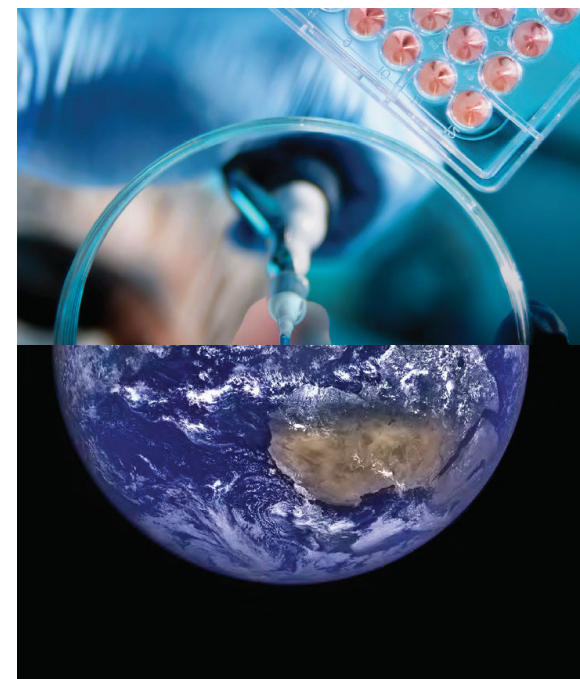
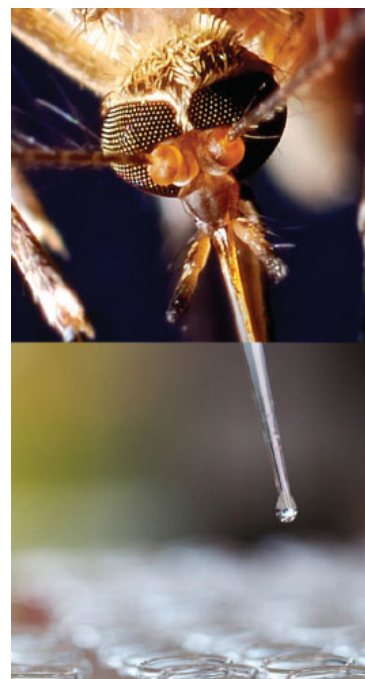
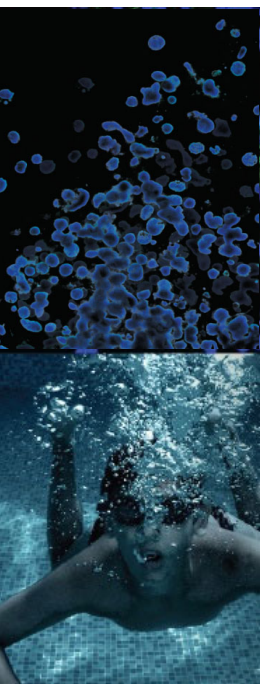




Creating a vaccine for the tick-borne Powassan Virus

Velasco Cimica, PhD
Scientist, ATCC

Credible Leads to Incredible™



About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for microbial culture – the “*gold standard*”
- Innovative R&D company featuring a novel genome portal, BSL-1 derivatives of infectious organisms, novel technologies for R_x and D_x development
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

Agenda

- Introduction
- Scientific approach
- Results
- Summary



<https://www.cdc.gov/ticks/gallery/index.html>

Powassan Virus

An emerging global infection

- Flavivirus like Zika virus and dengue virus
- Transmitted by the tick species *Ixodes scapularis* and *Ixodes cookei*
- Circulating in North America and the Russian Far East
- Causes encephalitis, meningitis, or encephalomyelitis
- Medical treatment available is only supportive
- There is not an FDA-approved diagnostic test
- Increase in the number of infections in the U.S. in the last decade

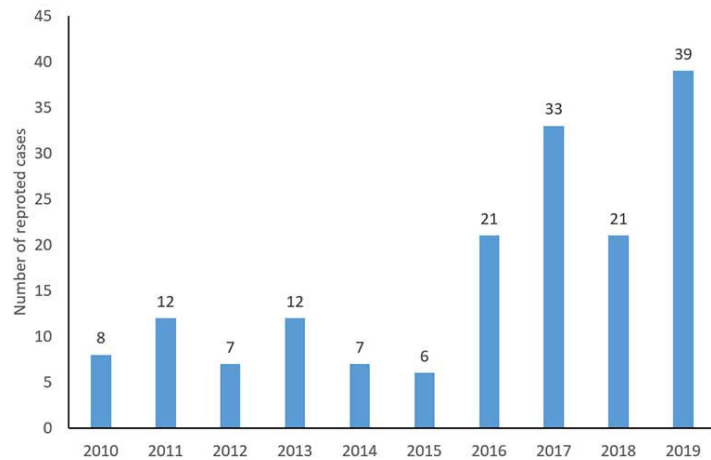


<https://www.cdc.gov/powassan/index.html>

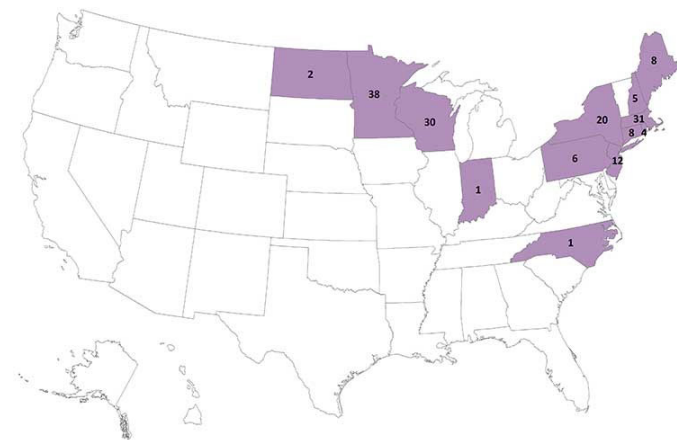
Powassan Virus Statistics (2010-2019)

Powassan and Emerging Infection in the US

Powassan virus neuroinvasive disease cases reported by year



Powassan virus neuroinvasive disease cases reported by state



<https://www.cdc.gov/powassan/statistics.html>

Factors important in the increase of Powassan infections:

- Increase in tick population due to global warming
- Reforestation, urban sprawl, and higher human density in rural area
- Possible adaptation of POWV in the tick vector

Ixodes scapularis main vector of Powassan Virus

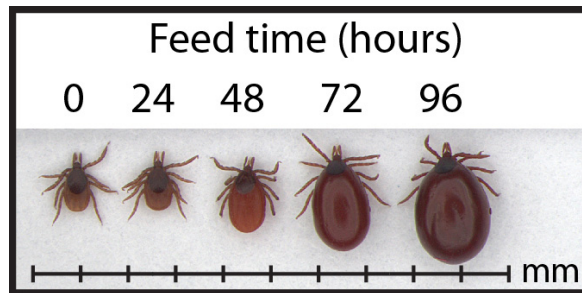


The tick vector population expansion in US

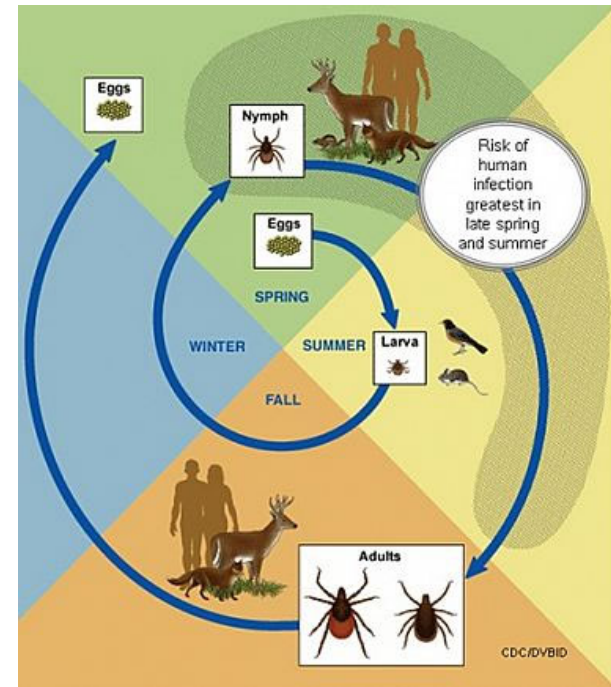
Ixodes scapularis, also called blacklegged tick, is present in the eastern part of U.S.



