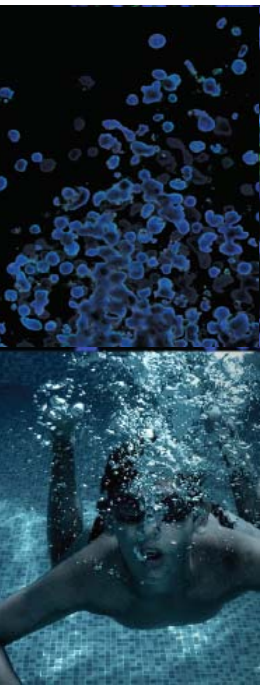
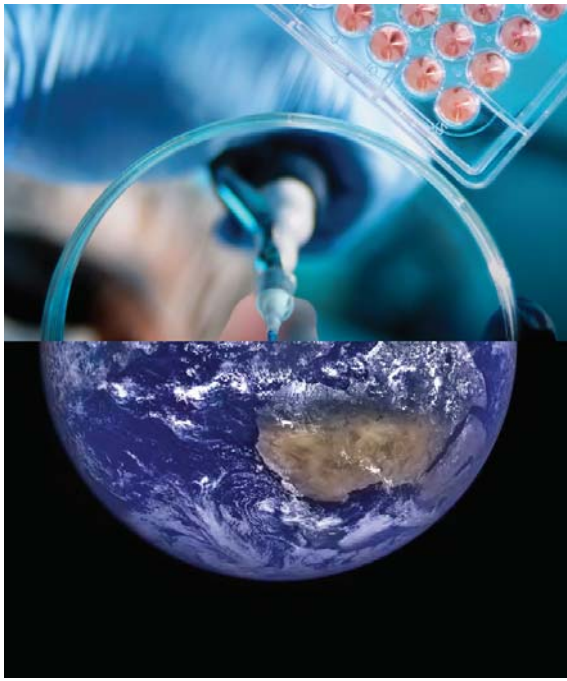
Building a Zika virus vaccine: from global health to Virus-like particles technology

Velasco Cimica, PhD
Scientist, ATCC

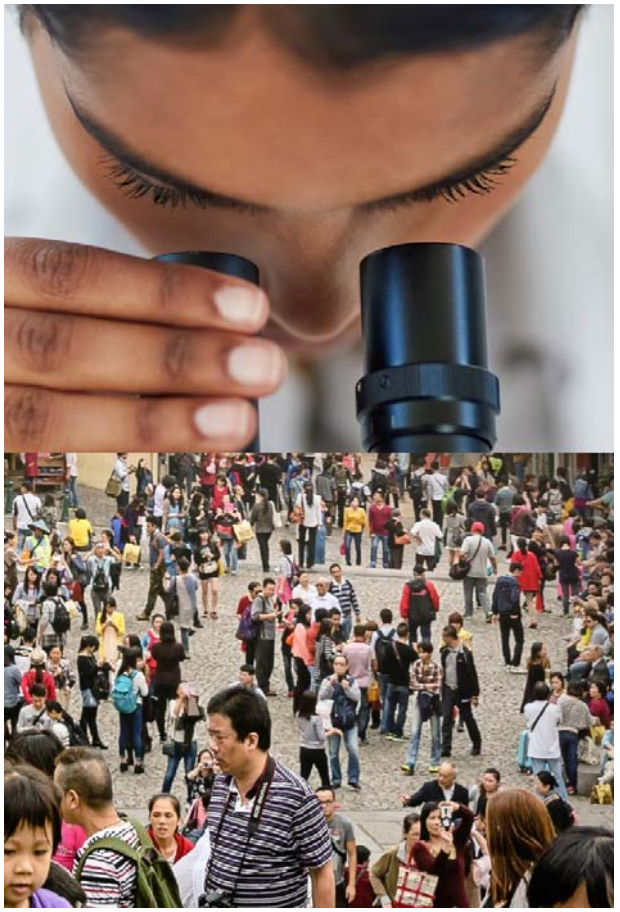




Credible Leads to Incredible™

ATCC – Credible leads to Incredible

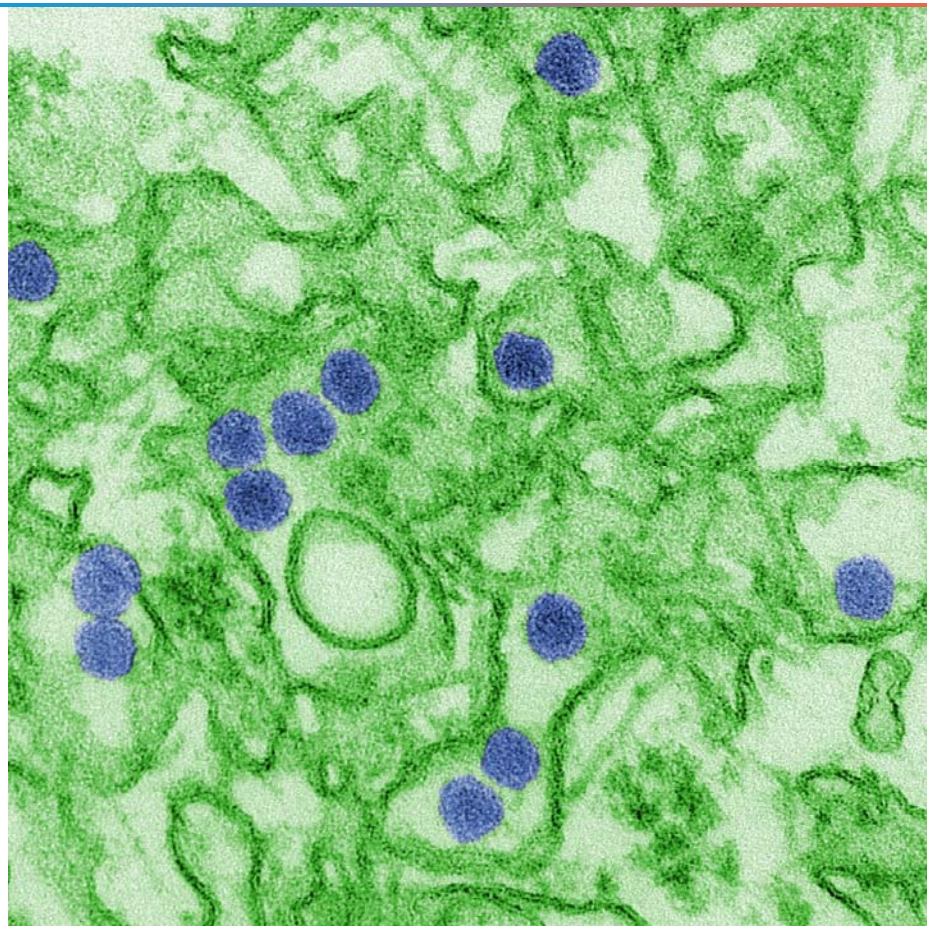
- ATCC has provided credible biomaterials for over 90 years
- We continue to cultivate collaboration
  - Among scientists across disciplines
  - Essential for accelerating innovative research
  - Leading to incredible, high-impact results
- Our Cultivating Collaboration pledge: We bring scientists together to discuss
  - Breakthroughs in the state of science
  - Multidisciplinary approaches to key areas of research
  - Breaking the silos that impede research
- Our partnership with you, the scientific community, allows us all to reach the incredible



ATCC

Agenda

- Introduction
- Scientific approach
- Results
- Summary

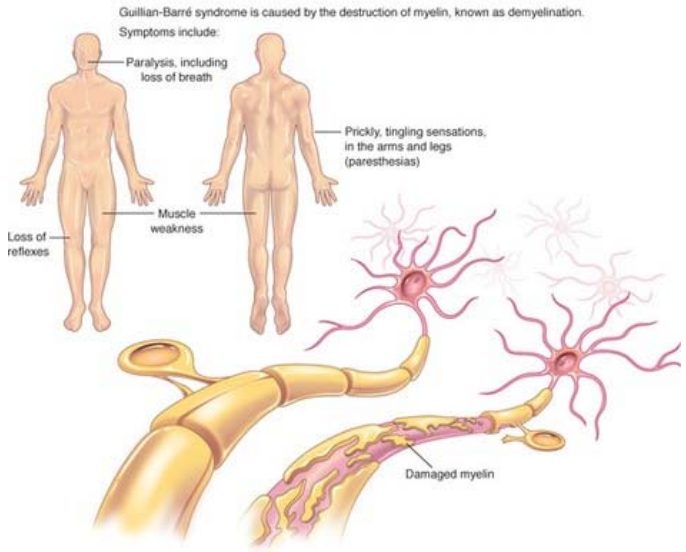


Zika virus

An emerging global infection

- Flavivirus such as dengue virus
- Transmitted by *Aedes* species mosquito
- Spread in the Americas in 2015
- Local cases of Zika in US (FL, TX) and US territories in 2016
- Causes microcephaly and Guillian-Barré Syndrome (GBS)

https://www.cdc.gov/pregnancy/zika/testing-follow-up/documents/Microcephaly_measuring.pdf



http://healthlive.co.in/2016/07/07/guillain-barre-syndrome-can-be-a-starting-point-to-test-for-zika-virus/

Zika virus history

Zika spread (1947-2016)

https://www.who.int/bulletin/online_first/16-171082/en/

1947
Discovery in Uganda

1947-Present
Sporadic cases in Africa and Asia

2007
YAP Outbreak

2012-2014
French Polynesia Outbreak

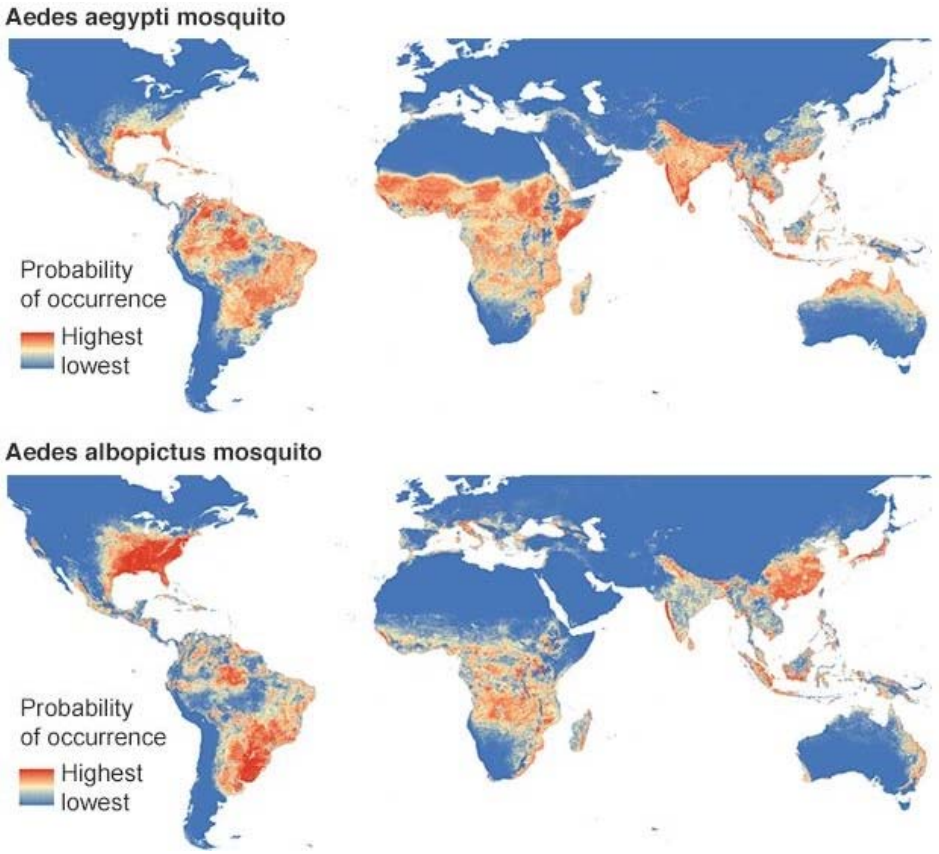
2015-2016
Epidemics in Brazil and the Americas

