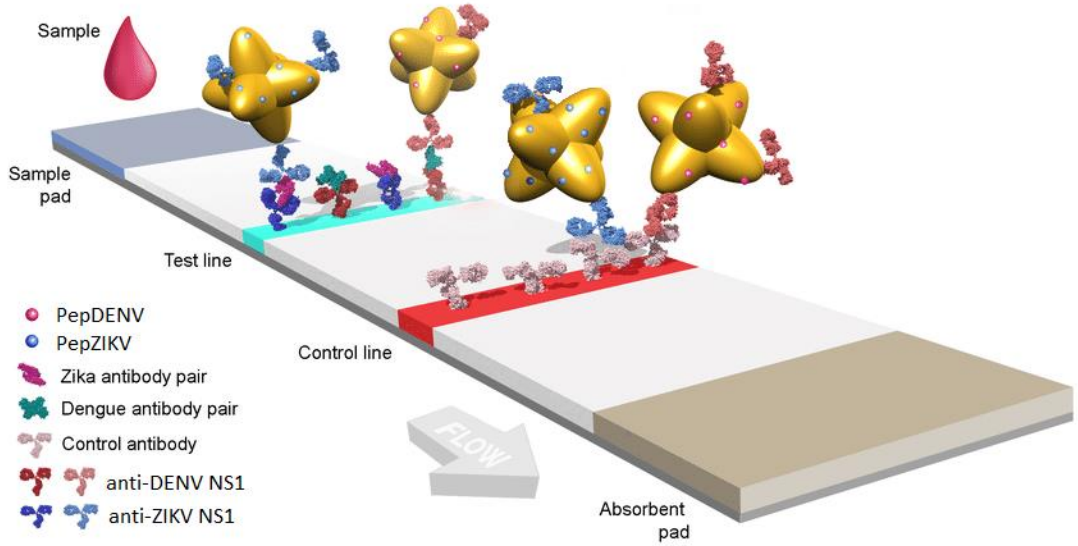
Cite this: DOI: 10.1039/c7me00052a

Design of SERS nanotags for multiplexed lateral flow immunoassays†

Maria Sánchez-Purrà,[a] Biel Roig-Solvas,[b] Alice Versiani,[cd] Cristina Rodriguez-Quijada,[a] Helena de Puig,[e] Irene Bosch,[dg] Lee Gehrke[df] and Kimberly Hamad-Schifferli[*ae]



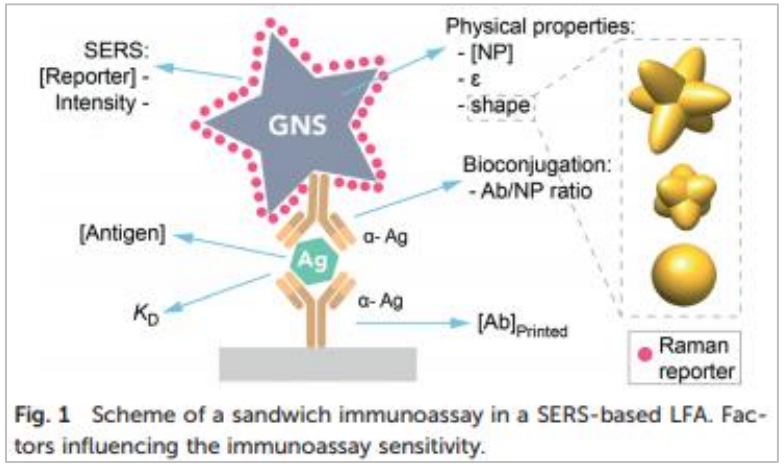
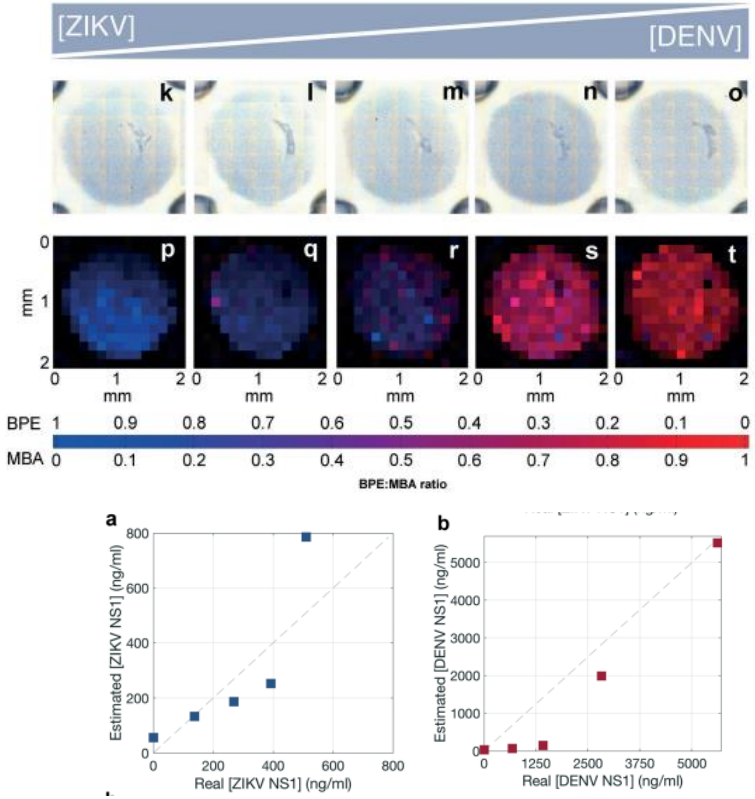
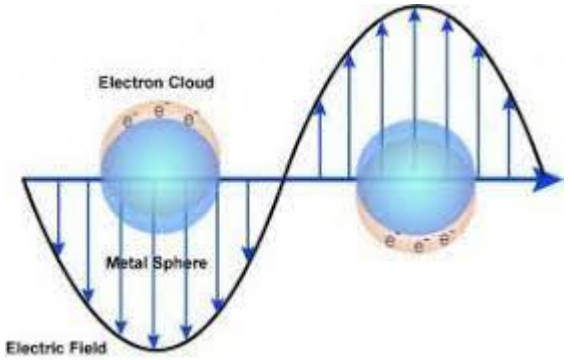


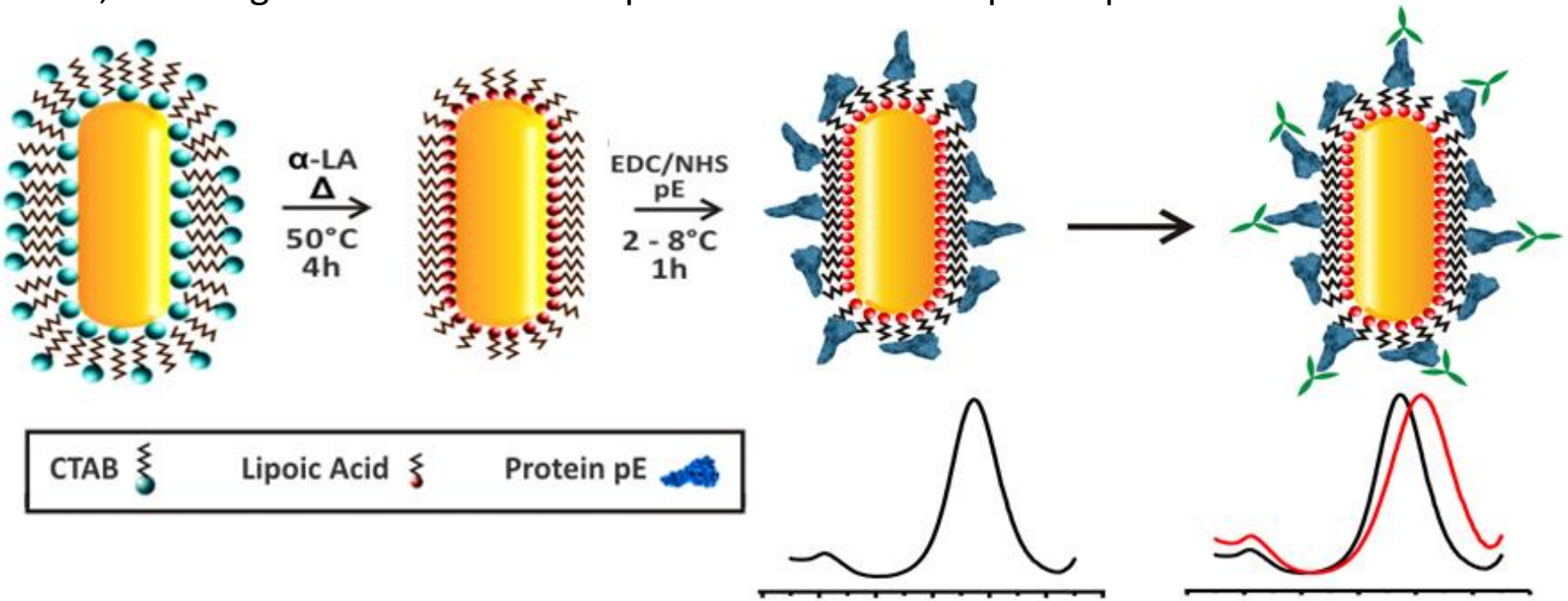
Fig. 1 Scheme of a sandwich immunoassay in a SERS-based LFA. Factors influencing the immunoassay sensitivity.