Stages of tick engorgement



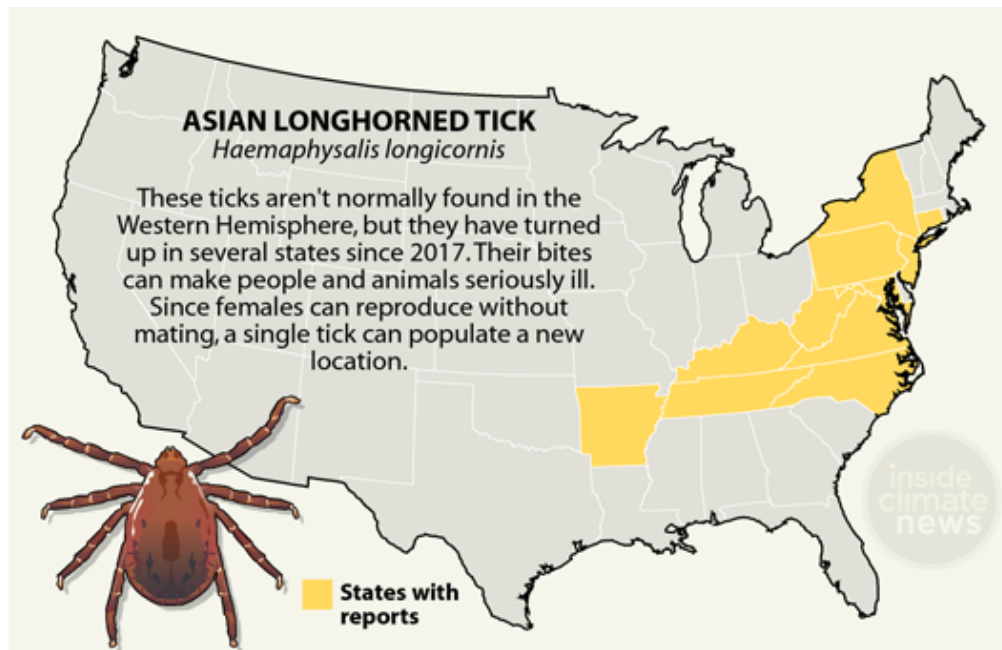
Ixodes scapularis has a life cycle divided in 4 stages



<https://www.cdc.gov/ticks/surveillance/index.html>

Asian Longhorned Ticks: A Potential New Vector

Asian longhorned ticks have spread to the U.S. and can potentially transmit Powassan virus



SOURCE: Centers for Disease Control and Prevention

PAUL HORN / InsideClimate News

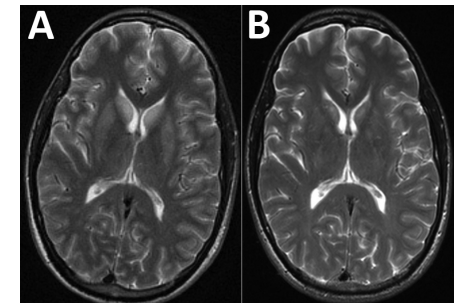
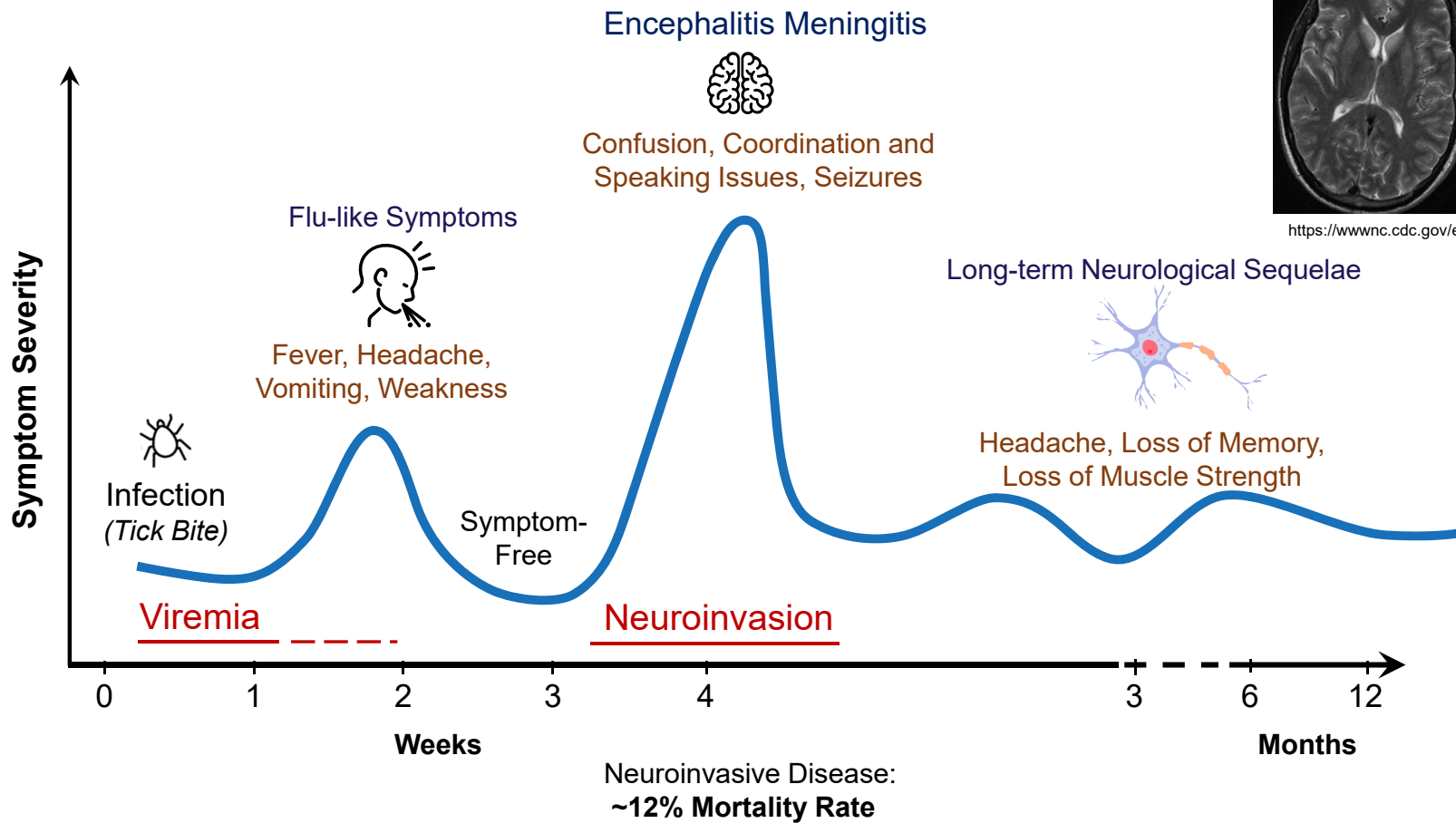
<https://insideclimatenews.org/news/05072019/tick-disease-danger-species-longhorned-lonestar-climate-change/>