Zika Spread

ATCC

Global distribution of Aedes mosquitos

Aedes mosquitos are responsible for the spread of Zika

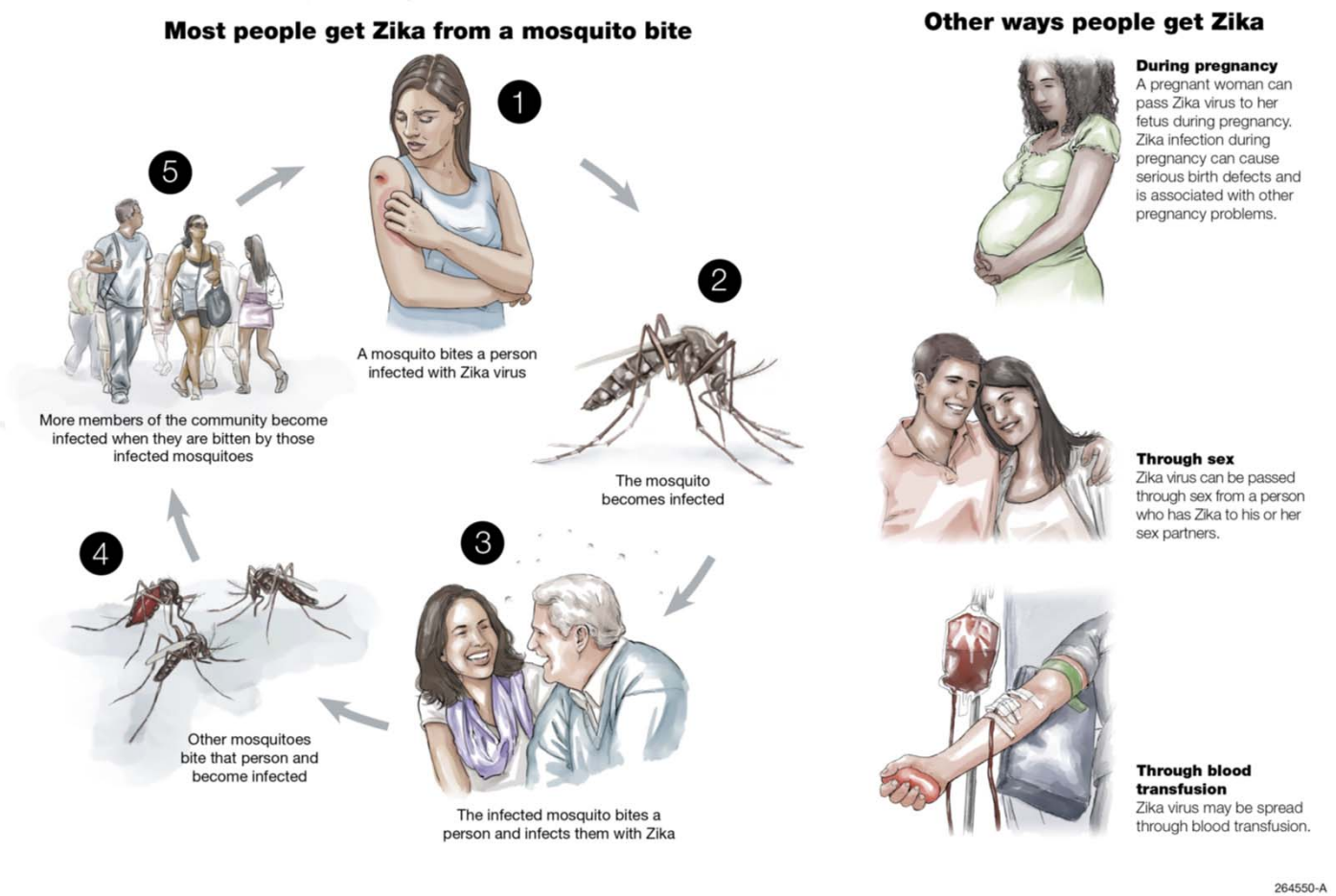


elifesciences.org/content/4/e08347, File:Global Aedes aegypti distribution.gif

ATCC

Zika transmission

Zika virus is passed through different modalities



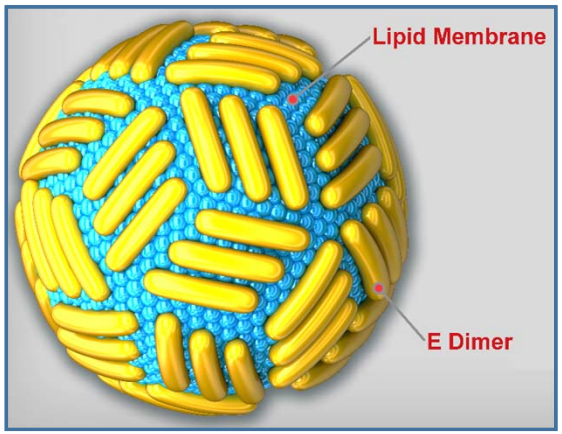
- 3-7% of Zika infections occur via sexual intercourse

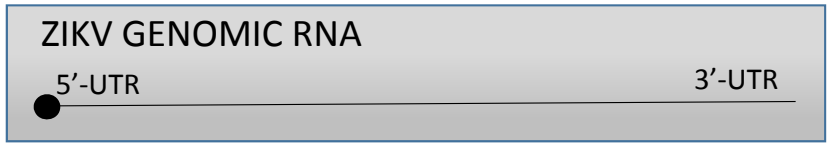
https://www.cdc.gov/zika/prevention/transmission-methods.html

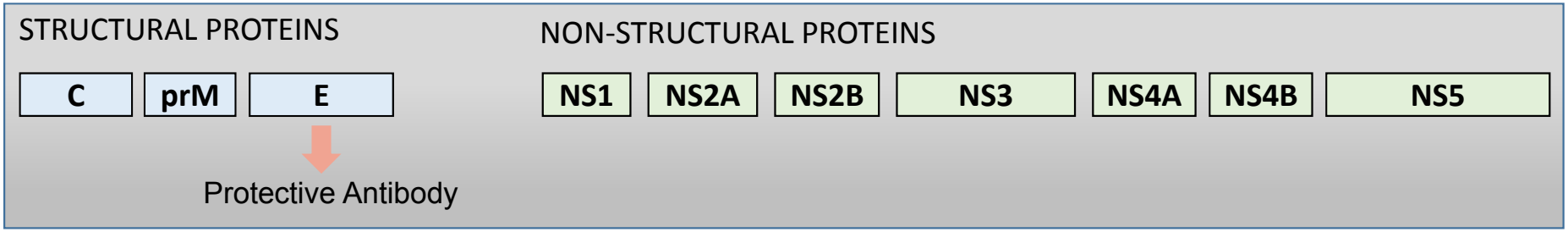
Zika virus organization

Zika virus genome

- Zika virus genome is a single-stranded positive RNA(10.7kb)
- Zika genome codes a polyprotein that is cleaved in structural and non-structural proteins
- Envelope (E) protein is the main Zika immunogen







Zika vaccine in clinical trial

Multiple vaccine approaches for Zika vaccines

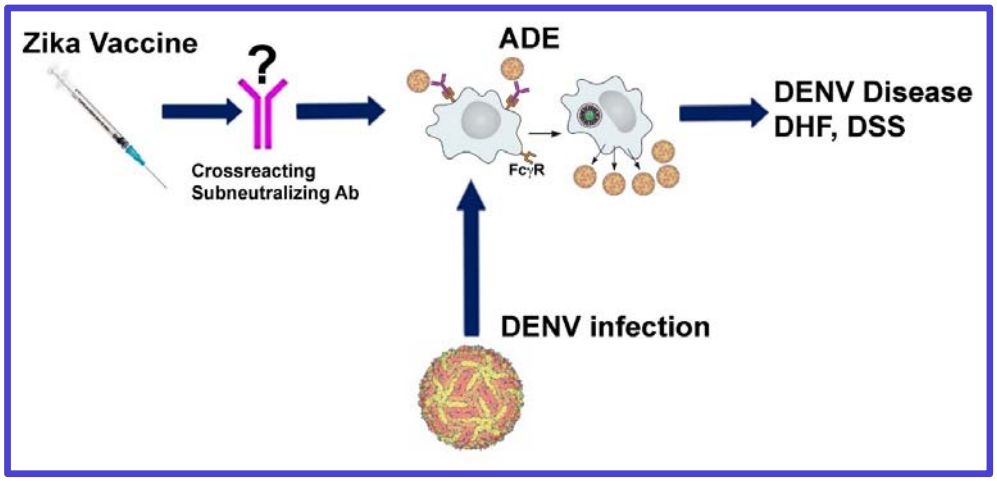
Platform	Immunogen	Adjuvant	Vaccine Candidate	Administration	Main Sponsor	Phase I	Phase II
DNA	prM-E	None	VRC-ZKADNA090-00-VP	IM or PharmaJet	NIAID	NCT02996461	NCT03110770
			VRC-ZKADNA085-00-VP	IM	NIAD	NCT02840487	-
			GLS-5700	ID	GeneOne Life Science	NCT02887482 NCT02809443	-
mRNA	prM-E	None	mRNA-1325		Moderna Therapeutics	NCT03014089	
Adenovirus Viral Vector	M-E	None	Ad26.ZIKV.001	IM	Janssen Vaccines	NCT03356561	-
Measles Viral Vector	prM-E	None	MV-Zika		Themis Bioscience	NCT02996890	-
Inactivated Virus	Whole Virus	Alum	VLA1601	IM	Valneva	NCT03425149	-
			PIZV	IM	TAKEDA	NCT03343626	-
			ZPIV	IM	NIAD	NCT03008122 NCT02963909 NCT02952833 NCT02937233	-
			MR8766	IM	Bharat Biotech	CTRI/2017/05/008539	-
Live Attenuated	Whole Virus	None	rZIKV/D4Δ30-713	SI	NIAID	NCT03611946	-