Brazilian experience



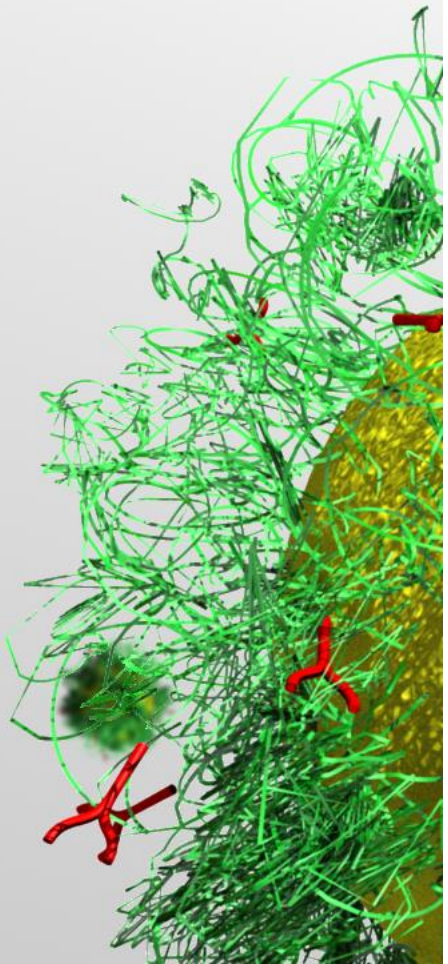
- LSPR Nanosensors
  - *Localized surface plasmon resonance: w*hen the plasmon frequency is of the same as the incident light, a resonance phenomenon occurs and results in a noticeable optical absorption and generates a sharp electric field on the surface of the metallic nanoparticles. Therefore, any modifications around the nanoparticle, including alterations in their surface, the solvent and particle aggregation, will determine changes in the electronic properties of the nanoparticle's surface, resulting in alterations in the patterns of the absorption spectrum.



Versiani et al, 2020. Sci Report.

Brazilian experience

- DENV LSPR Nanosensors:



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nature research
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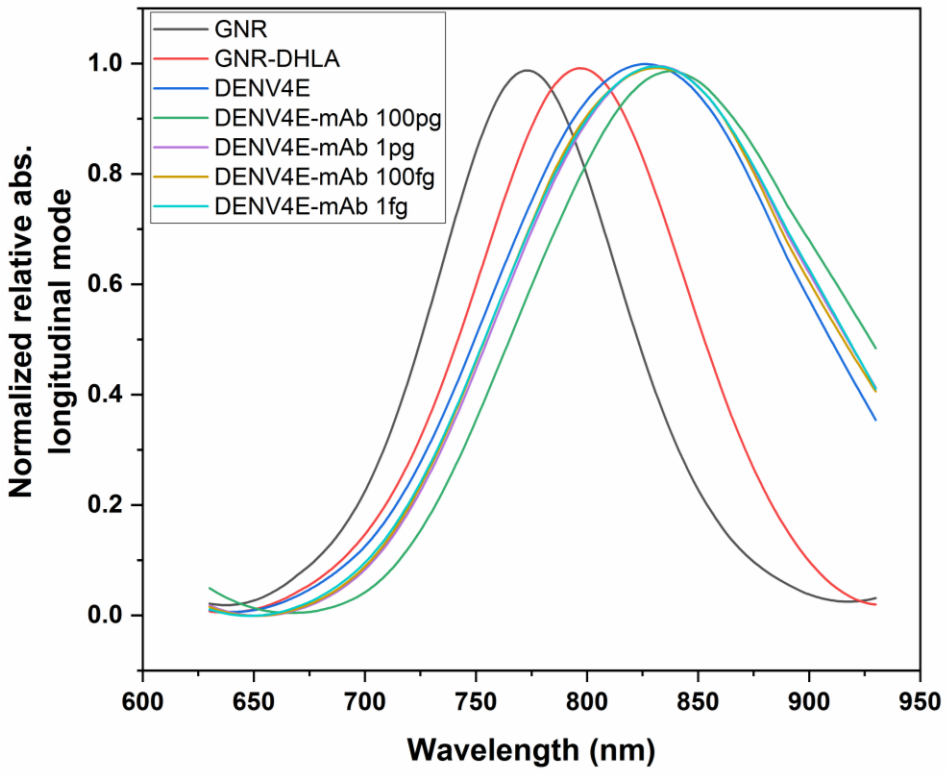
OPEN

Nanosensors based on LSPR are able to serologically differentiate dengue from Zika infections

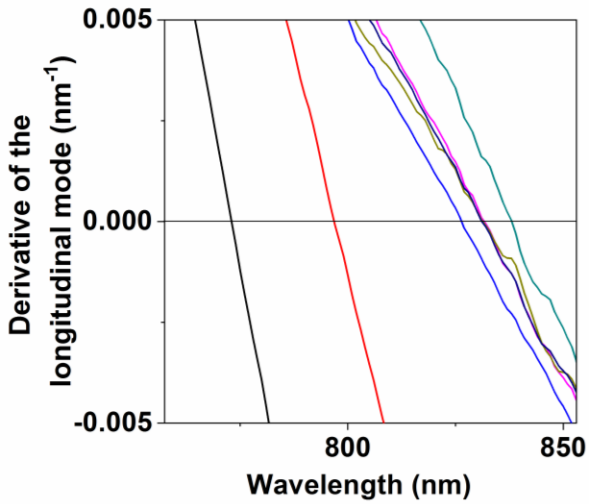
Alice F. Versiani[1,6,8], Estefânia M. N. Martins[2,6], Lidia M. Andrade[3,6], Laura Cox[1], Glauco C. Pereira[4], Edel F. Barbosa-Stancioli[1], Mauricio L. Nogueira[5], Luiz O. Ladeira[3,6] & Flávio G. da Fonseca[1,7]

DENV1-4 monoclonal antibody

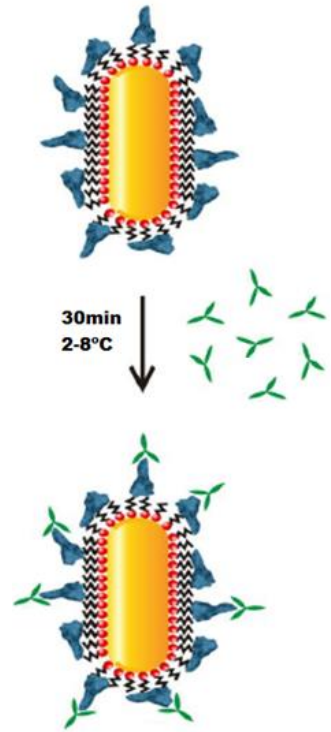
[A]



[B]



Sample	Relative shift (nm)
GNR	-54
GNR-DHLA	-30
DENV4E	0
DENV4E-mAb 100pg	11
DENV4E-mAb 1pg	5
DENV4E-mAb 100fg	4
DENV4E-mAb 1fg	4

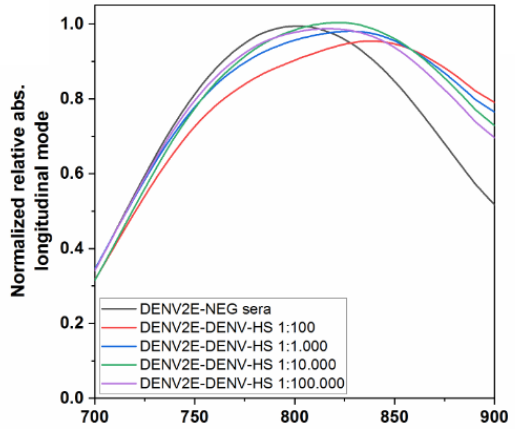


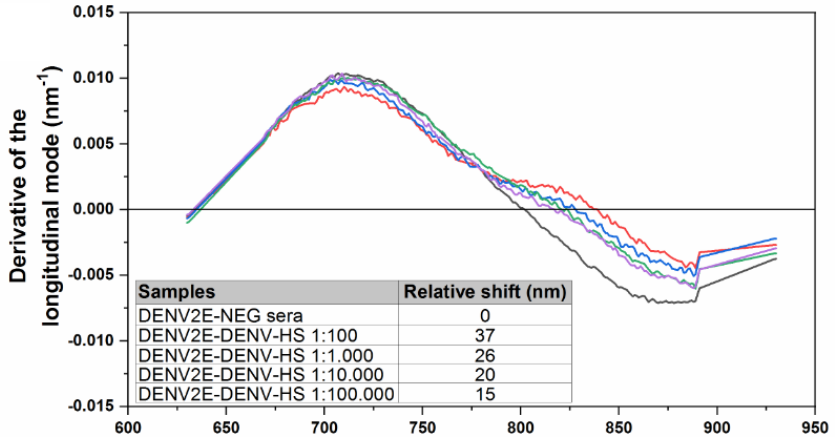
Versiani et al, 2020. Sci Report.