<https://www.cdc.gov/ticks/longhorned-tick/index.html>

Powassan Virus Disease

Powassan virus-induced encephalitis and meningoencephalitis

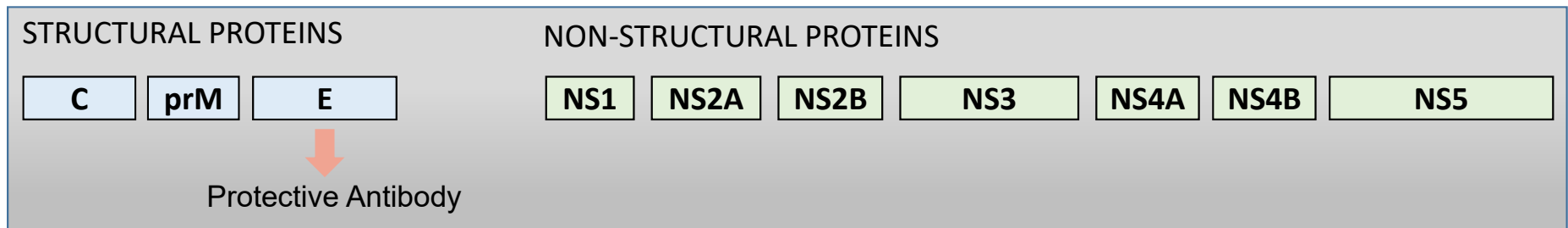
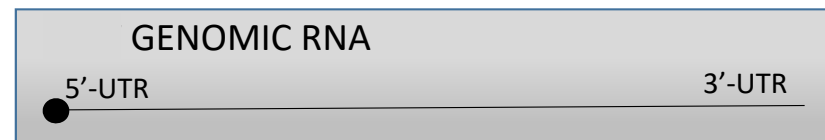
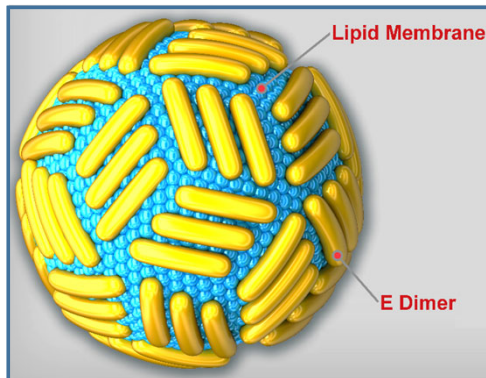


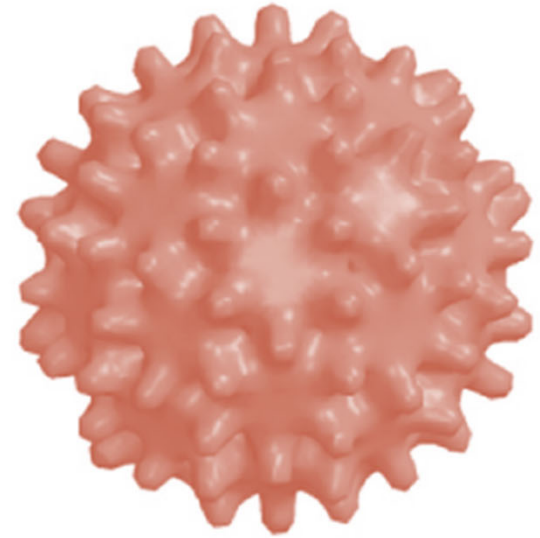
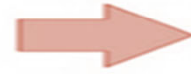
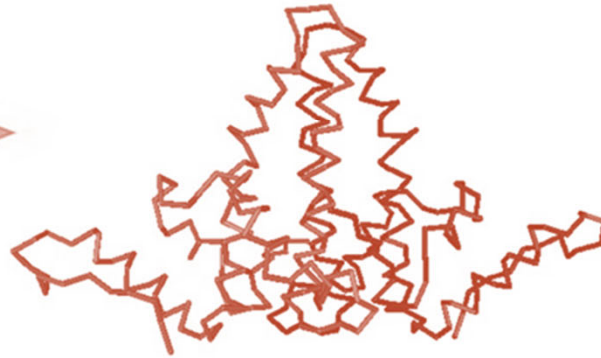
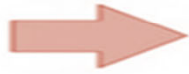
<https://wwwnc.cdc.gov/eid/article/25/10/18-1262-f1>

Powassan Virus Organization

Powassan virus genome

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





Scientific Approach

ATCC Powassan Resources

ATCC® No.	Product Description
VR-1262™	Powassan virus LB strain
VR-3273SD™	Quantitative Synthetic Powassan virus lineage I RNA
VR-3275SD™	Quantitative Synthetic Powassan virus lineage II RNA
VR-1262AF™	Powassan virus immune ascitic fluid [V-518-711-562]
VR-1262CAF™	Powassan virus control ascitic fluids
CRL-11973™	Tick cell line, IDE8
CRL-11974™	Tick cell line, ISE6

www.atcc.org/vectorborne

ATCC® VIROLOGY GUIDE
Tips and techniques for propagating virus in tissue culture and embryonated chicken eggs

SYNTHETIC NUCLEIC ACIDS FOR THE DEVELOPMENT AND EVALUATION OF IN VITRO DIAGNOSTIC DEVICES DESIGNED TO DETECT DENGUE, CHIKUNGUNYA, AND ZIKA

Table 1. Possible Symptoms of Dengue, Chikungunya, and Zika Infections

Symptom	Dengue	Chikungunya	Zika Infection
Fever	X	X	X
Myalgia	X	X	X
Headache	X	X	X
Arthralgia	X	X	X
Conjunctivitis	X	X	X
Exanthema	X	X	X
Diarrhea	X	X	X
Urticaria	X	X	X
Other	X	X	X

DENGUE, CHIKUNGUNYA, AND ZIKA can be diagnosed through viral isolation, serological tests, molecular subtyping, and next-generation sequencing. The identification of an acute infection and a secondary infection. However, this method is laborious and can only be performed by personnel with access to the appropriate facility level infrastructure. In-house serological assays that are easy to use, sensitive, and specific are needed for the diagnosis of these diseases. However, these assays are not available in many laboratories. The ATCC has developed a series of diagnostic assays for the diagnosis of these diseases. The ATCC has developed a series of diagnostic assays for the diagnosis of these diseases. The ATCC has developed a series of diagnostic assays for the diagnosis of these diseases.

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AP Notes

COMPREHENSIVE GENE EXPRESSION ANALYSIS AND NEUROTOXICITY TESTING OF HUMAN IPSC-DERIVED NEURAL PROGENITOR CELLS AND NEURONS

Abstract
Neurotoxicity models are critical to understanding the physiology of the nervous system and for assessing the neurotoxicity of pharmaceutical and environmental compounds. In this study, we assessed the ability of a novel method to stimulate neural progenitor cells (NPCs) to differentiate into multiple types of neurons. To determine the capability of the NPC culture system for use in neurotoxicity studies, we investigated the neurotoxicity of the NPC and dopamine neurons for various known toxic compounds.