ATCC

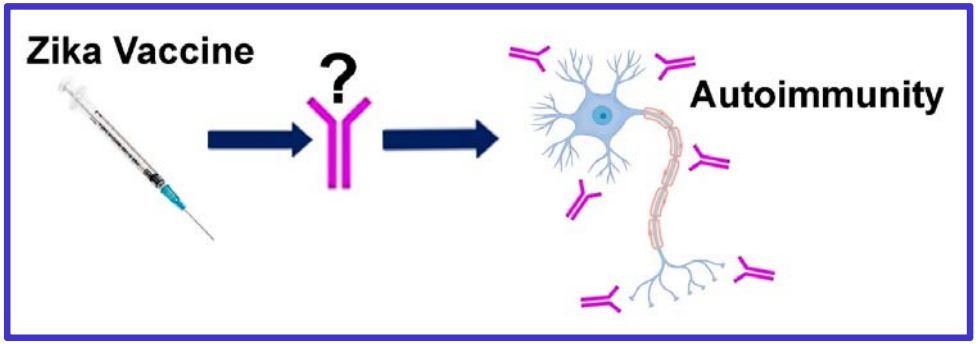
Challenges for Zika vaccines

ZIKV antibody response can trigger ADE and GBS

Antibody Dependent Enhancement

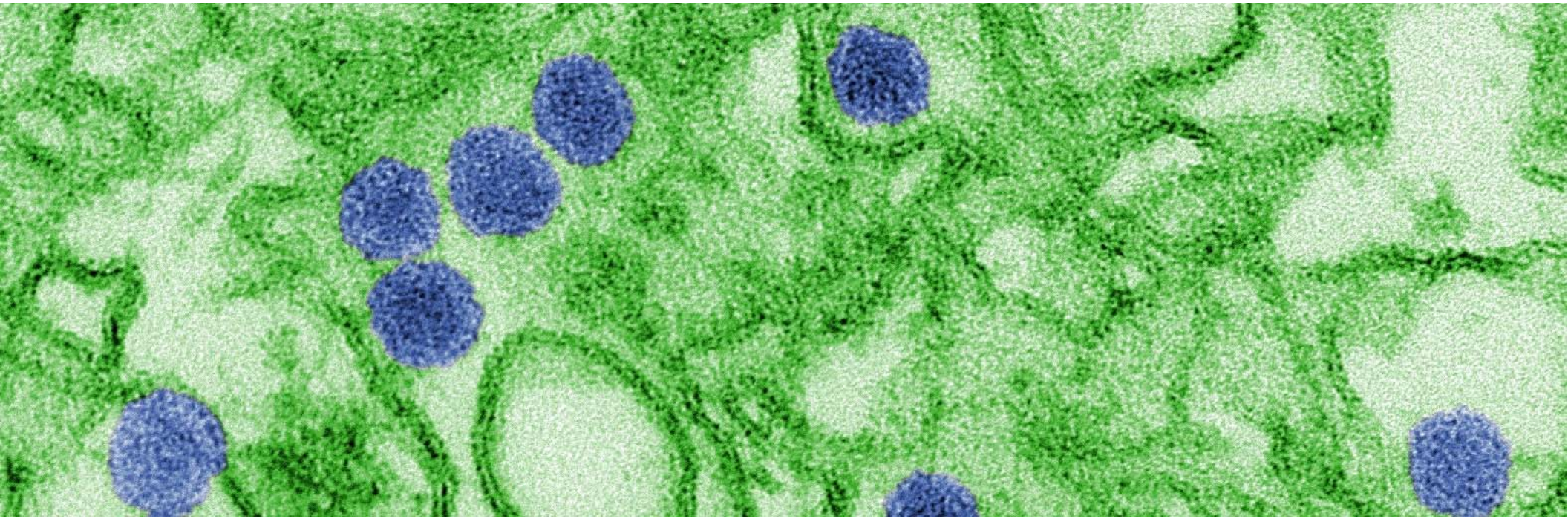


Guillain-Barré syndrome



Adapted from Richner JM, Diamond MS. Curr Opin Immunol 53:130-136, 2018.

ATCC



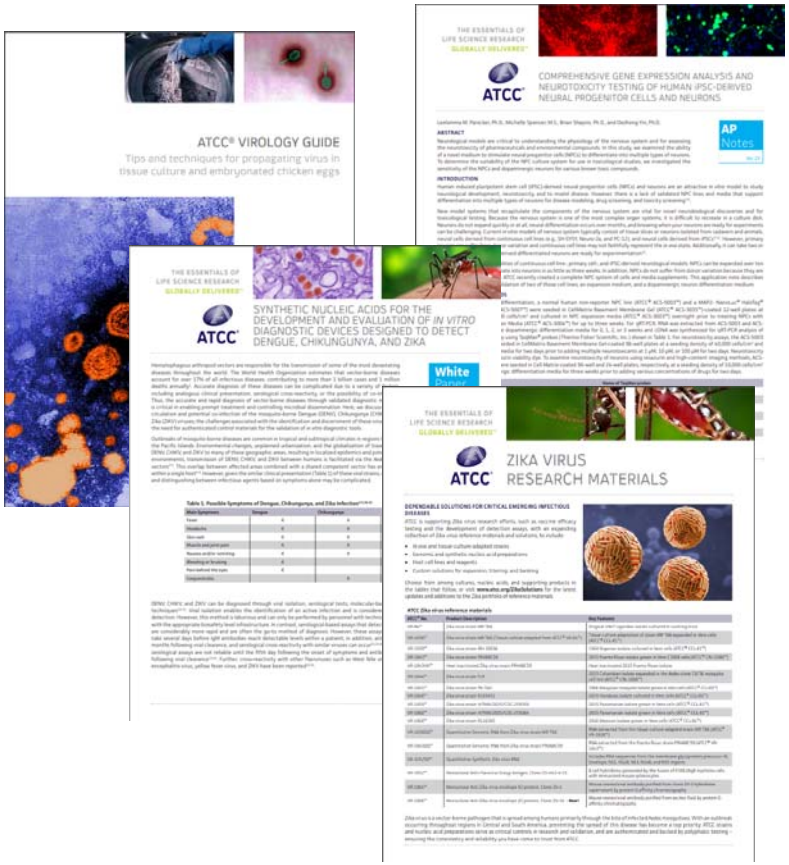
Scientific Approach

ATCC®

ATCC Zika Resources

www.atcc.org/zikasolutions

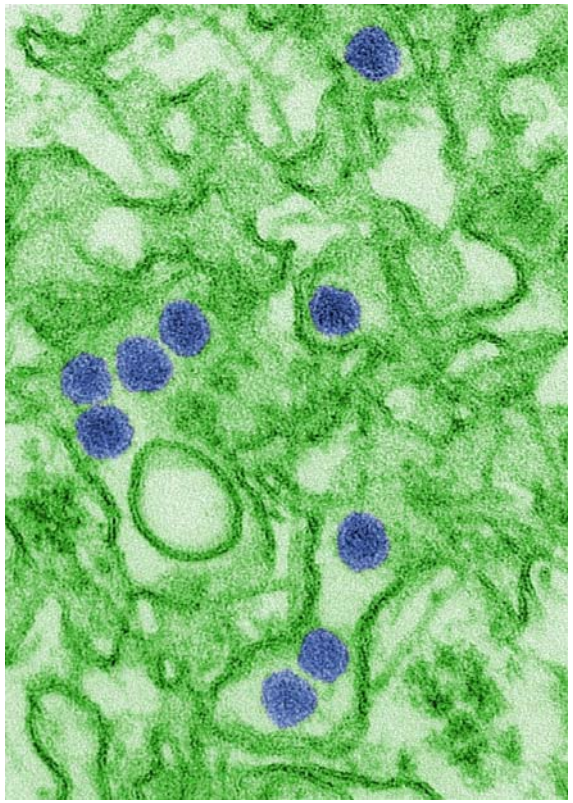
ATCC® No.	Product Description
VR-84™	Zika virus strain MR 766
VR-1838™	Zika virus strain MR 766 (Tissue culture-adapted from ATCC® VR-84™)
VR-1839™	Zika virus strain IBH 30656
VR-1843™	Zika virus strain PRVABC59
VR-1843HK™	Heat inactivated Zika virus strain PRVABC59
VR-1844™	Zika virus strain FLR
VR-1845™	Zika virus strain P6-740
VR-1848™	Zika virus strain R103451
VR-1859™	Zika virus strain H/PAN/2015/CDC-259359
VR-1860™	Zika virus strain H/PAN/2015/CDC-259364
VR-1868™	Zika virus strain R116265



Principles for vaccine design

Strategies for developing a novel, efficacious, and safe Zika vaccine

- Rational design for Zika antigen for improving vaccine immune response
- Virus-like particle (VLP) technology for delivering highly immunogenic Zika antigens
- Intranasal administration for inducing:
  - Systemic protection
  - Mucosal protection
- Non-invasive ZIKV-specific vaccine that provides protection against:
  - Sexual transmission
  - Vector-borne transmission