Brazilian experience

- DENV LSPR Nanosensors:

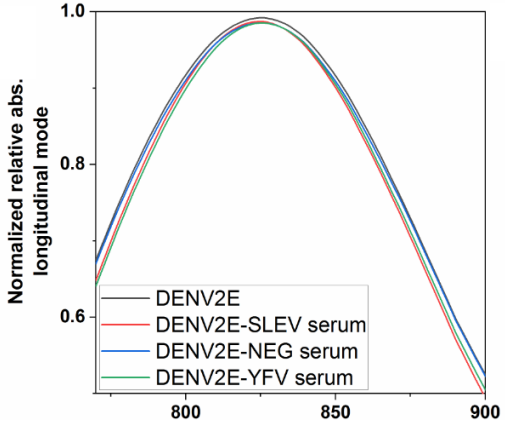
DENV+ HUMAN POOL SERA

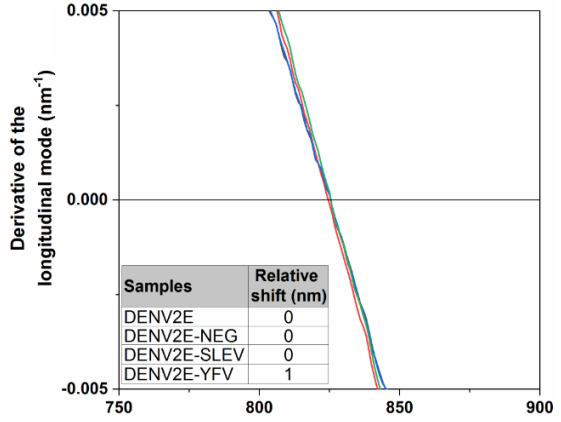




Samples	Relative shift (nm)
DENV2E-NEG sera	0
DENV2E-DENV-HS 1:100	37
DENV2E-DENV-HS 1:1.000	26
DENV2E-DENV-HS 1:10.000	20
DENV2E-DENV-HS 1:100.000	15

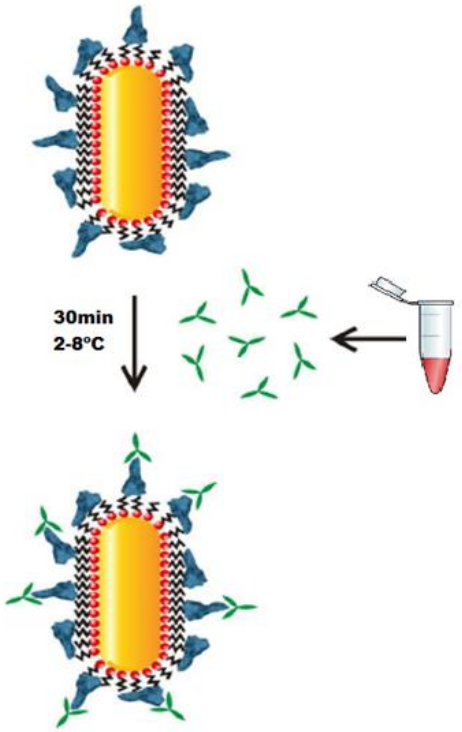
CONTROLS HUMAN POOL SERA





Samples	Relative shift (nm)
DENV2E	0
DENV2E-NEG	0
DENV2E-SLEV	0
DENV2E-YFV	1

Controls:
YFV
SLEV
TN



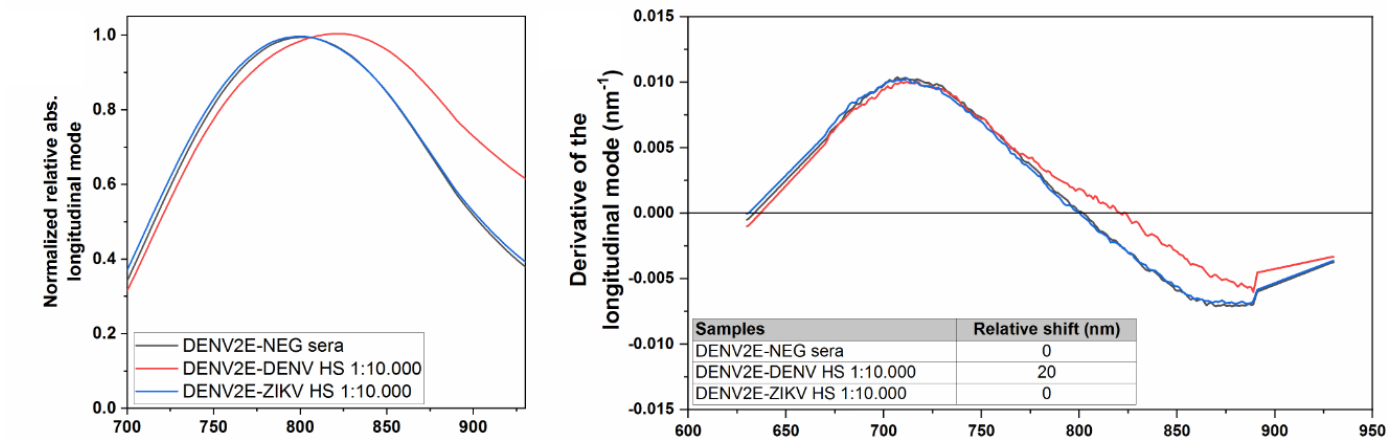
Versiani et al, 2020. Sci Report.

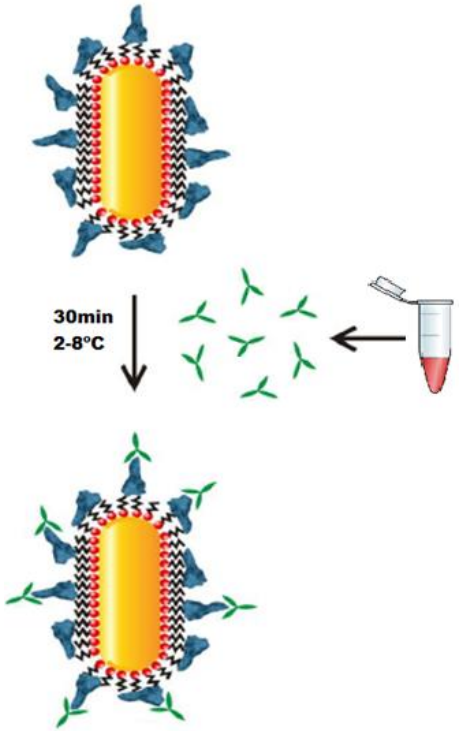
Versiani et al, 2020. Sci Report.

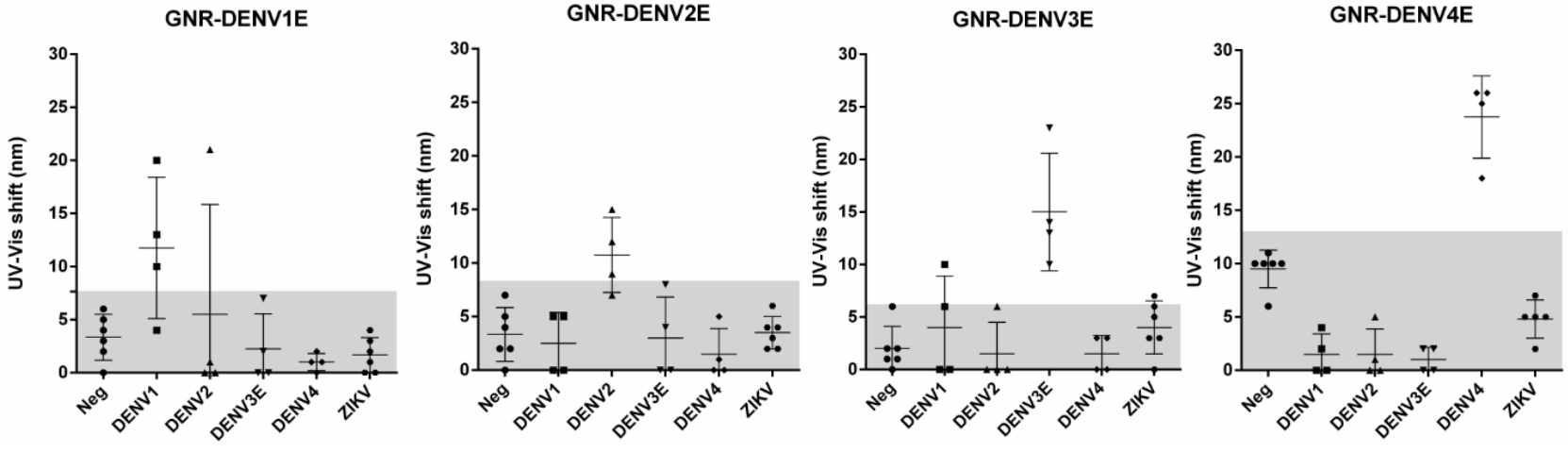
Brazilian experience

- DENV LSPR Nanosensors:

DENV+ HUMAN SERA x ZIKV+ HUMAN SERA



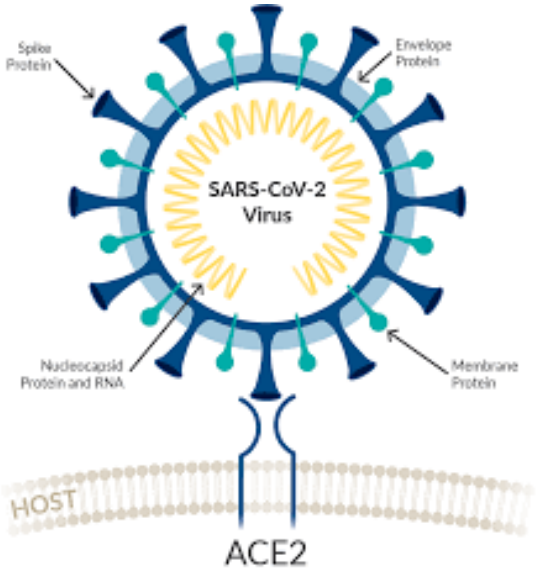


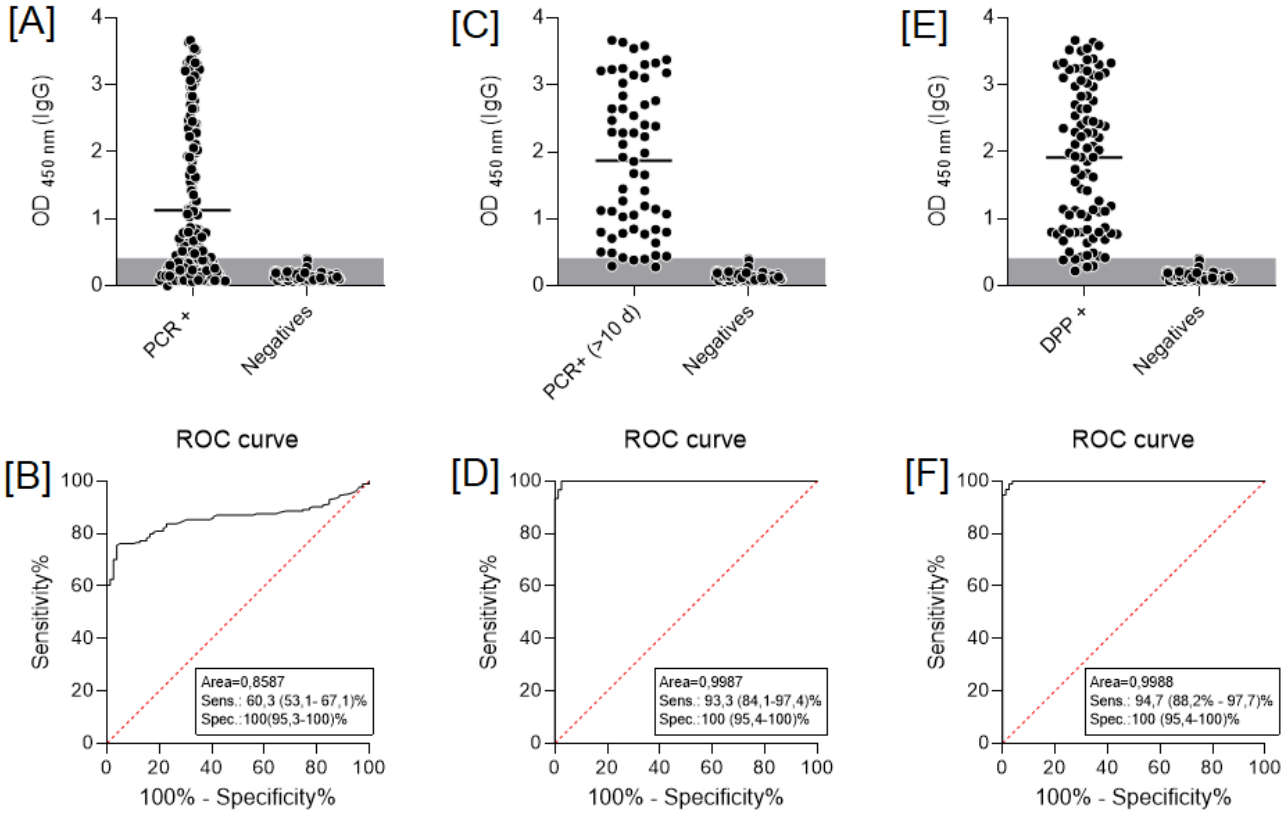


Brazilian experience

- COVID-19 ELISA and LSPR Nanosensor:

IgG ELISA anti-N validation



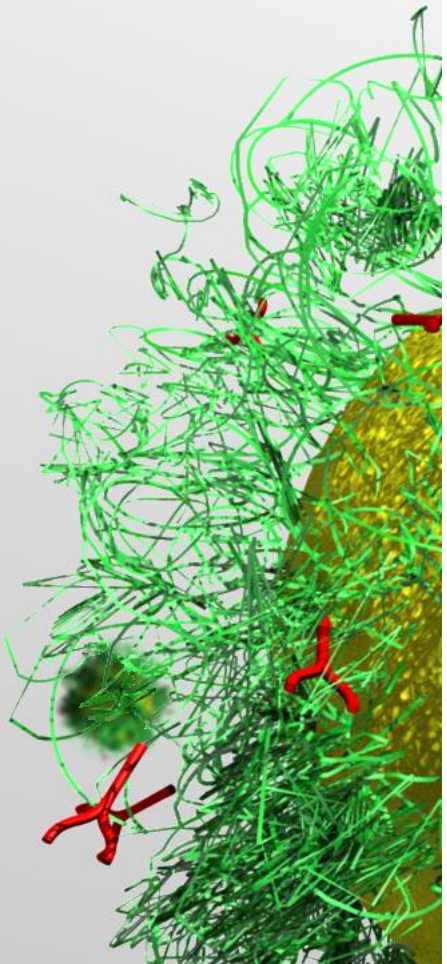


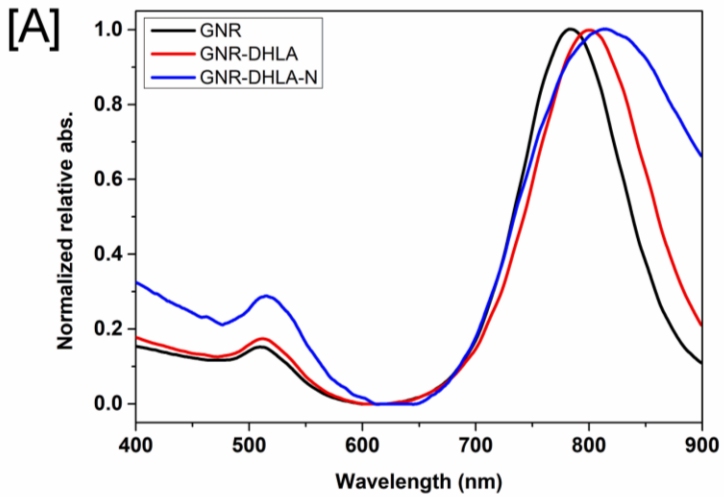
Versiani et al, 2021. Manuscript in preparation