Introduction
Human induced pluripotent stem cell (iPSC)-derived neural progenitor cells (NPCs) and neurons are an attractive in vitro model to study neurological development, neurotoxicity, and to model disease. However, there is a lack of validated NPC lines and media that support differentiation into multiple types of neurons for disease modeling, drug screening, and toxicity screening.

Methods
We used a novel system that incorporates the components of the nervous system and cell for neurobiological discovery and for toxicological testing. Because the nervous system is one of the most complex organ systems, it is difficult to recreate in a culture dish. Neurons are specialized cells and their differentiation from stem cells is a complex process that requires precise timing and signaling. We have developed a novel system to generate a diverse population of neurons in vitro. This system is based on the use of a defined set of growth factors and signaling molecules that are known to be essential for the differentiation and maturation of neurons in vivo. We have developed a novel system to generate a diverse population of neurons in vitro. This system is based on the use of a defined set of growth factors and signaling molecules that are known to be essential for the differentiation and maturation of neurons in vivo.

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White Paper

ZIKA VIRUS RESEARCH MATERIALS

Responsible Solutions for Critical Emerging Infectious Diseases
ATCC is supporting Zika virus research efforts, such as in-house efficiency testing and the development of detection assays, with an expanding collection of Zika virus reference materials and solutions, to include:

- In-vivo and tissue culture-adapted strains
- Genomic and synthetic nucleic acid preparations
- Host cell lines and reagents
- Custom solutions for expansion, titration, and banking

Choose from among cultures, nucleic acids, and supporting products in the table that follows or visit www.atcc.org/zika for the latest updates and additions to the Zika portfolio of reference materials.

Zika Virus Reference Materials

ATCC#	Product/Description	Key Features
VR-1262™	Zika virus strain VR-1262	Original human isolate cultured in Vero cells
VR-1262AF™	Zika virus immune ascitic fluid [V-518-711-562]	Human immune ascitic fluid containing high concentrations of virus
VR-1262CAF™	Zika virus control ascitic fluids	Human immune ascitic fluid containing no virus
VR-3273SD™	Quantitative Synthetic Powassan virus lineage I RNA	Quantitative synthetic RNA for diagnostic testing
VR-3275SD™	Quantitative Synthetic Powassan virus lineage II RNA	Quantitative synthetic RNA for diagnostic testing
CRL-11973™	Tick cell line, IDE8	Human embryonic kidney (HEp-2) cell line
CRL-11974™	Tick cell line, ISE6	Human embryonic kidney (HEp-2) cell line



Principles for Vaccine Design

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

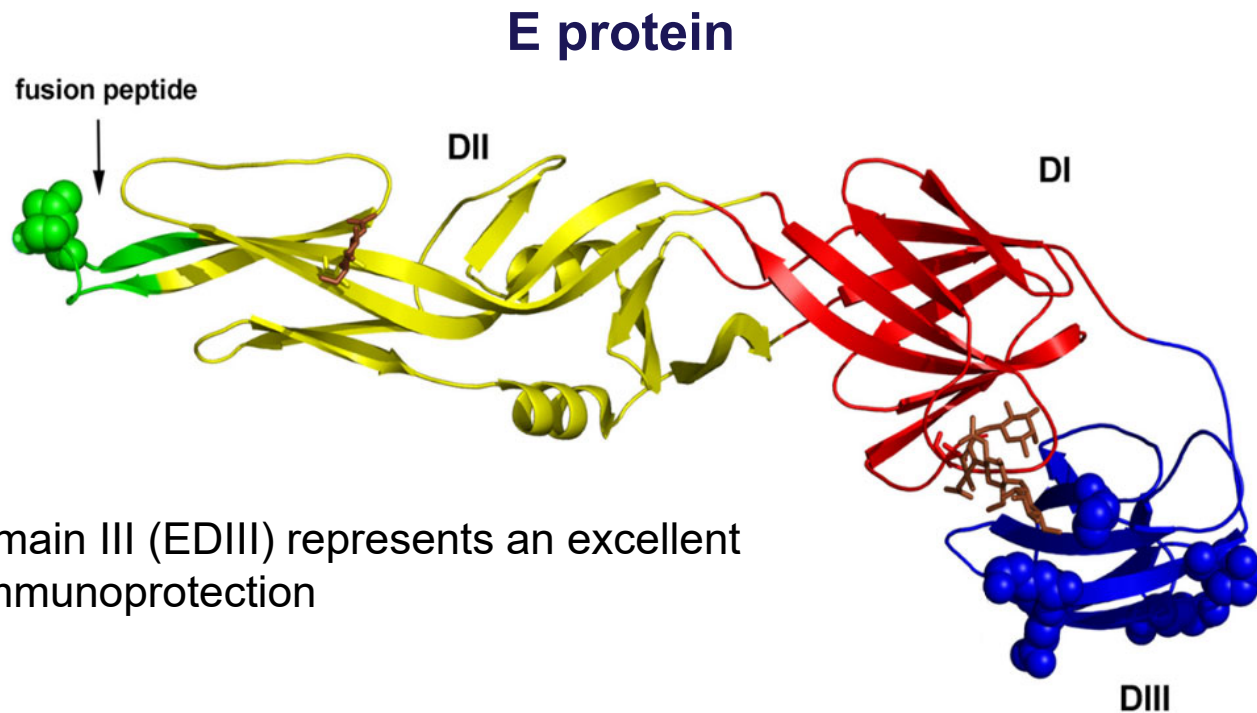
Virus-like particle (VLP) technology for delivering highly immunogenic Powassan antigens

Non-invasive Powassan-specific:

- Protection from vector-borne transmission
- Sterilizing immunity
- High level of safety and tolerability
- Immunogenicity in immunocompromised
- Stable for standard storage and distribution
- Easy to manufacture

Rational Design

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

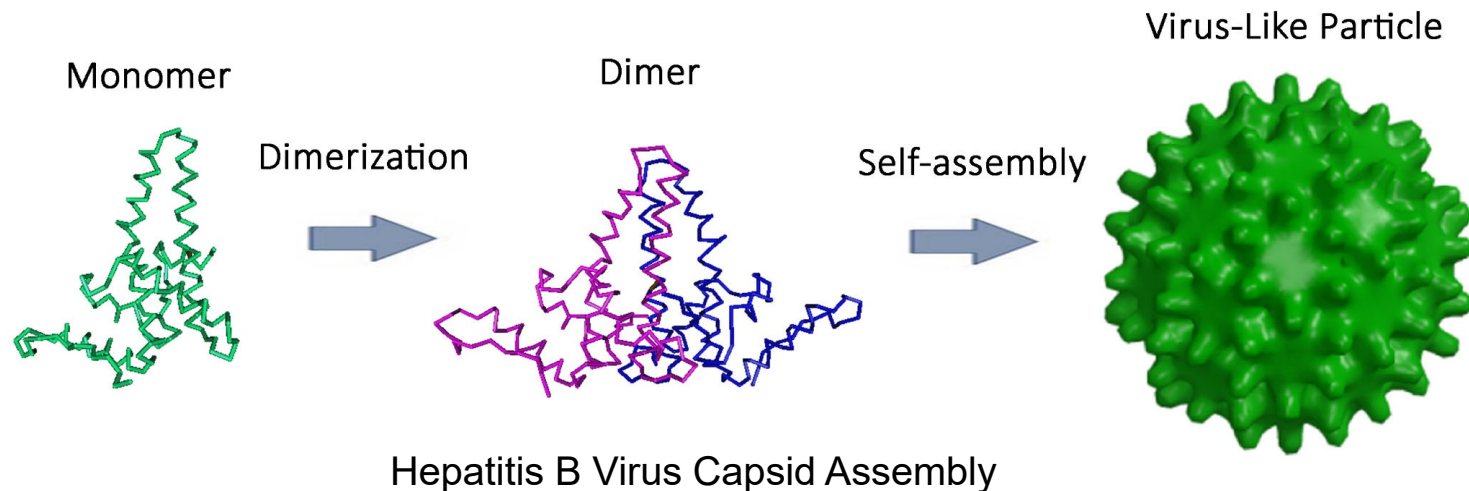


E protein Domain III (EDIII) represents an excellent antigen for immunoprotection