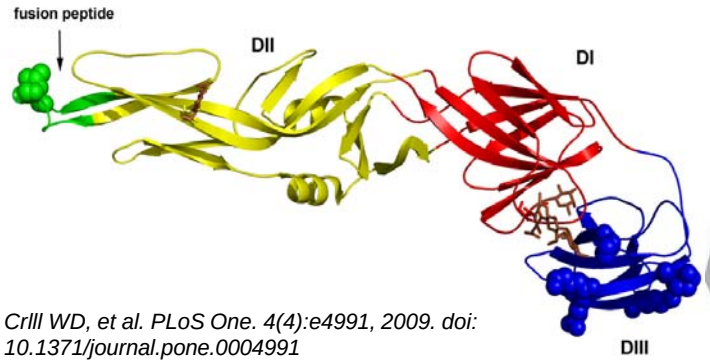


ATCC

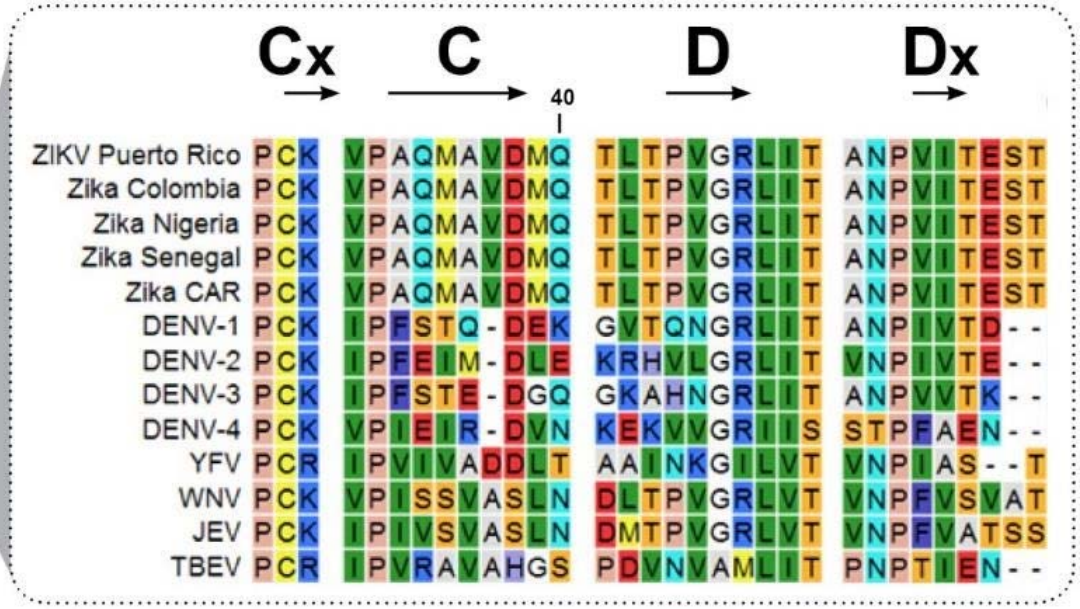
Rational design

Aims to improve vaccine immunogenicity, specificity, and inter-strain cross-protection

E protein



Crlll WD, et al. PLoS One. 4(4):e4991, 2009. doi: 10.1371/journal.pone.0004991



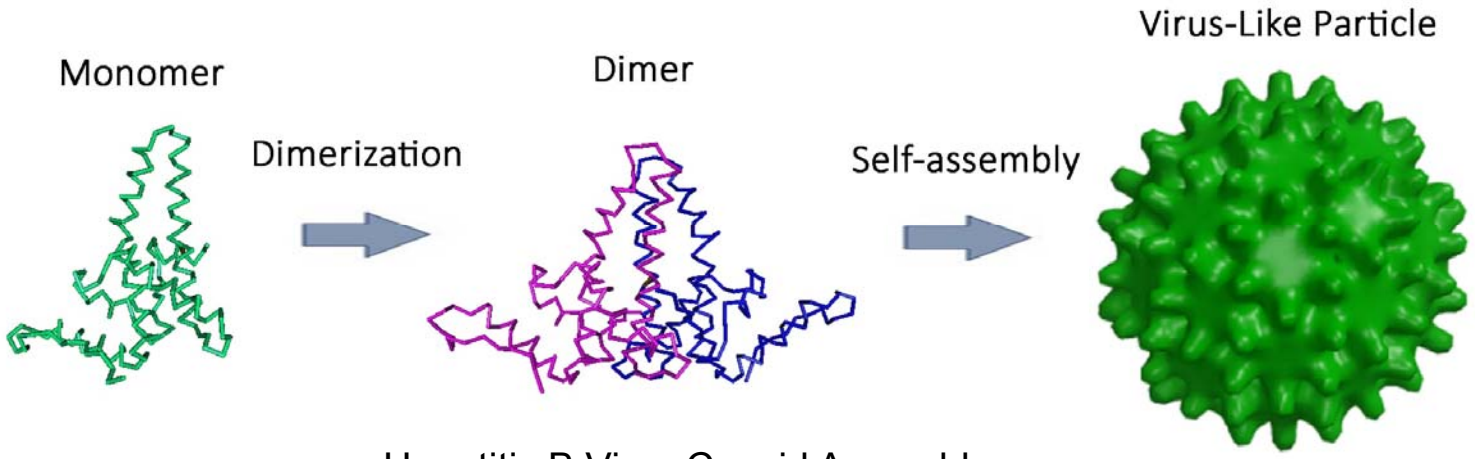
- A specific ZIKV antigen preserved between Zika-strains is selected as a vaccine immunogen

ATCC

Virus-like particle (VLP) technology

VLP technology demonstrated a strong impact in vaccinology

- Multimeric assembly of viral protein in 20 to 200 nm particle diameter
- Highly immunogenic for mimicking viral morphology
- Very safe because of the lack of virus genetic material
- Chimera VLPs can be used for a foreign antigen delivery



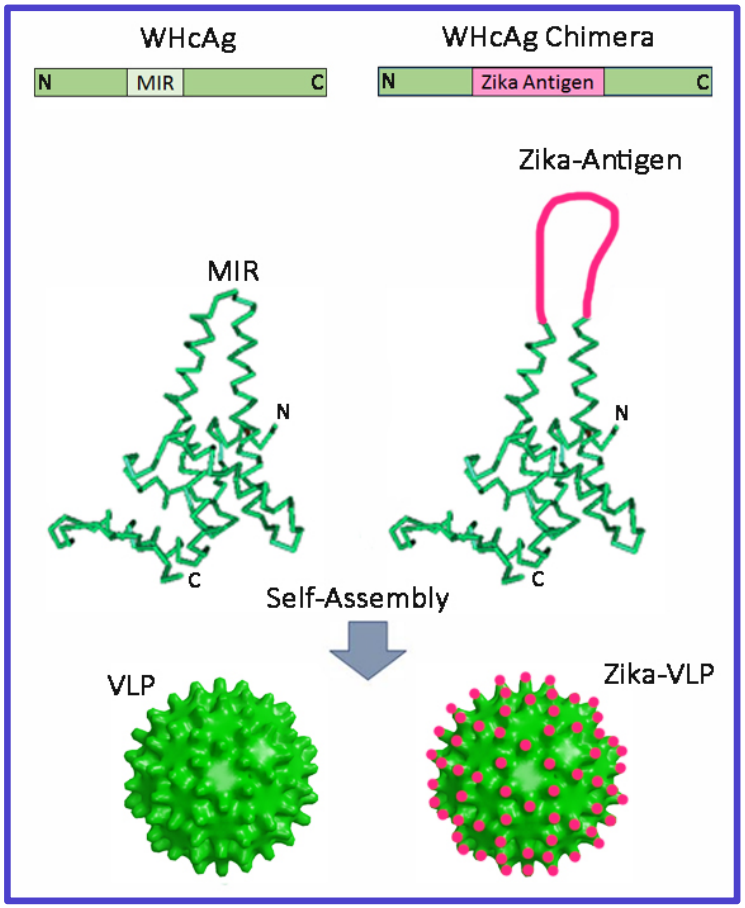
Hepatitis B Virus Capsid Assembly

Adapted from Peyret H, et al. PLoS One 10(4), 2015. doi:10.1371/journal.pone.0120751

ATCC

WHcAg VLP chimeric system

Zika antigen are delivered by engineered VLPs

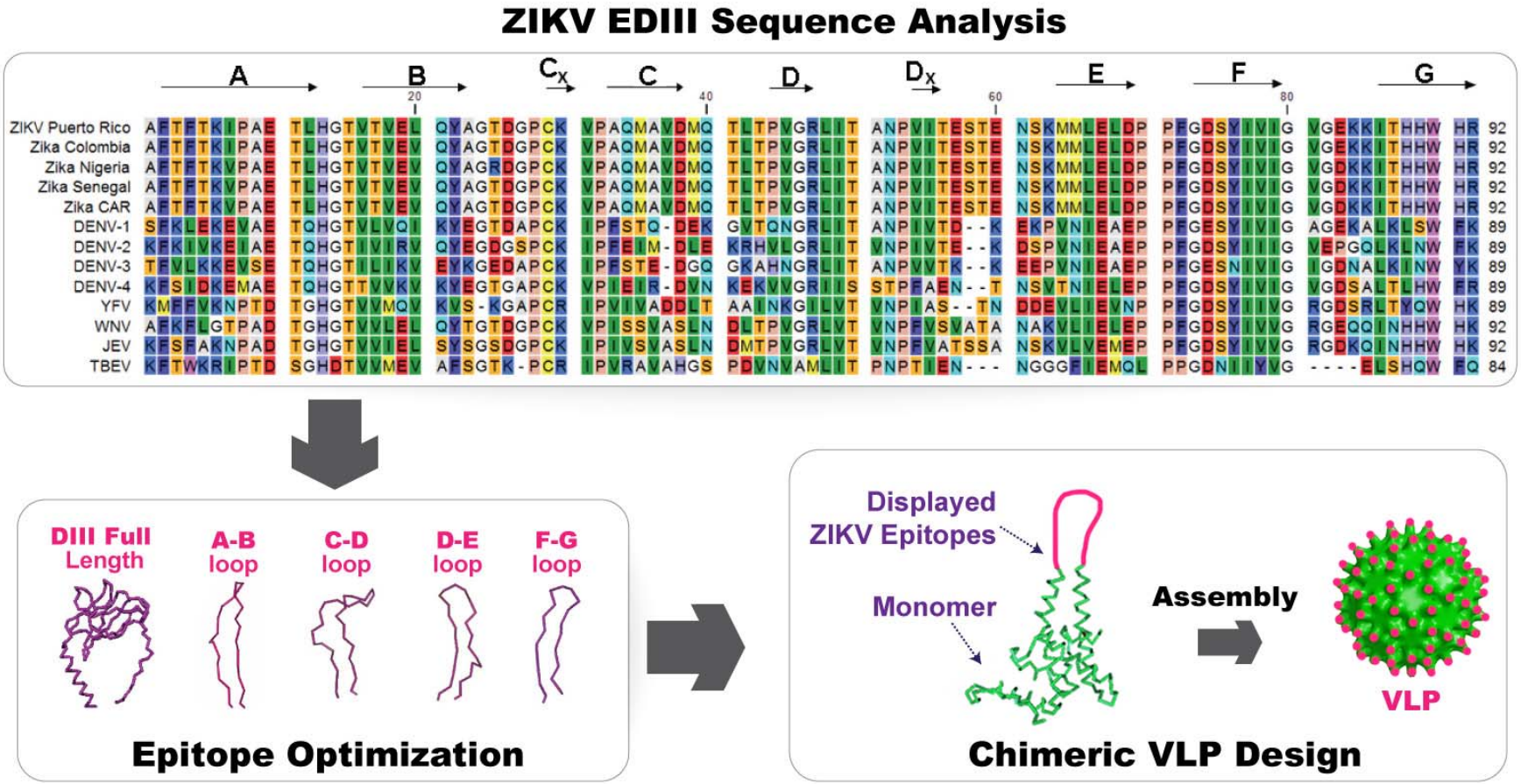


- Woodchuck hepatitis core antigen protein (WHcAg) is a capsid protein from the woodchuck hepatitis virus
- WHcAg VLPs can be produced using DNA recombinant technology
- WHcAg chimeric system allows delivery of specific Zika antigens

