

NON-HUMAN PRIMATE BIOMEDICAL RESEARCH FOR TACKLING EMERGING INFECTIOUS DISEASES (II): ZIKA VIRUS

Special series on Laboratory Animal Science

SPECIAL SERIES ON LABORATORY ANIMAL SCIENCE

Education and Training of Persons Conducting Animal Experiments

Interdisciplinary Technical Journal Club: special series on Laboratory Animal Science recognized by the Veterinary Office of the Canton of Zurich

CONFIRMATION OF ATTENDANCE

Name: Surname:

Date	Title	Speaker	Duration	Organizer signature
10.01.2017	Genetic humanization and the generation of human antibodies from transgenic animals	Dr. Susy Senatore	1h	
07.02.2017	Tissue engineering by bio-printing: current state of futuristic technology	Dr. Vijay Chandrasekar	1h	
07.03.2017	Non-human primate biomedical research to study neurological diseases	Dr. Yingjun Liu	1h	
04.04.2017	Non-human primate biomedical research for tackling emerging infectious diseases (I): Ebola virus	Dr. Regina Reimann	1h	
02.05.2017	Non-human primate biomedical research for tackling emerging infectious diseases (II): Zika virus	Dr. Karl Frontzek	1h	—
06.06.2017	Recent development in microbiome research: effect of diet and microbiota composition on the development of diseases	Dr. Katrin Frauenknecht	1h	

Organizers: Prof. A. Aguzzi, Dr. Silvia Sorce

Signature byAdriano Aguzzi
Karl Frontzek
Silvia Sorce

NON-HUMAN PRIMATE BIOMEDICAL RESEARCH FOR TACKLING EMERGING INFECTIOUS DISEASES (II): ZIKA

- Mtracution So Zika virus
- Rapid development of a DNA vaccine for Zika virus
- Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components



INTRODUCTION TO ZIKA VIRUS

THE ZIKA VIRUS



Dick et al., Trans R Soc Trop Med Hyg (1952)

Distribution of cumulative confirmed cases of microcephaly, Brazil (Epi Week 7) Rio Grande BRAZIL Grosso Golos Parana Microcephaly case count Municipalities with confirmed cases



Baby with Typical Head Size



Baby with Microcephaly

SCIENTIFIC AT THE OU ZIKA VIRU

PUBLICATIONS BREAK OF ◆ Full-length requesting and greenic characterisation of Bugus, Kindoupou, and Ellus viscous ■ One-sep REPOR for detection of Z ha virus

■ Z this value continues processors US to larger travellabel to pregnant women. · Inmaka advises scottom to arrold prognancy on Zilia virus approaches ♠ Interior Guidelines for Program Women During a Zika Vera Outbreak - United States, 2016 ■ Zika visus a report on those cases of human infection during an epidemic of jaundor in Nigeria • Stoty womb on . . . Disavirus ▲ Ziku virus infection experim enaity induced in a human volumeer ■ ZEKA virus circulates in new regions. ■ Electron micrographs of errefrecess from Swin abino mice indexed with Elsa visus ◆Owner REPORTs ne of 2 has street . A simple sechnique for infection of an expirate with viruse; manufactor of Z for single Vap Mand, Federaled States of Microsonia minings of nome on some viruses included in Uganda, Vallow Sevor, Bith Valley Sevor, Breamba Sevor, West Nile, Mongo, Sandiki Sevor, Branyan wess, Napo, Uganda Seard Educ viruses ■ Zika Virusin die Americas — Tie Another Advocine Three ◆ Therefore institutions of Zikur virus from Andre (Negomysig africanus (Therhold) sitem in and above a Urgania forest ■ Zifu virus a provincely stew punchmix spreads rapidly through the American pid great of energing Z ha virus in the Patific area ◆The flight activity of some East African monophics (Diptera, Calichian). I. Studen on a high steel tower in Zilia Forest, Vigorda • Installers of 2 has virus from Assite angept in requires in Malaysia
• Detection of Visco-specific Analysis in the Nuclei or Nucleii of Calls Infe ▲ Interior Cubbillion for Pergussi Women During a Zilia Virus Outlenak — United Nates, 2011 es of Zika virus associated with an epidemic, Vap State, Micrometia, 2007 $_{\Phi}\text{Comparison by electron microscopy of the Napa and Zilia virsum$ ◆ A pollow fever episosic in Zilu Fores, Uganda, during 1972; Ren 2: Munkey sensings: ologie: Zilia Viras Dita Virus Infection in Man Z ha virus infections in Nigerice virological and serrogichenistingical investigations in Cyc Stan tor-bosse transmission of Edua virus, Columba, USA • Notice from and Zilkurina episonics and especials in Capada aracterization of Zika virus strates; geographic expansion of the Adam linear