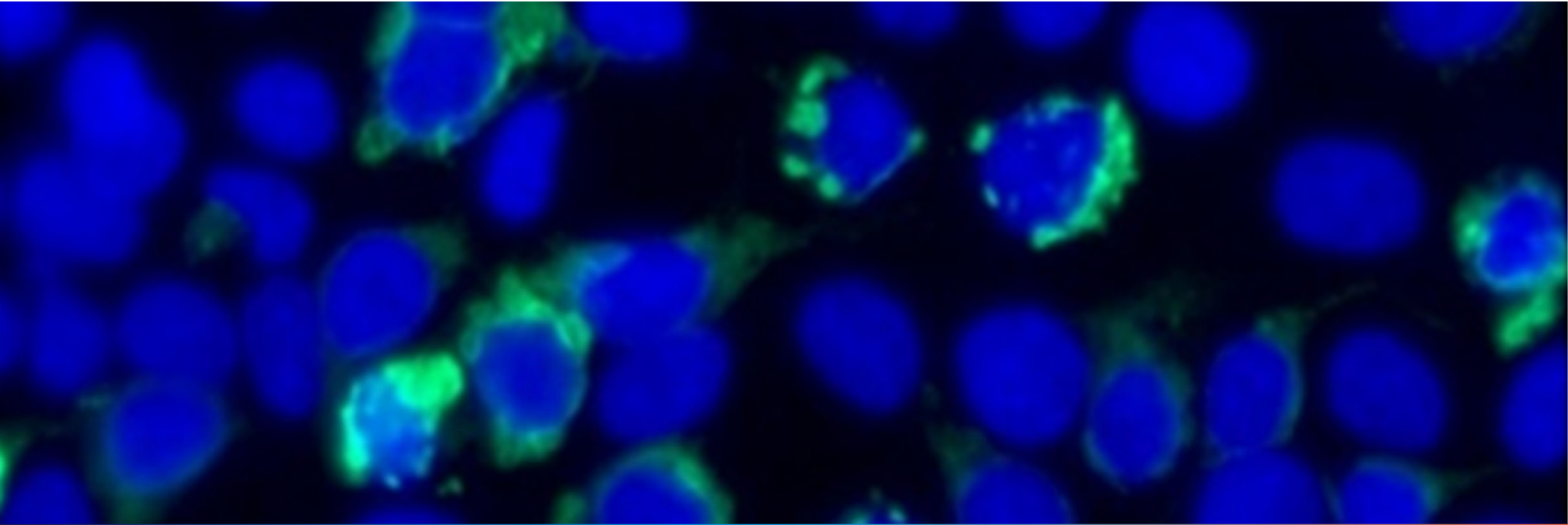
Virus-like Particle (VLP) Technology

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
- FDA-approved vaccines against Hepatitis B virus and Human papillomavirus



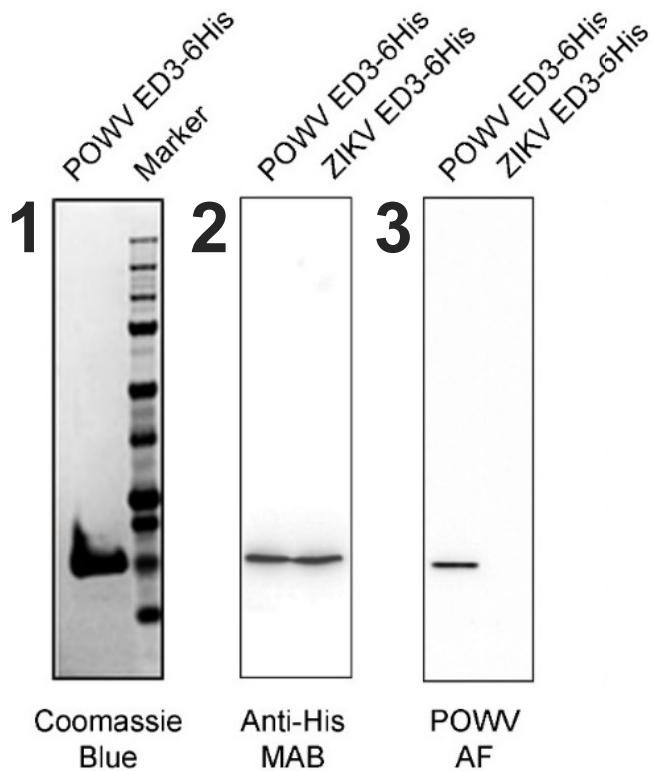
Adapted from Peyret H, et al. PLoS One 10(4), 2015. doi:10.1371/journal.pone.0120751



Results

POW-EDIII Recombinant Protein Standard

POW-EDIII testing by biological and immunological assays



- POW-EDIII was produced in yeast and purified using chromatography
- SDS-PAGE Coomassie staining demonstrate high purity of POW-EDIII protein (Panel 1)
- POW-EDIII reacted with anti-histidine tag antibody and migrated with similar size of Zika-EDIII recombinant protein standard (Panel 2)
- POW-EDIII reacted specifically with Powassan monoclonal antibodies (Panel 3)

Design of POW-VLP

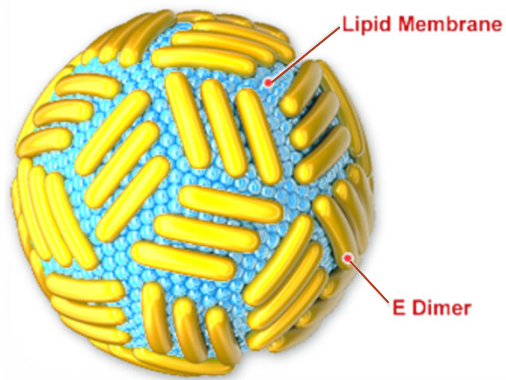
Powassan VLPs were generated using expression of structural proteins prM-E