ATCC

Structural vaccinology

Conformational epitopes are selected and optimized for enhancing vaccine immunogenicity



Intranasal delivery for Zika-VLP vaccine

Intranasal delivery route is a convenient and powerful system for immunization

Mucosal Atomization Device (MAD)

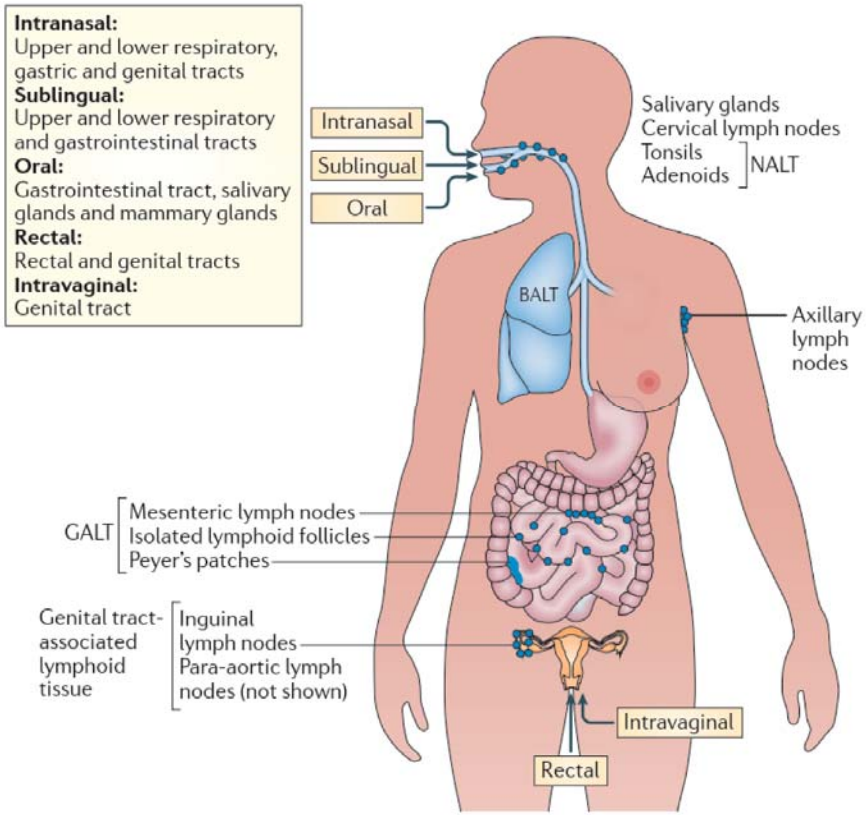
BMC Pediatr 14(67) 2014. doi: 10.1186/1471-2431-14-67.

- Inexpensive
- Less invasive and less discomfort
- Safe due to needle-free delivery
- Increases vaccine compliance
- Easy to deliver for mass administration
- Broader antibody induction:
  - Mucosal
  - Systemic
- Induction of mucosa-associated lymphoid tissues (MALTs)
- Protection for both mosquito and sexual transmission

ATCC

Mucosal protection from nasal to genital tract

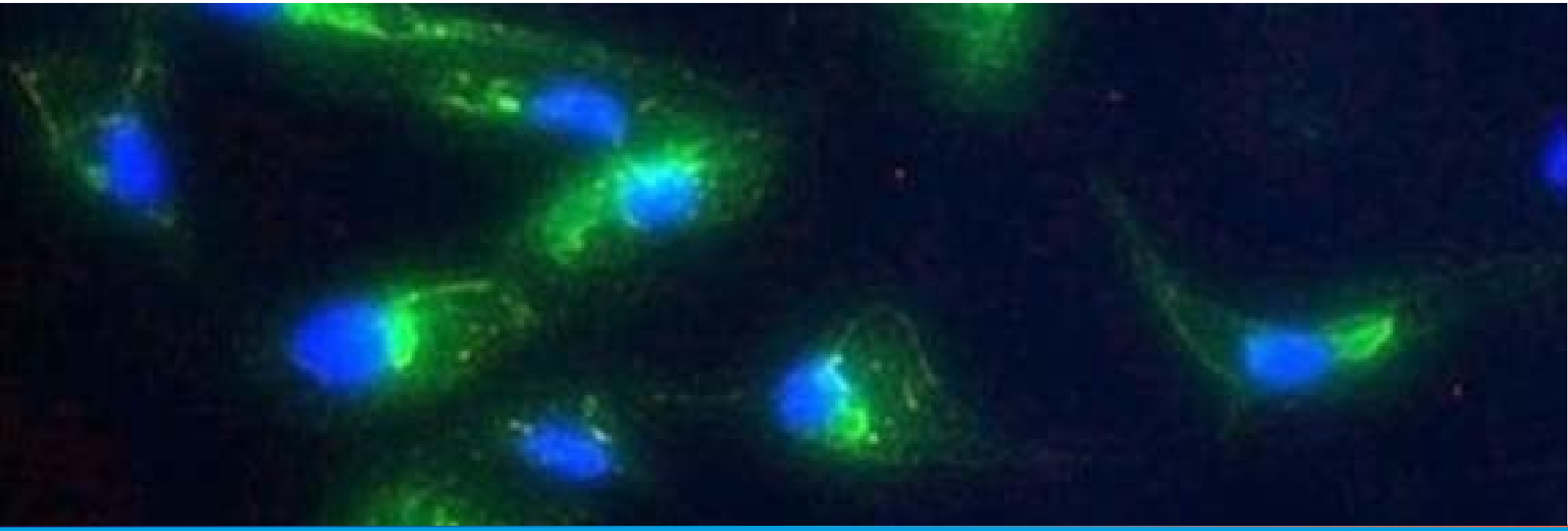
The nasal and genital tract mucosae are connected immunologically



Singh, B. et al. Int J Mol Sci 19, 3639, 2018.

- Nasal immunization stimulates a very strong mucosal immune response
- This response localizes to the genital-vaginal tract mucosa

ATCC



Results

Multiple methodologies for vaccine development

ATCC®

Zika-VLP bio-production

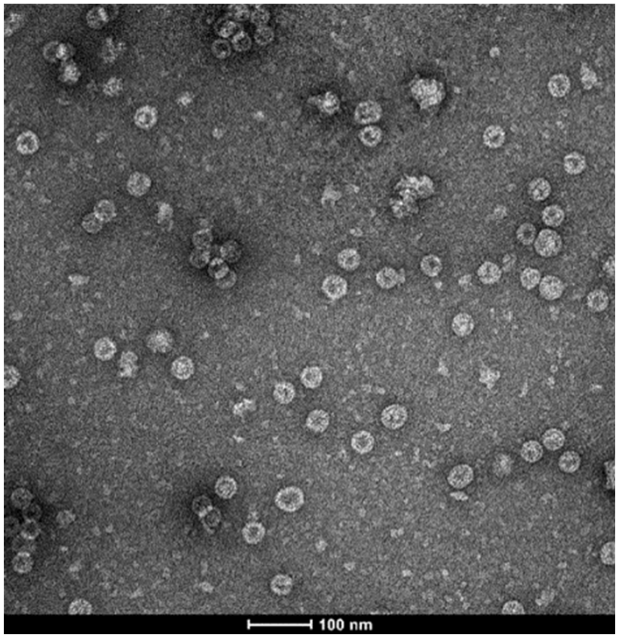
A yeast strain from ATCC® is used for Zika-VLP bioproduction

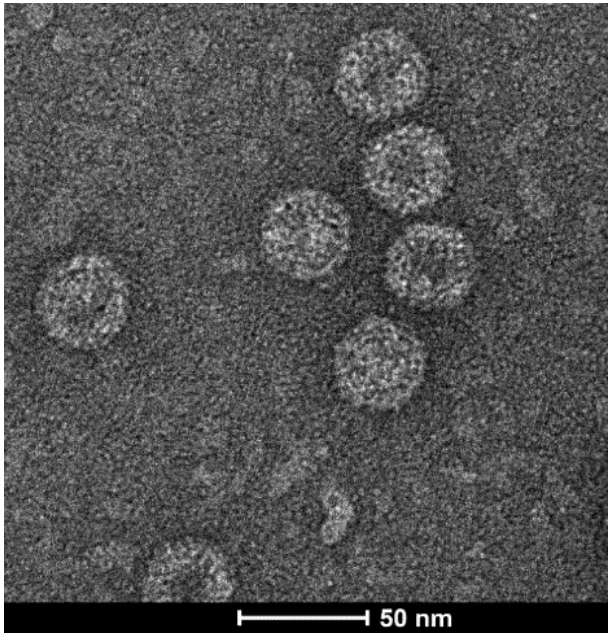
Komagataella phaffii clones

- *Komagataella phaffii* Kurtzman (deposited as *K. pastoris*; ATCC® 76273™)
- Low cost and high-scalability
- Bio safety level 1 organism
- Most of FDA approved VLP-based vaccines are manufactured in yeast