Infection Zitu visit particle in female Bi.

Acute more little due to Zilka virus indextion.

■ Timesegenic offices of the Zika virus and the role of the placema. Ziku Virus Infacts/Human Control Natural Progenitors and Attenuates Their Consolin ▲ Zika Virus Inflorior in Program Women in Ric-de Asseins — Perfemisary Report

Tita-microsophaly paper quarter data-during confusion

■ One succeptibility of Singapore Andre (Singapore pix) segret (Limowol to Tiba viru.

■ Countries on a since PCE describe of Zika-visco and replaction with field cought moraphism Antire (Segentyia) altropictus (Sieure): a potential wester of Zilia virus in Singapore ▲Mospiton of Zita Force, Vigantic species composition and relative abundance. ★ First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013. ■ Two case of Zilla fever imported from French Polymeia to Agon, December 2010 to January 2014; ■ Zilha virus in Culton (Contral A Broat) - 2007: a new storal from Andreas Amprilana.

■ Ziku viso infection complicated by Guillain-Burn condition—case report, French Palvania, December 2013

■ Michaellar characterisation of those Zitar Revisionan obtained from release or magniture in the Control African Resolution.

■ Ziku ninu infenies importal in Balty diskul, immunological and strological findings, and public builds implication

■ Existence of periodic transmission of Zika virus, French Polymeia, December 2013 and Pebruary 2014.

■ Principal for Ethy visco transmission decough blood transferance decoupled during an exchange in French Polymeric, November 2013 to Entropy 2014

■ Conserved conferrable of distance, obtaining an and Edge viscosisfections on an associated existency wave of consequences for the Sectle 2012 2014 (Sectle 2012).

. Disa Yirus Associated with Microsophuly . Tike's long, stronge trip into the limelight ▲ A plea for open science on Tike Opening discovery for the Zikarvina · Anticipating the international ground of Zilia virus from Brazil

◆ Ella virus Fernit polynesis, South partile: 2013 . Imported also visus infection from the cook inlands into according 2014. ■ Complete coding sequence of olia virus/trees a French polynomia sudromic in 2013. Elka vitus infection after most to Table, December 200.3 Current Etha virus epidemiology and reconstruiden in . Andre benefit as a potential vector of Chileoppress and Zika viruse ■ Route served of emerging 2 the view in the Pacific area

Zitu virus, French Polonosia, Smith Pacific, 2013.

 Prantial would transmission of Z flux virus. ■ 2 thu virus telection, (Notington, 2012)

Tita virus cultivalo in the America.

◆ First case of Z ika virus infection in a returning Canadian travelor. . Detection of Tike virus in wine

◆ Co-infection with Elia and despay viruse in 2 patients, New Coledonia, 2014 ♠ Norm on Ziku virus—an emerging patrogen now present in the South Pacific

■ Acute Zita virus infection after travel in Malaysian Bonner, September 2014 ■ First report of autochdonous transmission of Z & v time in Brasil . The the new arteriors from the Late Ameri · Zika virus following the path of deeper and chikunganyo Description of Files Virus Induction in Thickard, 2012-2014 Biology of Zita Virus Infection in Human State Cells Etha Vinn in an American Recognised Travelor ◆ Ella Vine Transmission from Street Polymerican Brazil ■ Ellie Viror Outerals, Baltin Road

■ Ziba virus spreads across Americas an oncome mouse over birth defense

 First V.S. case of Ziku virus infection is intentified in Texas. Ziturvino infector in a traveller returning from the Maldrey, June 2013 ■ Title beer imported from Thailand to Japan, and diagnosed by JKE to the origin.