Cleavage
and
Maturation



Self-
Assembly

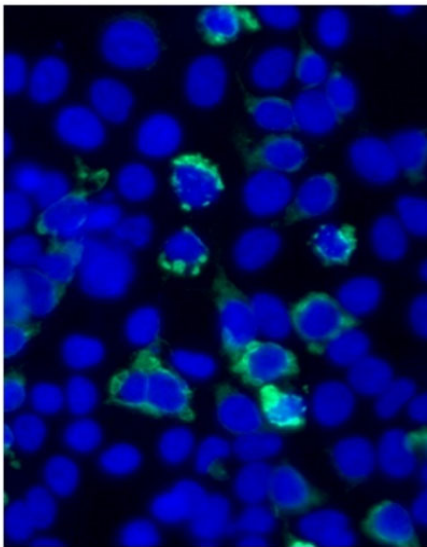


prM-E polyprotein is cleaved by host proteases and self-assemble in VLPs

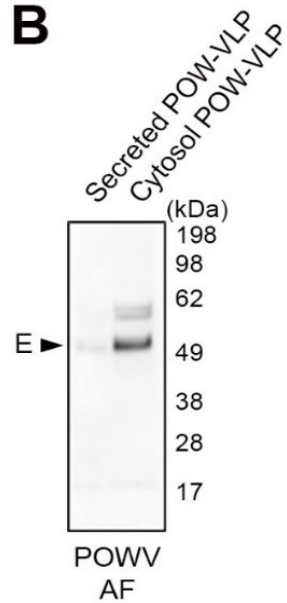
Production of POW-VLP

POW-VLP produced in mammalian cells 293T (ATCC® CRL-3216™)

A



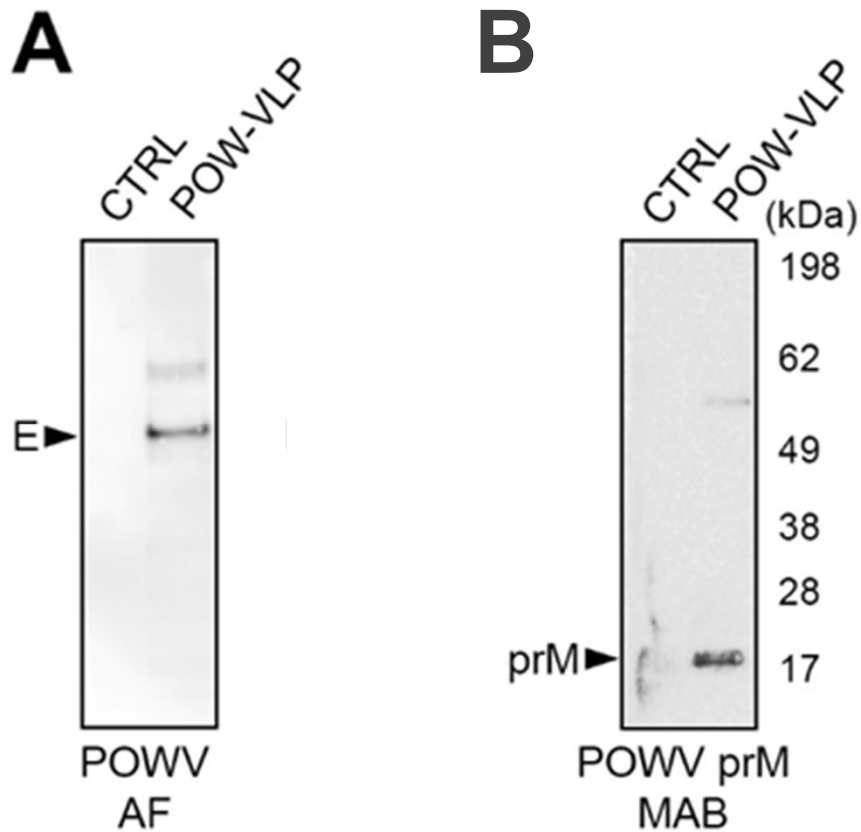
B



- Transfected cells show production of POW-VLP (Panel A: DAPI nuclear staining in blue; FITC green POWV E)
- POW-VLP were expressed and accumulated in the cytosol (Panel B)

POW-VLP antigenicity

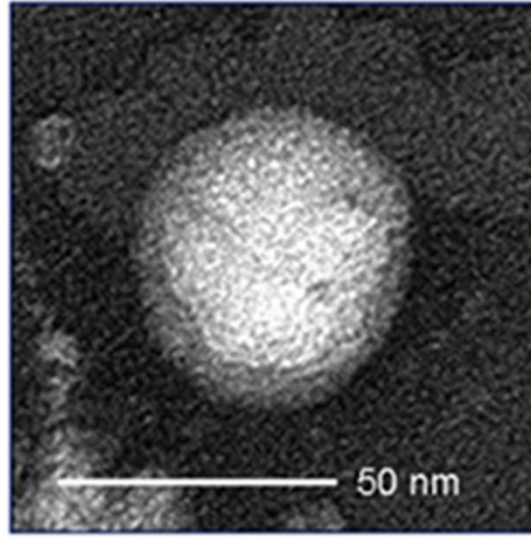
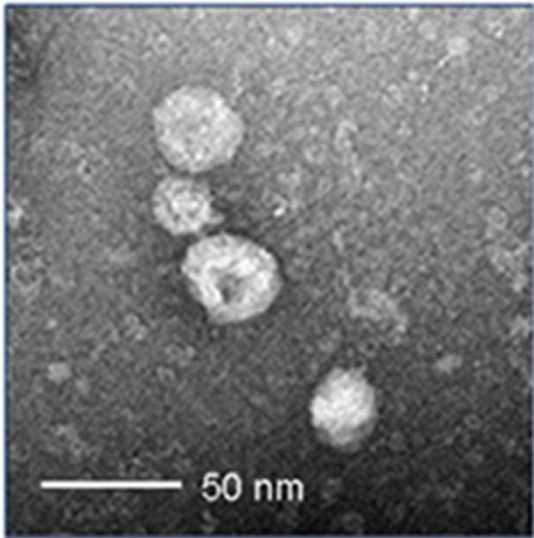
Immunological assays for testing POW-VLP antigenicity



POW-VLP showed a high level of antigenicity for Powassan antibodies against E (Panel A) and prM (Panel B) proteins