Zika-VLP morphology

Transmission electron microscopy (TEM) is used to study VLP morphology





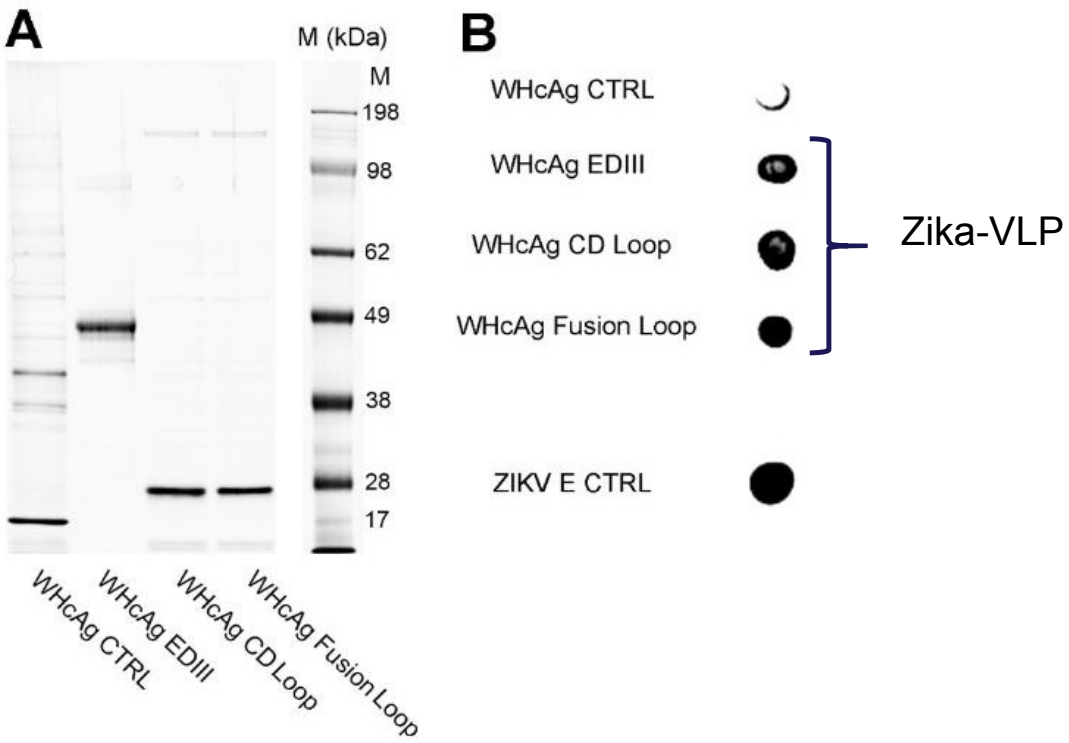
- Zika vaccine demonstrates VLP morphology

ATCC

Zika-VLP purity, antigenicity

Biochemical and immunological assays demonstrate the quality of Zika-VLPs

SDS Page Immunoblot

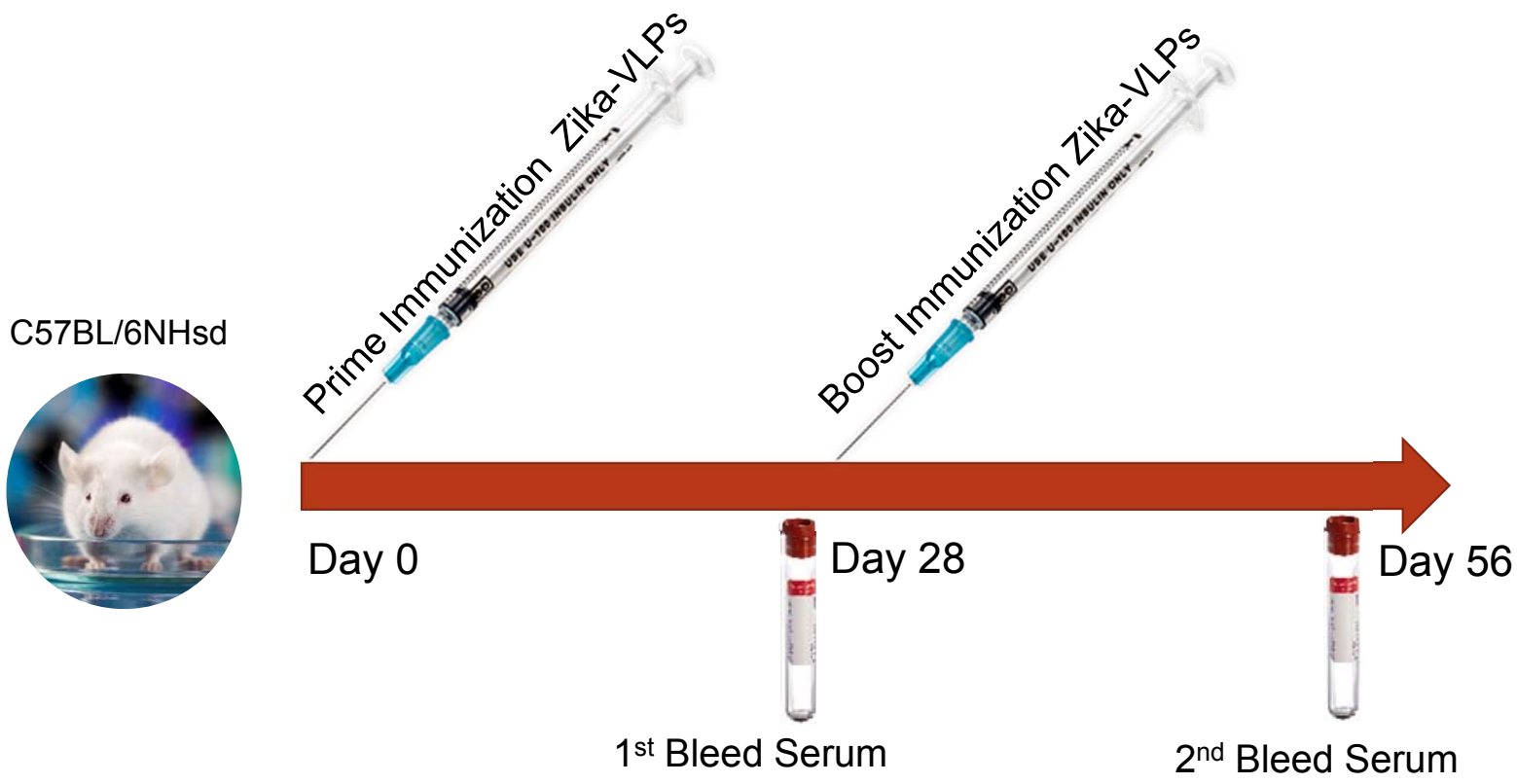


- Zika-VLP purity is assessed using SDS-PAGE
- Antigenicity is tested using immunoblot and mouse serum antibody against Zika virus

ATCC

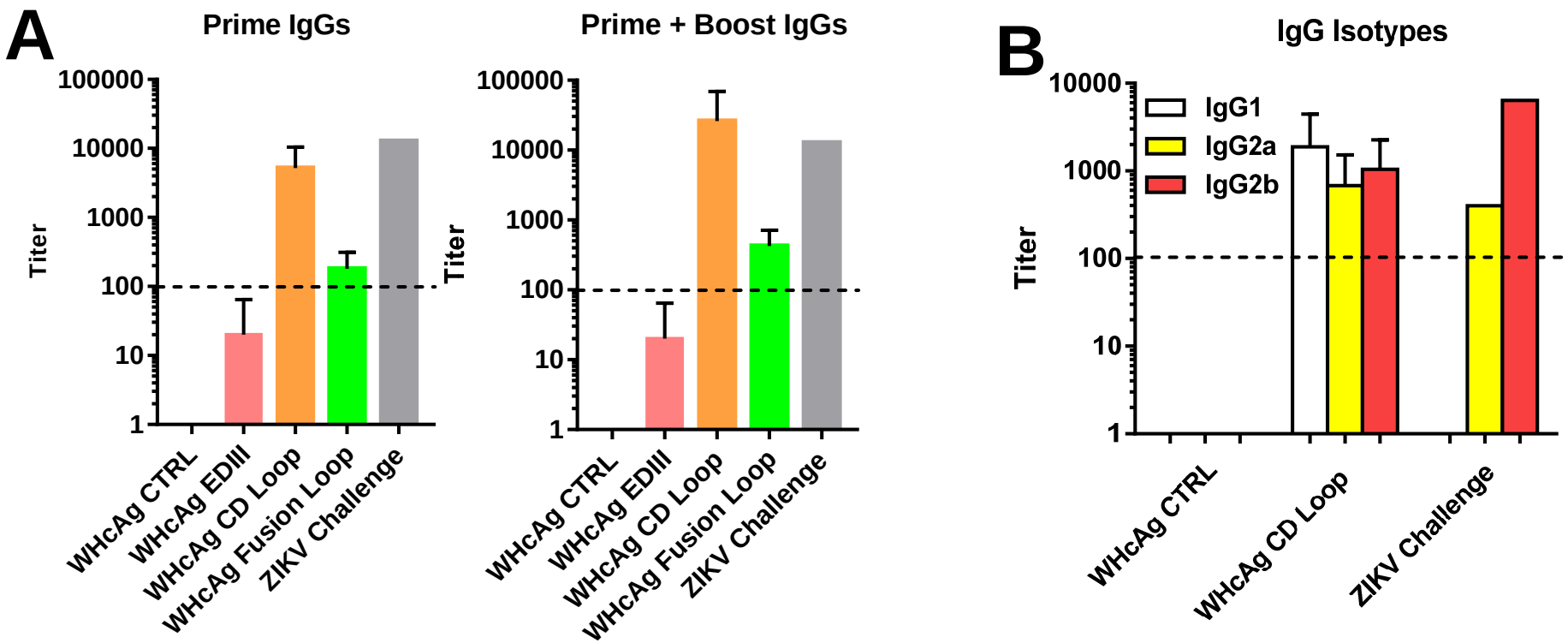
Mouse model for Zika-VLP testing

Safety and Immunogenicity is tested in a mouse model



Zika-VLP immunogenicity

ELISA method is used to assess mouse antibody response against Zika E coating antigen

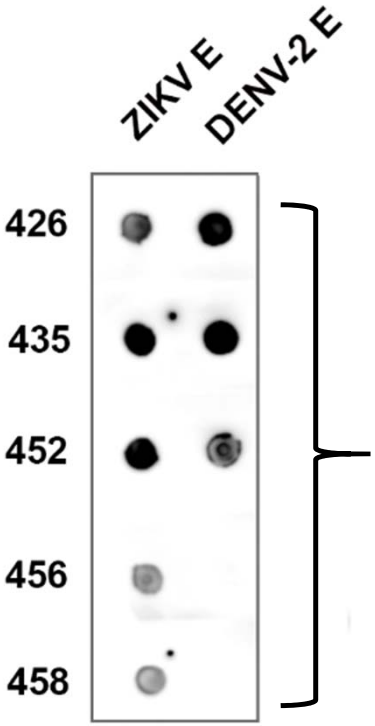


- Zika-VLP WHcAg CD Loop induces strong immune response via IgG1 and IgG2 production