■ Promisé of selected Senegaline Arrêm pp. mosquisses (Dignera Culturates) to transmit Zilar viru

Outrosit of Experiences of Street, Associated with Zika, Chilosoppess, and Desput Virgon, Salvator, Brasil

■ Tiles vine intracerine infection covers lead brain abnormality and microcophaly: ip of the industry ■ Inscripation of 2 key ring in plasma with assumption and altra toker A Burnington. ■ First detection of associations on Zilka vitra transmission in a HTV-indexed patient in Ris de Santon, Strait

■ Zikavirus infection, Cambrolia, 2010 • A diagnosis polymerare clain reaction away for Zita vitro

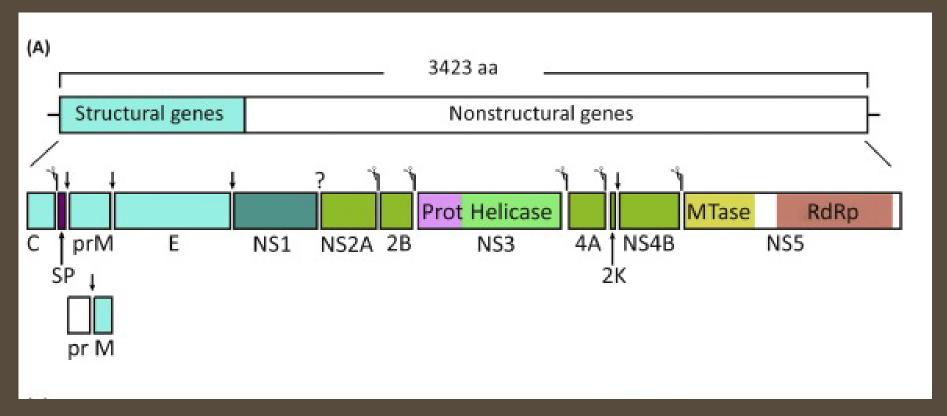
Ziko Virus Infection Among U.S. Begnant Travelers — August 2015-Ethnusry 2018

Cultish Barri Sondrome outbrook associated with Ziku virus infection in French Polymeia; a concentral start

 Experience of improved traditional Chinese and Western medicine in fine case of imported Pika virus disease in China. ■ Insertingtion of 2 key virus in plasma with assessments and alterations A Burnisation. Model-based projections of Ethy virus inductions in childhousing scotters in the America.

https://upload.wikimedia.org/wikipedia/commons/thum b/a/a4/Timeline_of_scientific_publications_about_Zika _virus%2C_based_on_Wikidata.svg/2000px-Timeline_of_scientific_publications_about_Zika_virus%2 C based on Wikidata.svg.png