POW-VLP morphology

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



Flavivirus morphology

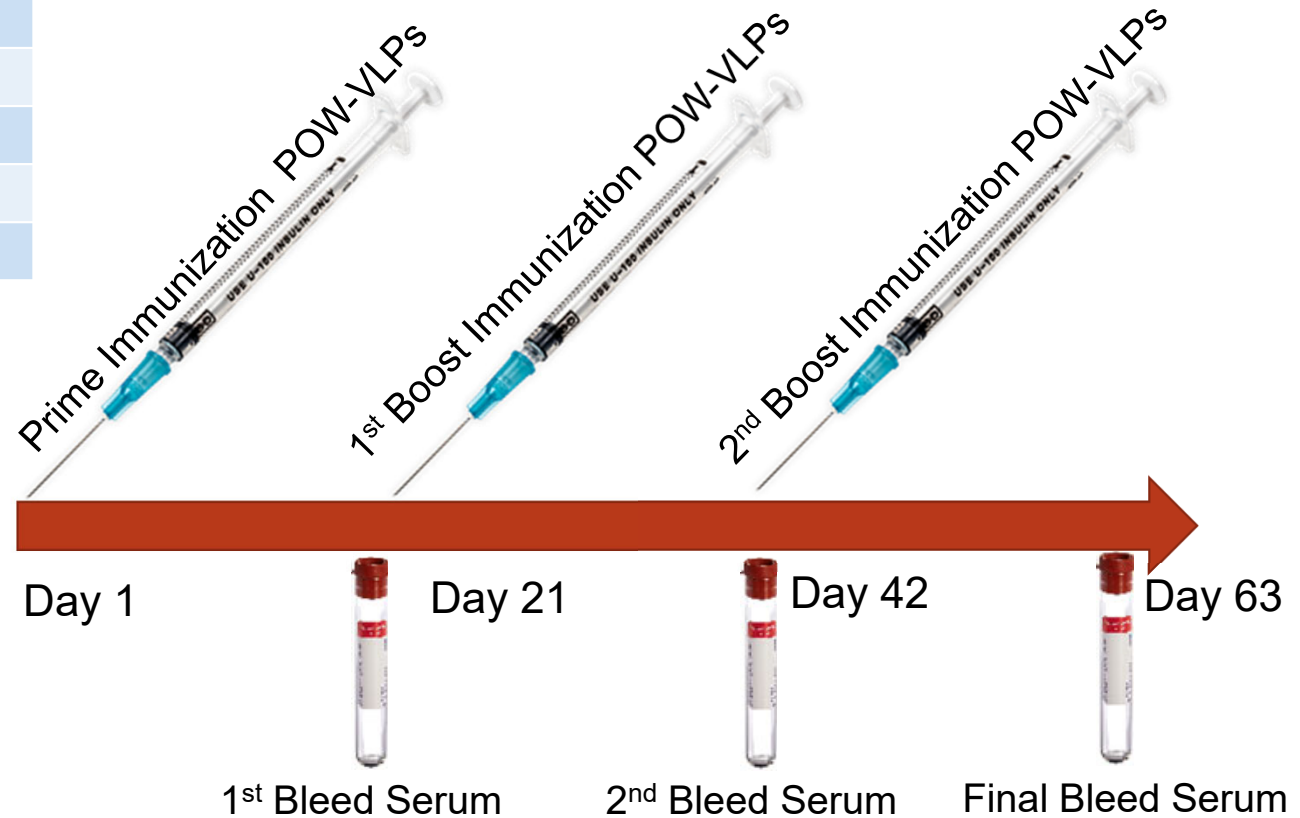


POW-VLP vaccine demonstrates flavivirus morphology

Murine model for POW-VLP testing

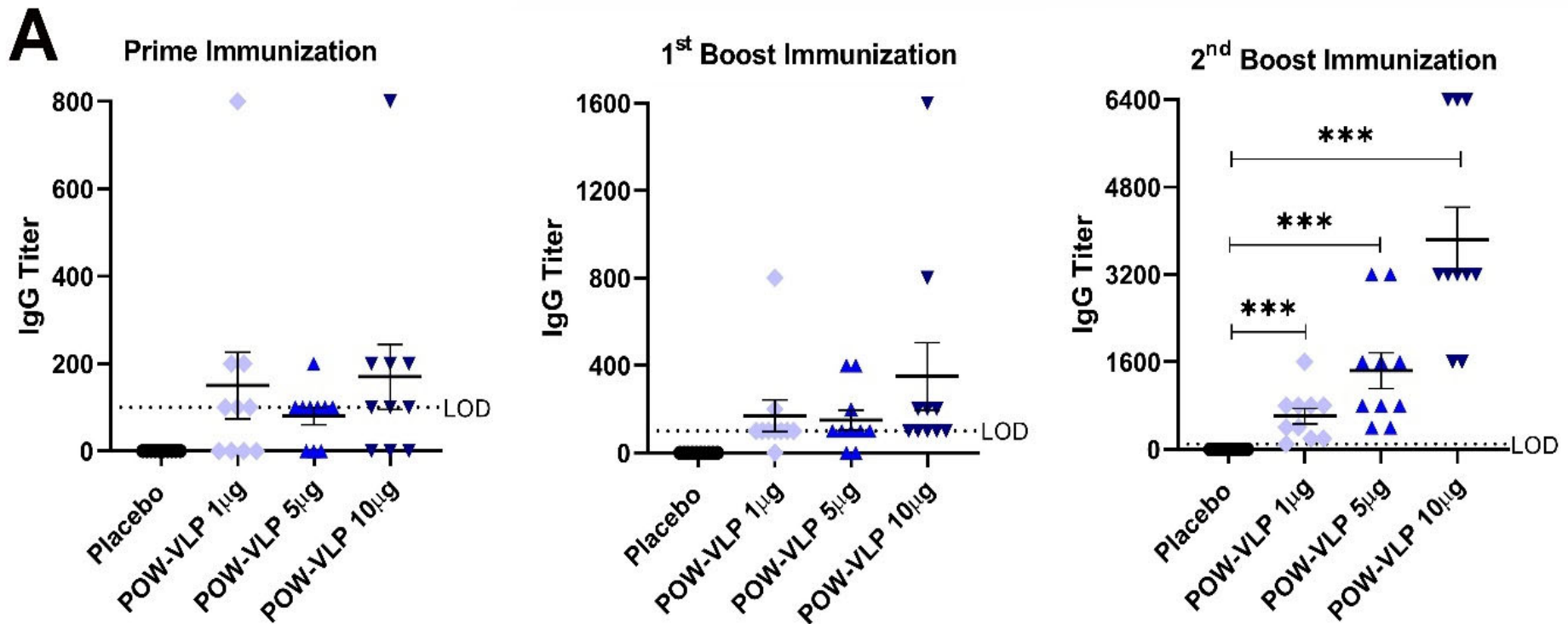
Safety and immunogenicity is tested in a mouse model

Group	Female	Male
Placebo	5	5
POW VLP 1 μ g	5	5
POW VLP 5 μ g	5	5
POW VLP 10 μ g	5	5
Total	20	20



POW-VLP immunogenicity

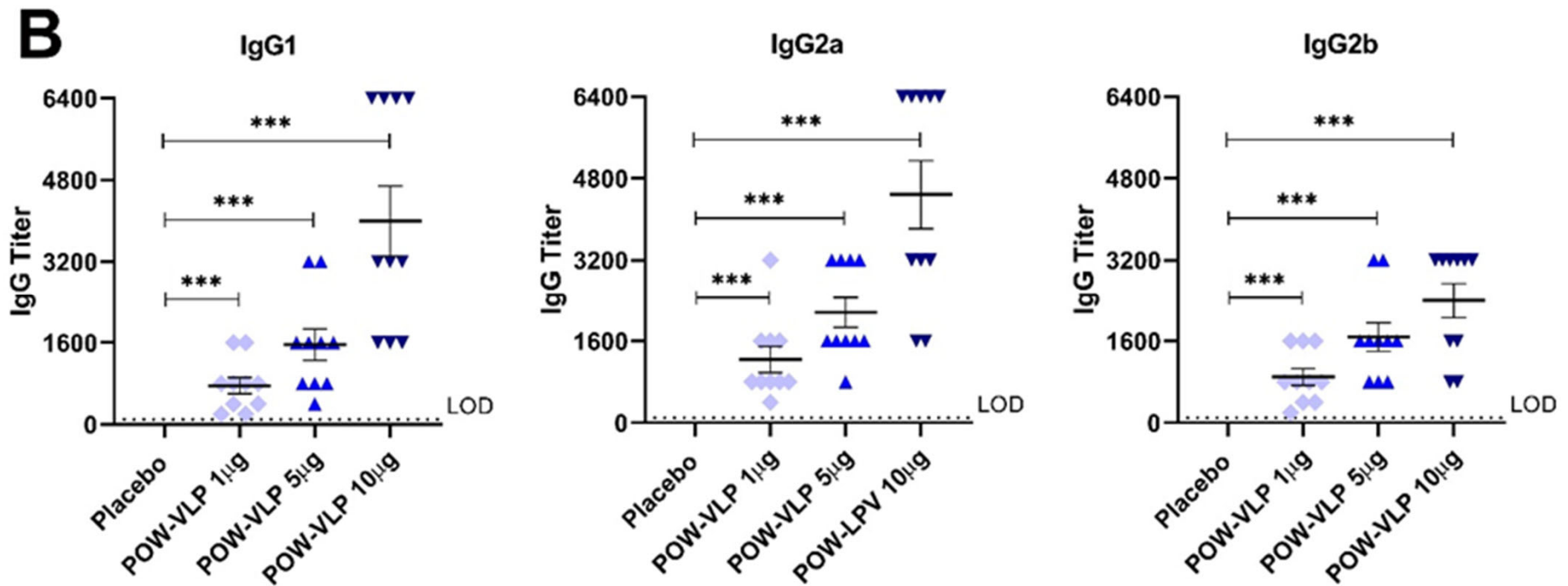
ELISA method is used to assess mouse POW-VLP antibody response