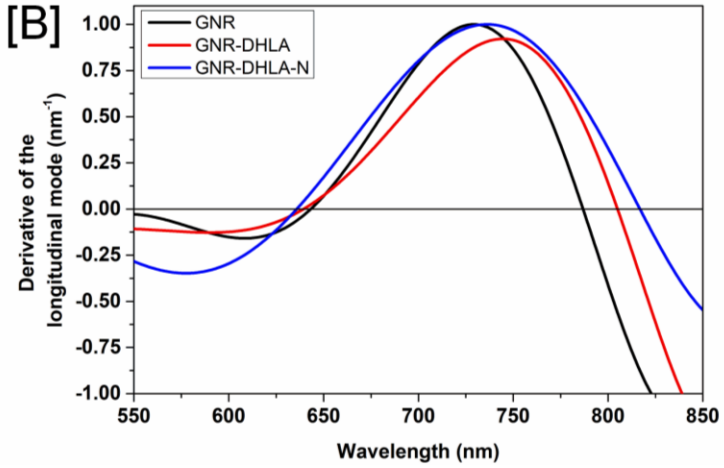
Brazilian experience

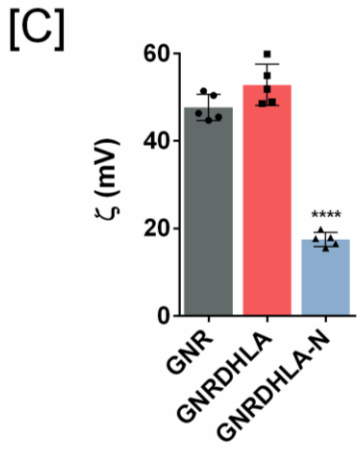
COVID Nanosensor characterization

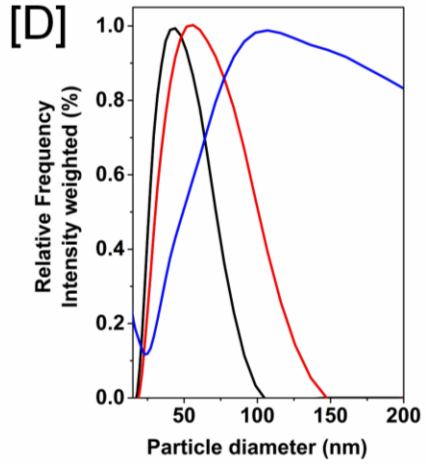
- COVID-19 ELISA and LSPR Nanosensor:

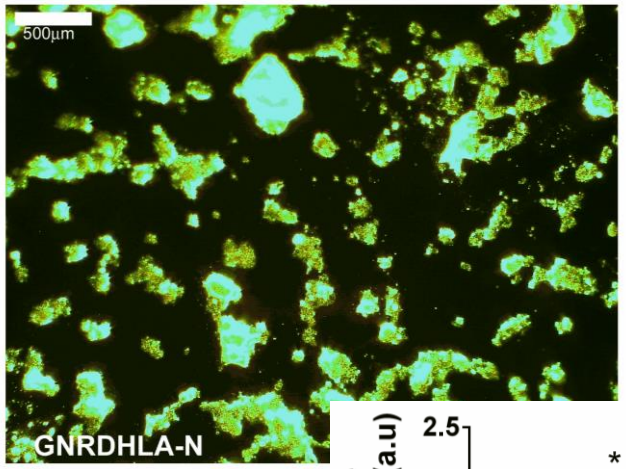


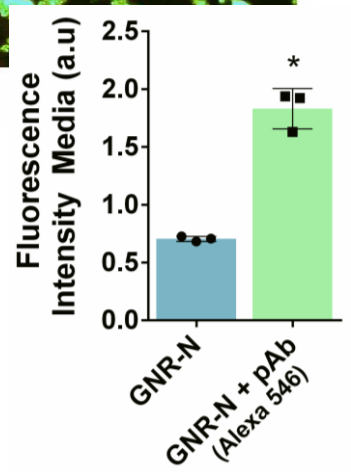










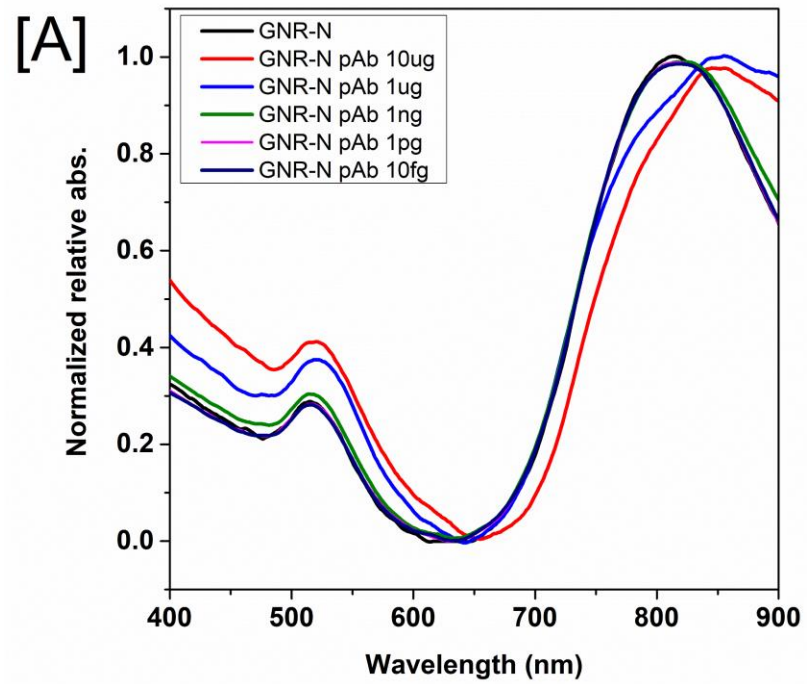


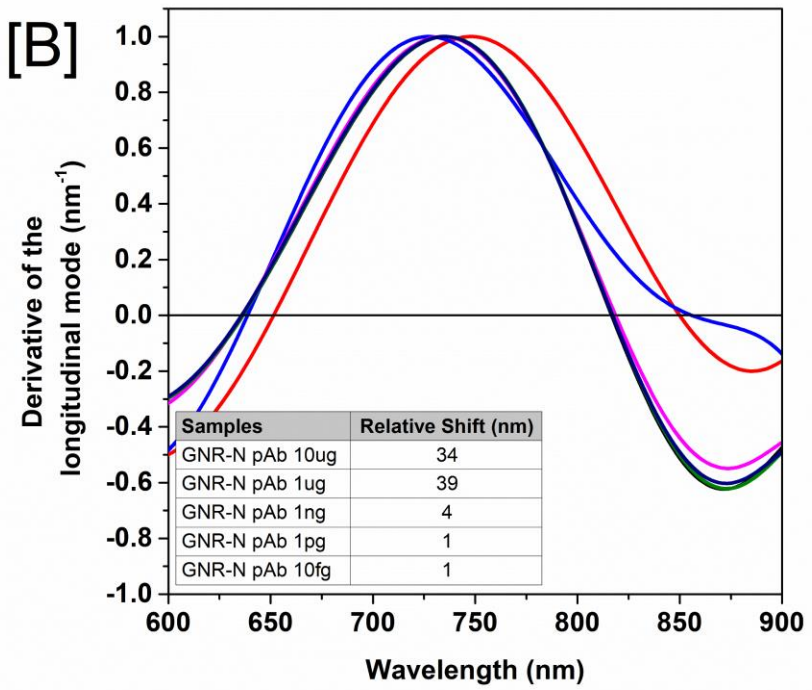
Versiani et al, 2021. Manuscript in preparation

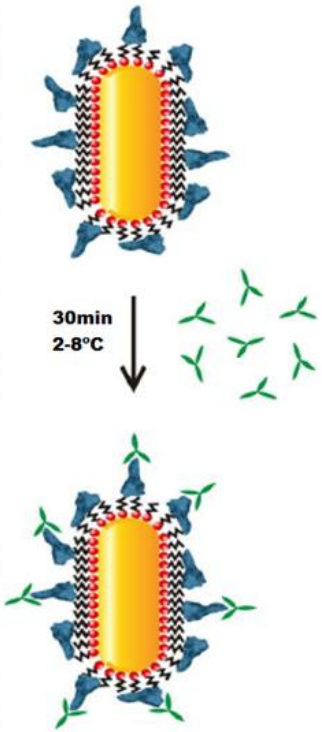
Brazilian experience

- COVID-19 ELISA and LSPR Nanosensor:

Anti-N polyclonal antibody





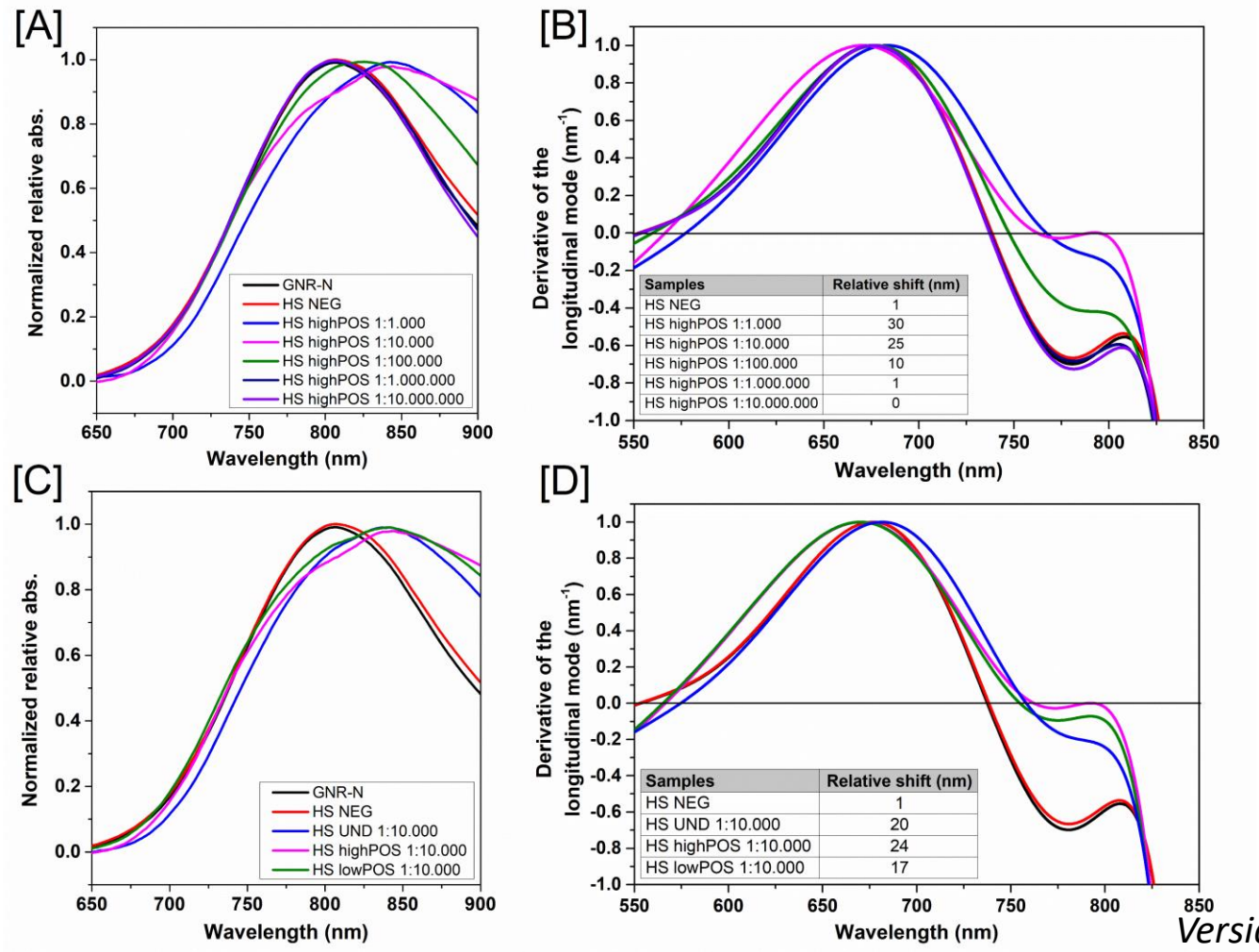


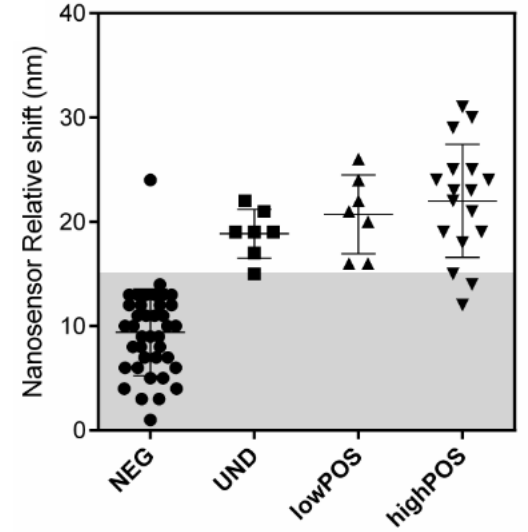
Versiani et al, 2021. Manuscript in preparation

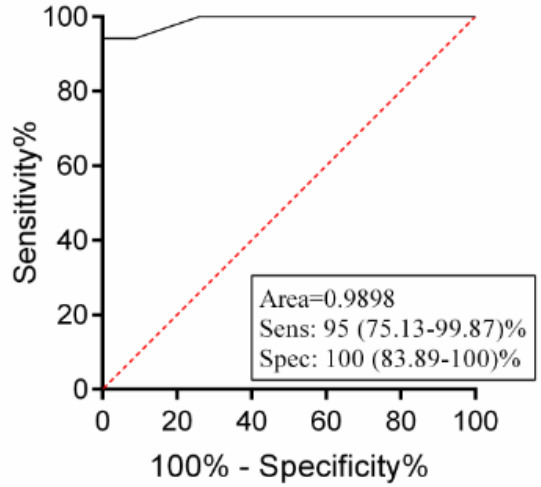
Brazilian experience

COVID-19+ HUMAN SERA

- COVID-19 ELISA and LSPR Nanosensor:



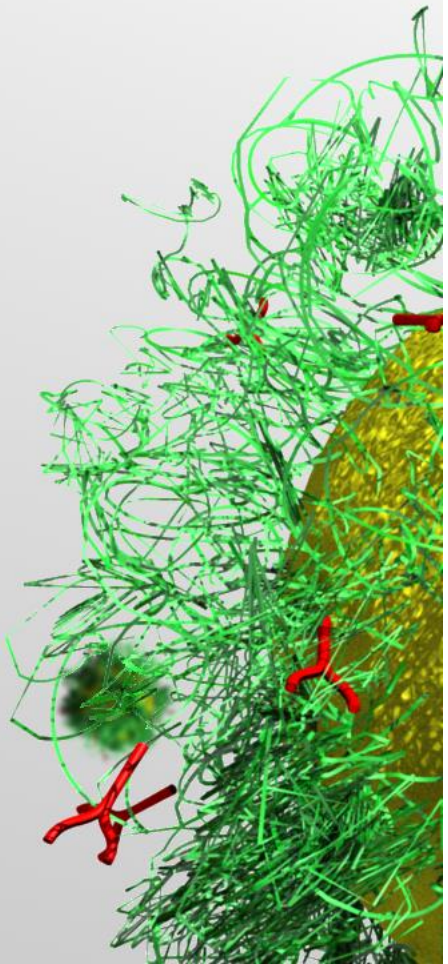




Versiani et al, 2021. Manuscript in preparation

Brazilian experience

- FACS signal enhancement:



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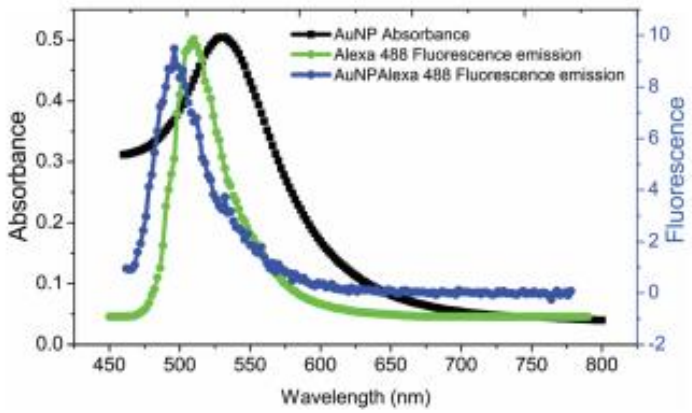
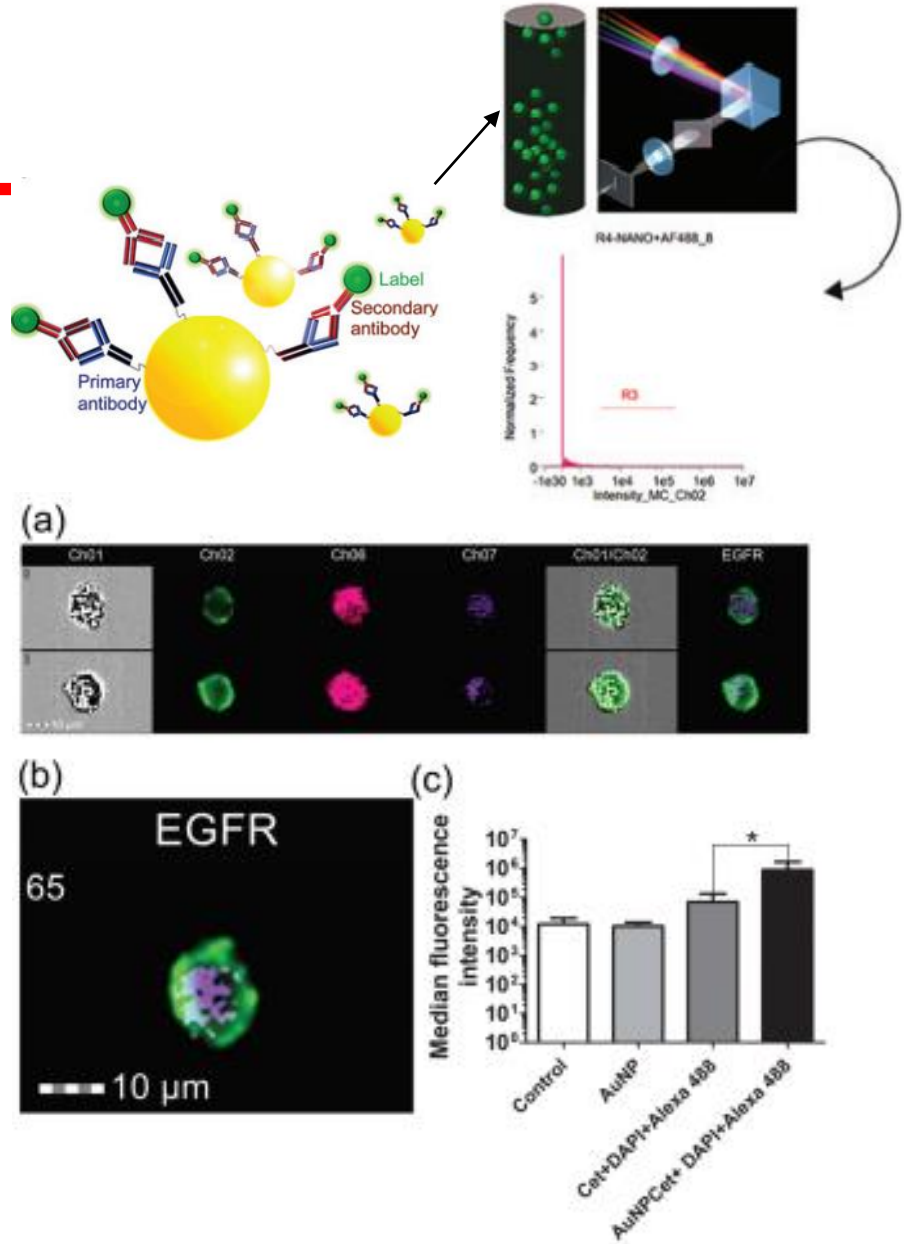
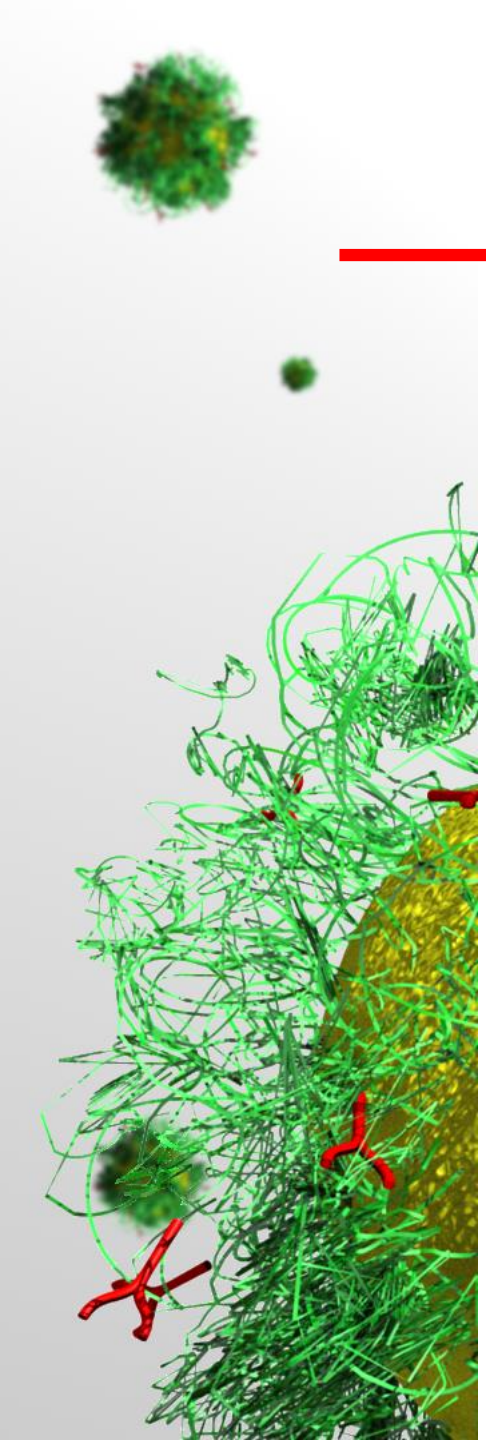
Gold nanoparticles enhance fluorescence signals by flow cytometry at low antibody concentrations†

Daniela S. Reis, [a] Vivian L. de Oliveira, [b] Misael L. Silva, [c] Roberto M. Paniago, [d] Luiz O. Ladeira [d] and Lidia M. Andrade [*d]



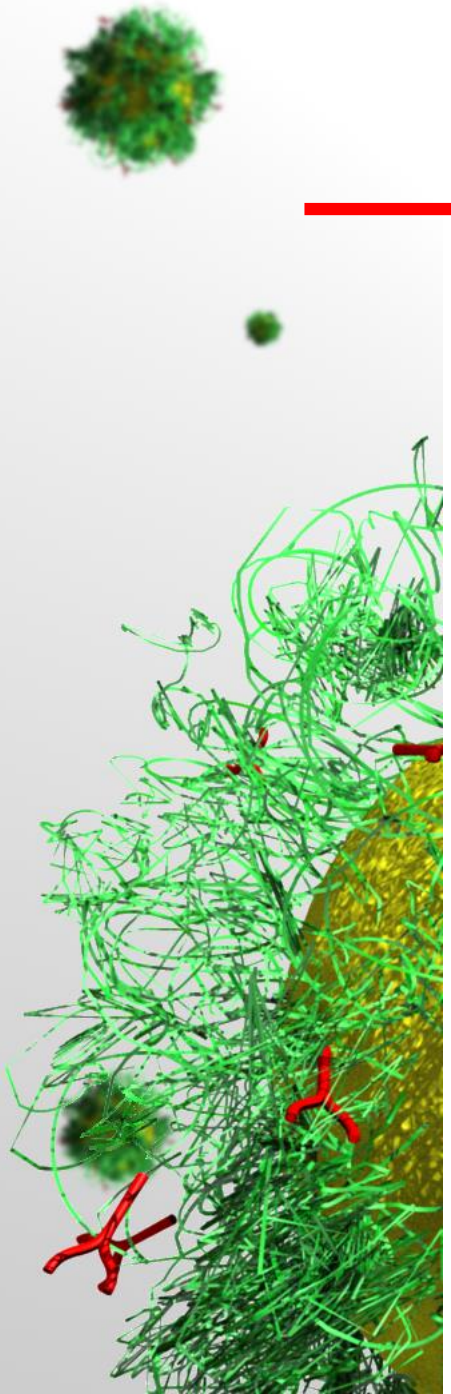


Sinergic effect of AuNP+Fluorophore



Diagnostic technologies

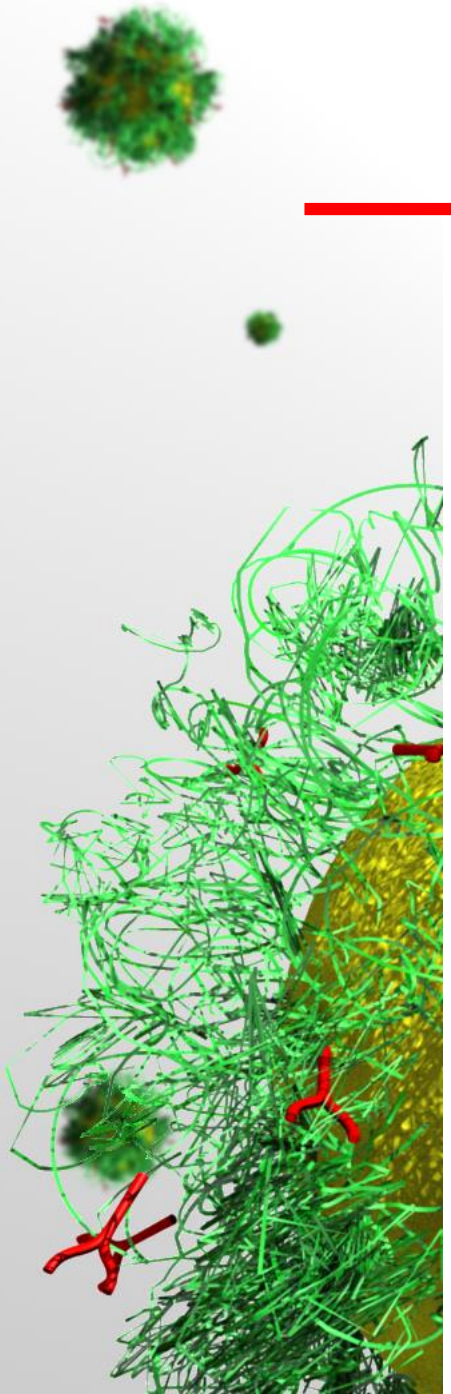
- How to choose your assay technology?
  - Lab infrastructure x Affected population
    - Cost
    - Time
    - Disease outcome: impact of false-negatives or false-positives results.
    - Co-circulation of similar pathogens that affects diagnosis
  - Interdisciplinary research group:
    - Allies technology and biological/medical background
  - New diseases
    - Opportunity to well-established academic techniques to gain market place