ATCC

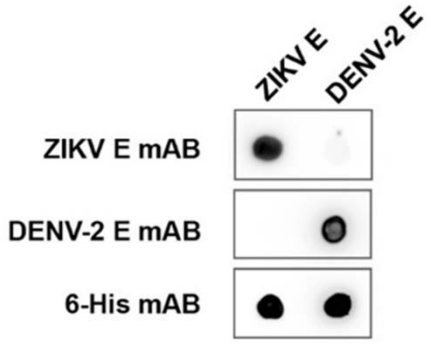
Zika virus immune response

Immunoblotting analysis for serum antibody of mice immunized with Zika virus



Mice infected with ZIKV

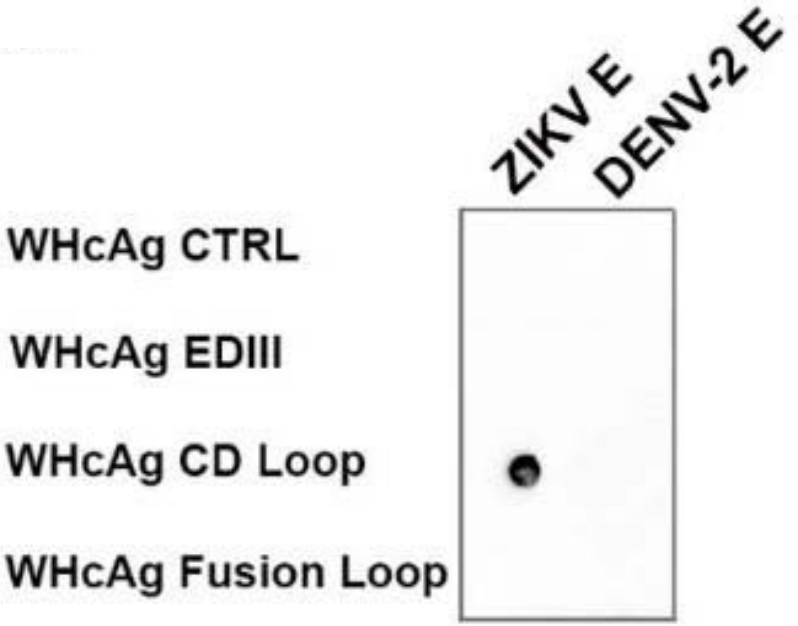
- Zika virus induces cross-reacting antibodies with dengue virus E
- Cross-reacting antibodies can potentially induce GBS and ADE



ATCC

Zika-VLP immune response specificity

Immunoblotting analysis for serum antibody of mice immunized with Zika-VLPs

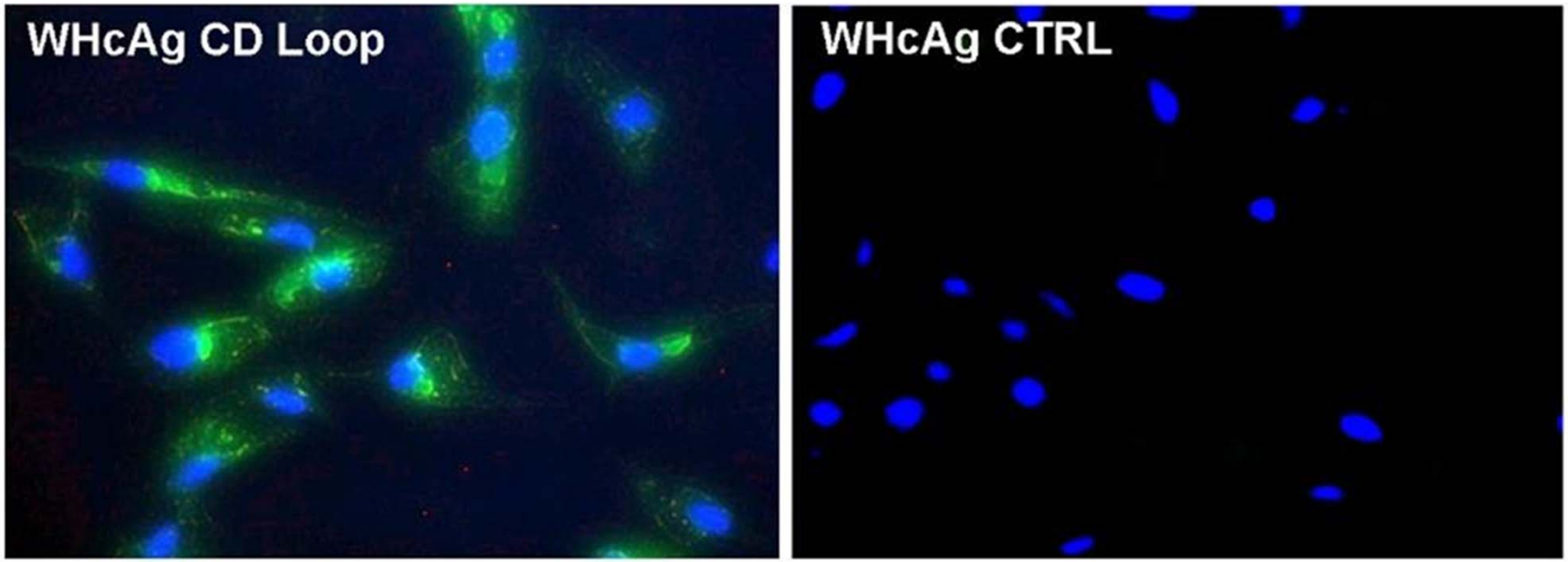


- Zika-VLP WHcAg CD Loop induces specific antibody response against Zika E antigen

ATCC

Zika-VLP immune response against Zika virus

Immunofluorescence analysis of Zika-infected Vero (ATCC® CCL-81™) using Zika-VLP WHcAg CD Loop immune serum



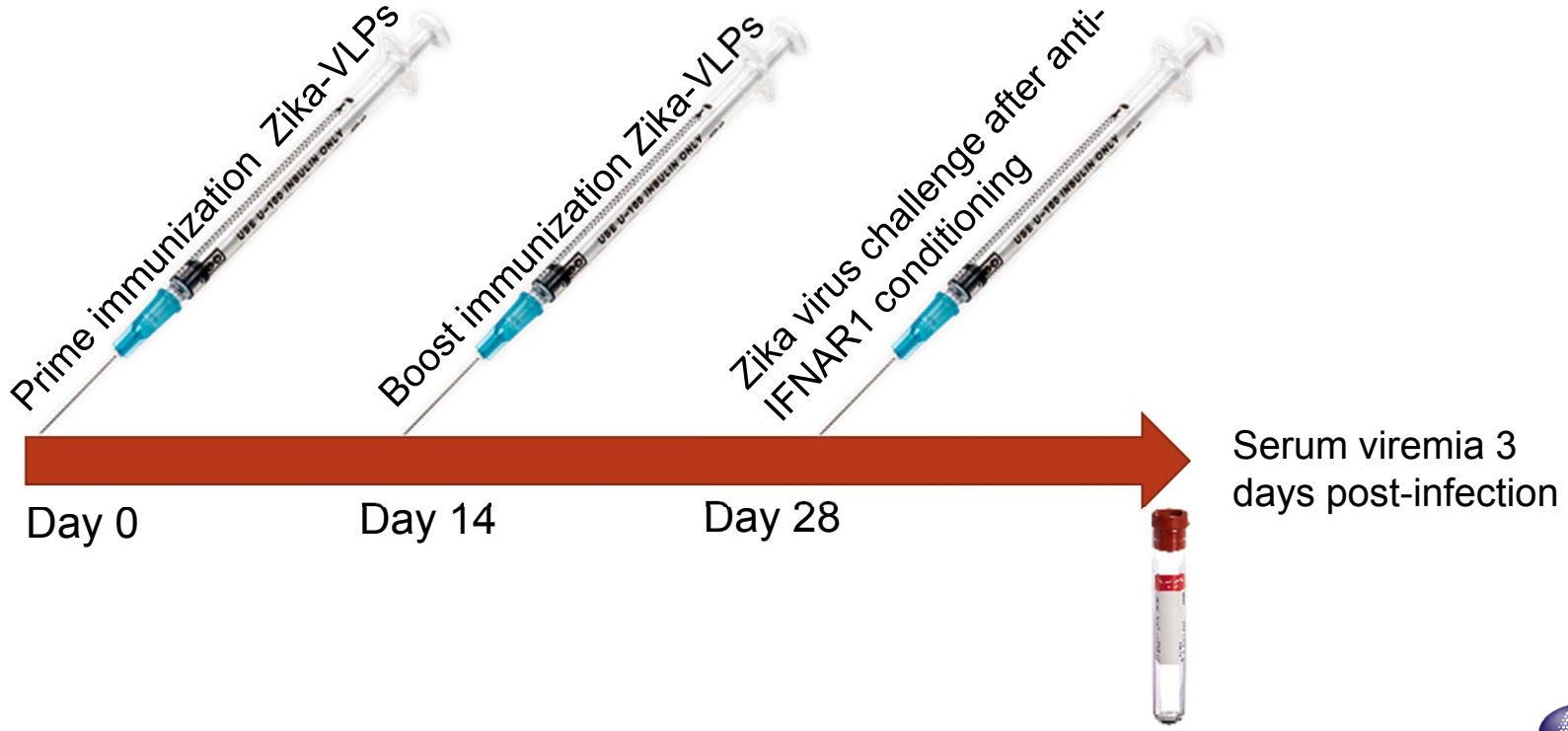
Green: Zika virus staining (FITC)
Blue: nuclear staining (DAPI)

- Zika-VLP WHcAg CD Loop elicits antibodies against Zika virus

Mouse model for Zika virus protection

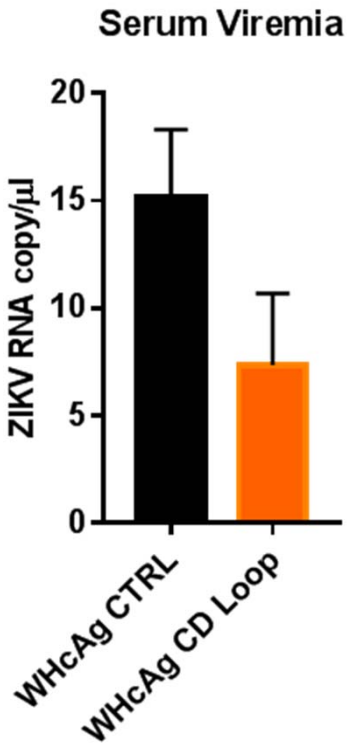
Safety and immunogenicity is tested in mouse model