POW-VLP afforded 100% seroconversion (***) = p value < 0.0005)

POW-VLP immunogenicity

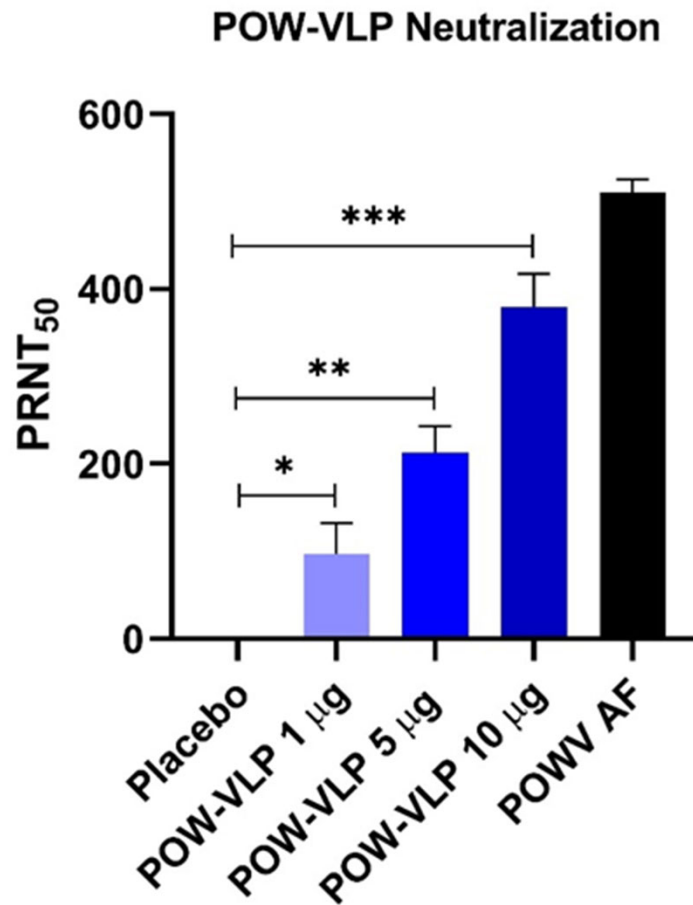
ELISA method is used to assess mouse POW-VLP antibody response



POW-VLP afforded high level of Th1- and Th2- mediated immune (***) (***) = p value < 0.0005)

Powassan virus immune response

Neutralization analysis of serum antibody of mice immunized with POW-VLP



- POW-VLP induces a high level of neutralizing antibodies (***) = p value <0.0005)
- Neutralizing activity is dose dependent

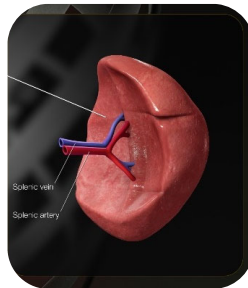
Antibody Generated by POW-VLP Immunization

Hybridoma were generated by POW-VLP vaccine candidate

Mouse immunized POW-VLP



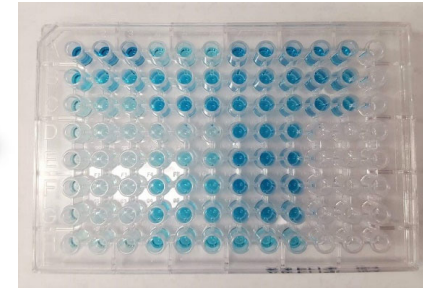
Splenectomy



335 Hybridoma Clones



ELISA Screening

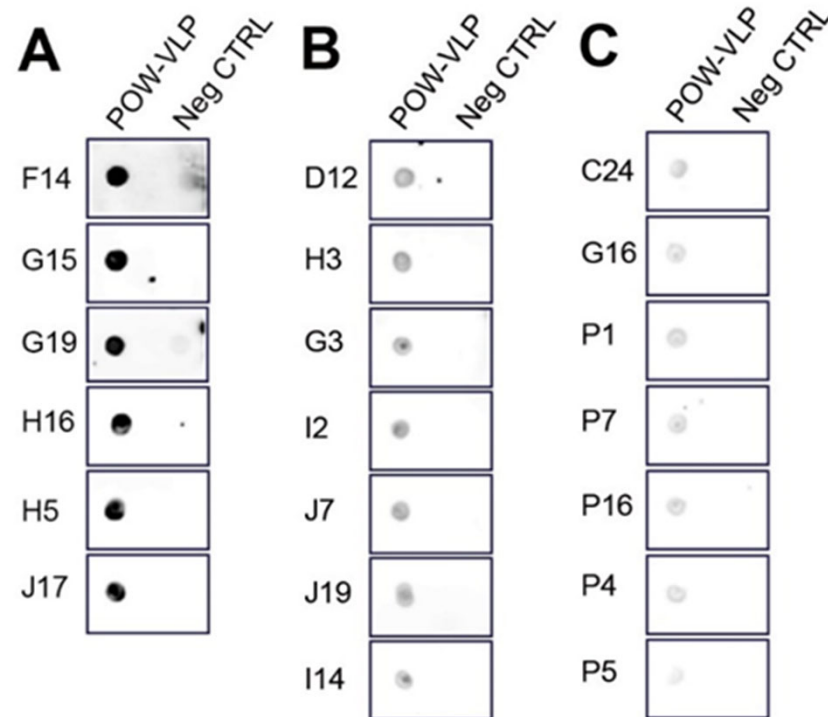


30 Hybridoma Candidates

https://commons.wikimedia.org/wiki/File:3D_Medical_Animation_Spleen_Anatomy.jpg
<https://commons.wikimedia.org/wiki/user:Ajpolino>

POW-VLP Monoclonal Development

Immunoblotting analysis of monoclonal antibody against POWV



Monoclonal with different level of reactivity have been generated using POW-VLP



Summary

Summary

Novel ATCC POW-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 Powassan vaccine candidate has demonstrated in murine models:
 - Safety
 - Immune response
 - Protection activity



Future Perspectives

Countermeasures against Powassan virus

- POW-VLP will be tested in mouse models for protection against tick-mediated Powassan transmission
- The Powassan vaccine candidate will be optimized for achieving complete sterilizing activity
- Development of neutralizing antibodies for a treatment against Powassan infection
- Testing broad spectrum anti-viral in vitro and in vivo



Acknowledgements

The ATCC Team

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Saleem Sahar, MS

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Jeb Suphankij, BS

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