Emerging or re-emerging pathogens

- How to deal with emerging or re-emerging pathogens?
  - Rapidly develop and deploy diagnostic testing methods;
  - Development of case definitions and testing criteria;
  - Engage public health partners to optimize response capacity and coordination;
  - Establish information sharing processes, procedures, and samples that supports surveillance of new pathogens;
  - Establish genomics and other omics approaches to further enhance infectious disease response capacity.

Establishment of an interdisciplinary research network



Emerging or re-emerging pathogens

Preparedness Latin American Network ZikaPLAN

- ZikaPLAN brings together 25 leading research and public health organizations in Latin America, North America, Africa, Asia, and Europe, taking a comprehensive approach to tackle the Zika threat

Centre for Arbovirus cadde Discovery, Diagnostics, Genomics & Epidemiology

- CADDE brings together multidisciplinary teams across Brazil and the UK to address critical questions in arbovirus epidemiology and public health in Brazil.

RedeVírus MCTI

- Brazilian committee that brings together specialists, government representatives, funding agencies, research centers and universities with the aim of integrating initiatives to combat emerging viruses.

RedeVírus MCTI

COVID-19 INITIATIVES

Genome sequencing

Corona BR MCTI

RedeVírus MCTI

10 granted vaccine projects

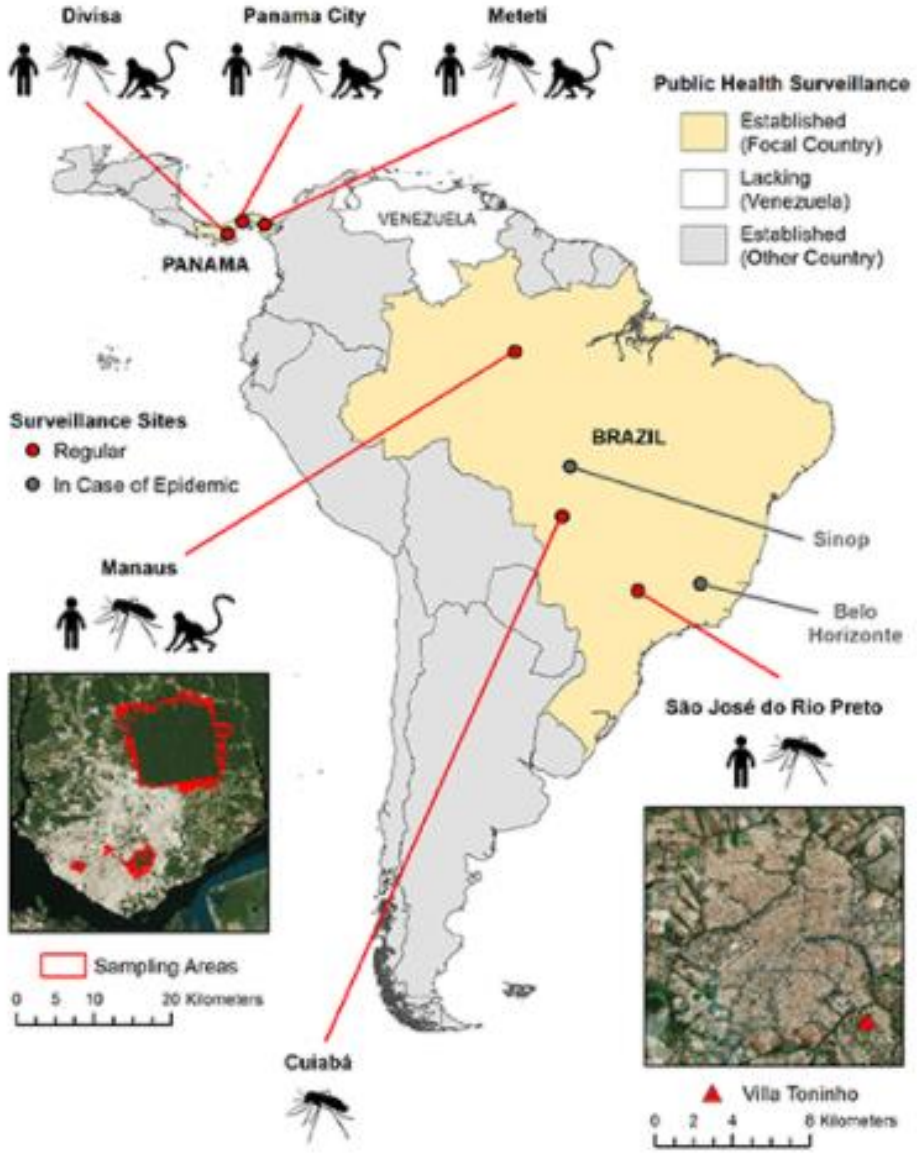
Protocols / Confirmed samples / virus distribution

Cross-validation

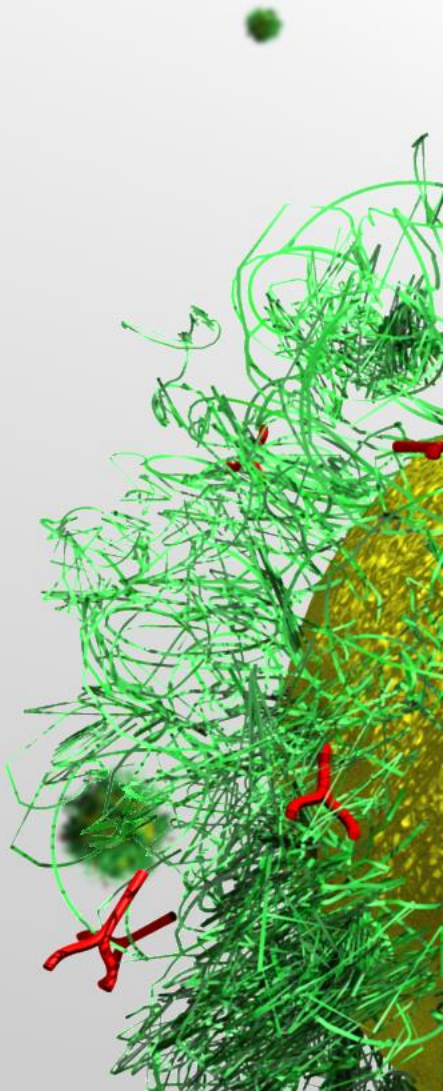
100% national serologic assays

CREATE-NEO

- The **C**oordinating **R**esearch on **E**merging **A**rboviral **T**hreats **E**ncompassing the **Neo**tropics (CREATE-NEO) project will provide a nimble and flexible network of surveillance sites in Central and South America coupled to cutting-edge modeling approaches in order to anticipate and counter emerging arboviruses. Importantly, CREATE-NEO can quickly redirect its mission to address any emerging zoonotic or vector-borne disease



Acknowledgments



- LVP/FAMERP group:
  - **Dr. Maurício L. Nogueira**
  - Dr. Nathalia Zini
  - Dr. Cássia Estofolete
  - Dr. Livia Sacchetto
  - Students & Hospital staff
- UTMB-Health group:
  - **Dr. Nikos Vasilakis**
  - Dr. Adam Hendy
- MIT group:
  - **Dr. Lee Gerhke**
  - Dra. Irene Bosch
- NBM research group:
  - Dr. Luiz Orlando
  - **Dr. Lídia Andrade**
  - **Dr. Estefânia Martins**
- LVBA/UFMG group:
  - **Dr. Flávio da Fonseca**
  - Dr. Edel F. Barbosa-Stancioli
  - Dr. Flávia Fonseca
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