C57BL/6NHsd



Zika-VLP WHcAg CD Loop protects against Zika infection

Quantitative Real-Time PCR is used to measure Zika genome copies in the serum

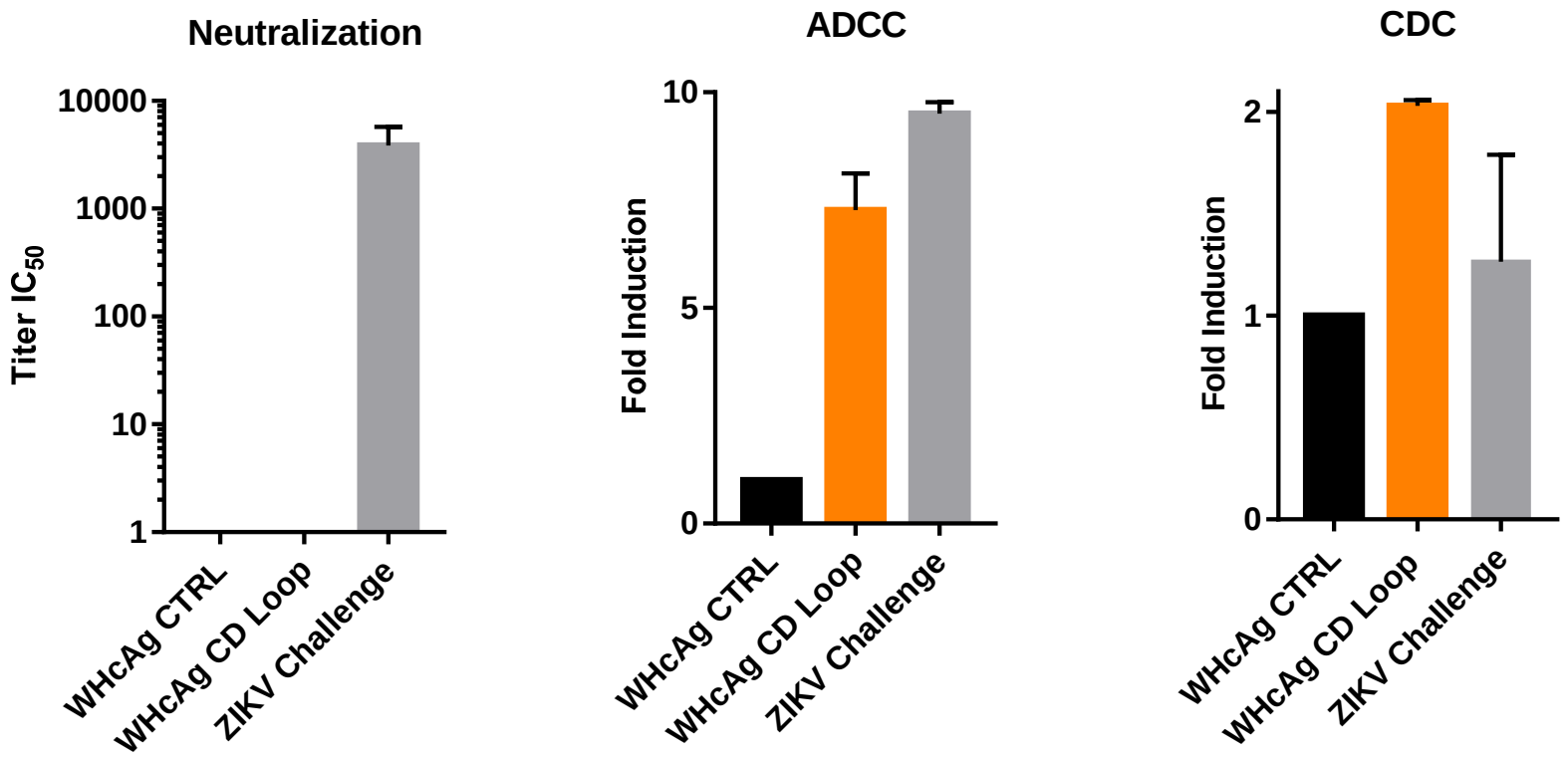


- Zika-VLP WHcAg CD Loop reduces serum viremia in mice after Zika challenge

ATCC

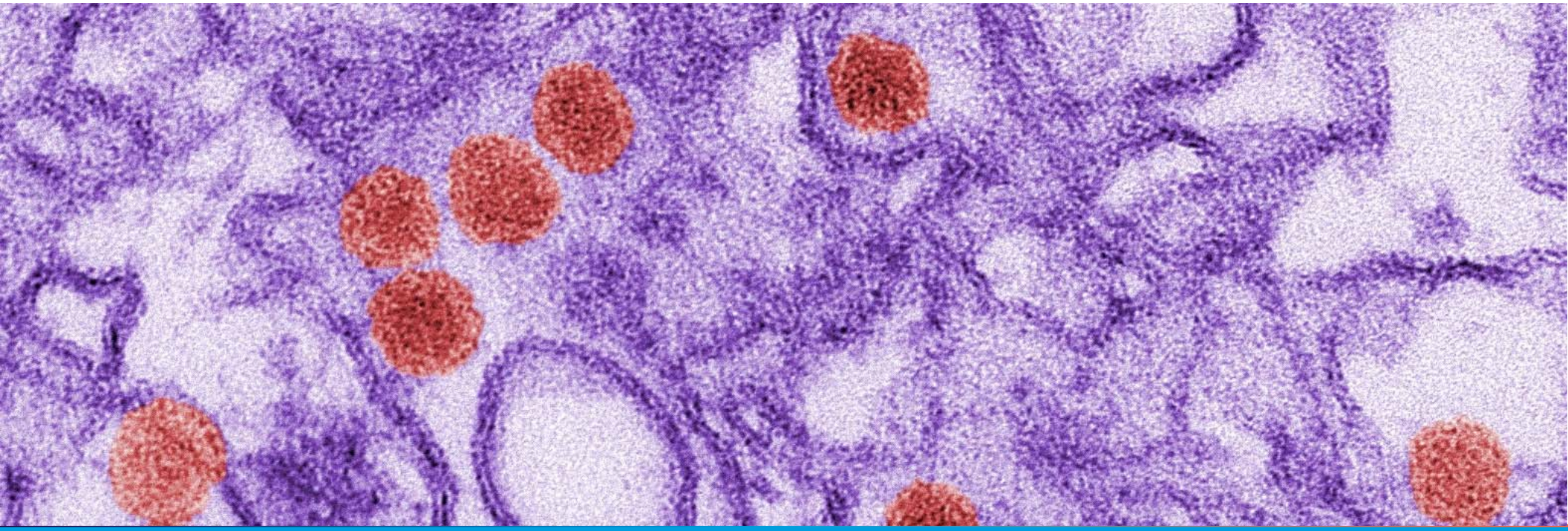
Zika-VLP mechanism of antibody protection

Viral and cellular assays are applied for testing antibody protection



- Zika-VLP WHcAg CD Loop induces antibodies with ADCC and CDC protective activities

ATCC



Summary

ATCC®

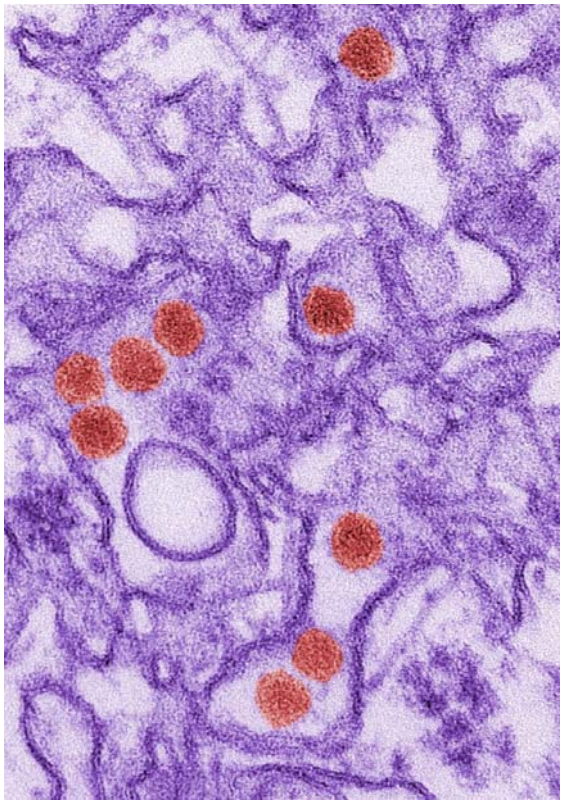
Summary

Novel ATCC Zika-VLP vaccine

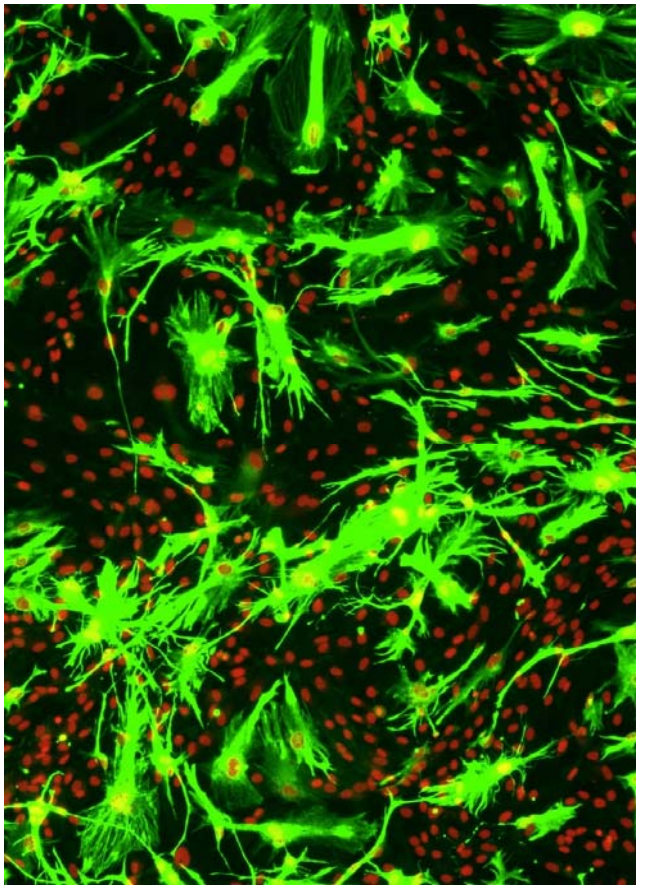
- Main study goal: Apply effective and safe immunization strategies for the generation of improved vaccines

- Complementary fields provided the foundation for a rational approach to creating novel vaccines
  - Structural biology
  - Virology
  - Adjuvant formulation
  - Immunology

- ATCC's novel Zika-vaccine candidate has demonstrated in murine models:
  - Safety
  - Immune response
  - Protection activity



Disclaimers



Cultivating collaboration to support global health

Let's continue to cultivate collaboration:

- ATCC is committed to making clinically relevant models available to the global scientific community
- Learn more about ATCC's partnerships with scientific community toward preventing and containing devastating epidemics

2020.atcc.org/supporting-global-health to learn more