GENOMIC ORGANIZATION OF ZIKA VIRUS

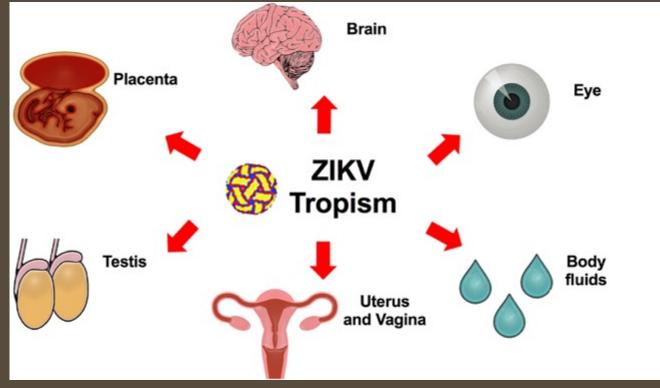


ZIKA VIRUS

○ Flavivirus (West Nile Virus, Dengue), genome of ~ 11 kb (+)-stranded RNA

o virion has a diameter of ~ 50 nm and contains a nucleoplasmid surrounded

by a lipid bilayer with membranal proteins



Modified from Miner & Diamond, Cell Host Microbe 2017



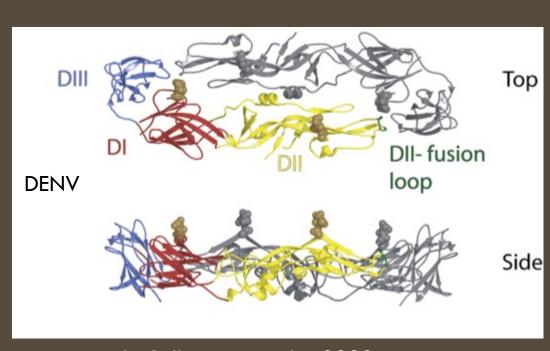
RAPID DEVELOPMENT OF A DNA VACCINE FOR ZIKA VIRUS

Dowd et al., Science 2016

GOAL OF THE STUDY

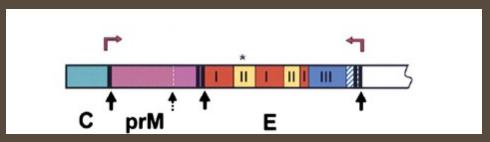
- To create & test a DNA vaccine against the current strains of the Zika virus epidemic
- Advantages of DNA-based vaccines:
 - rapid testing of a variety of batches
 - rapid production of GMP material
 - good tolerability in humans
 - established path in regulatory approval

ANTIGEN DESIGN



Pierson et al., Cell Host Microbe 2008

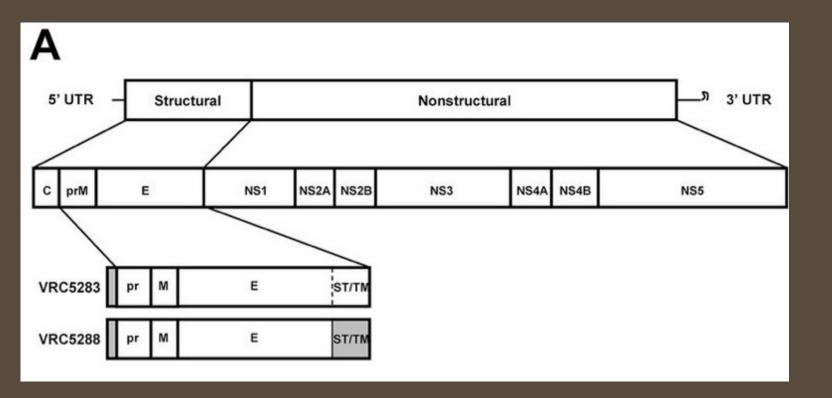
Subviral particles with similar antigenic properties compared to virions by expression of prM-E



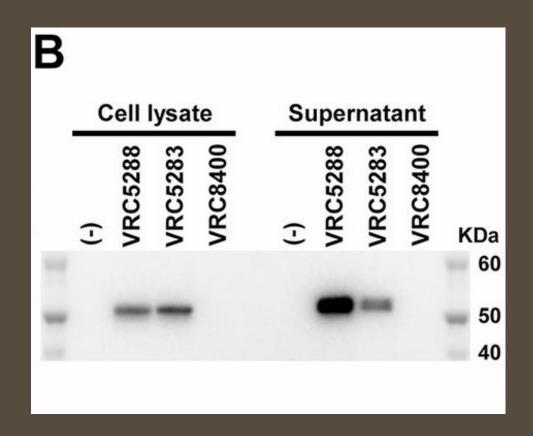
Ferlenghi et al., Molecular Cell 2001

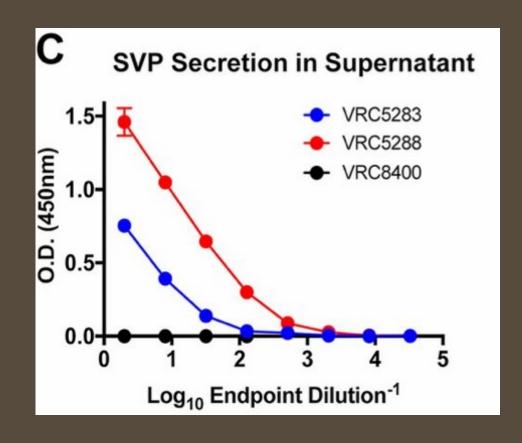
ANTIGEN DESIGN

 strain H/PF/2013 from French Polynesia similar to the one circulating in the Americas

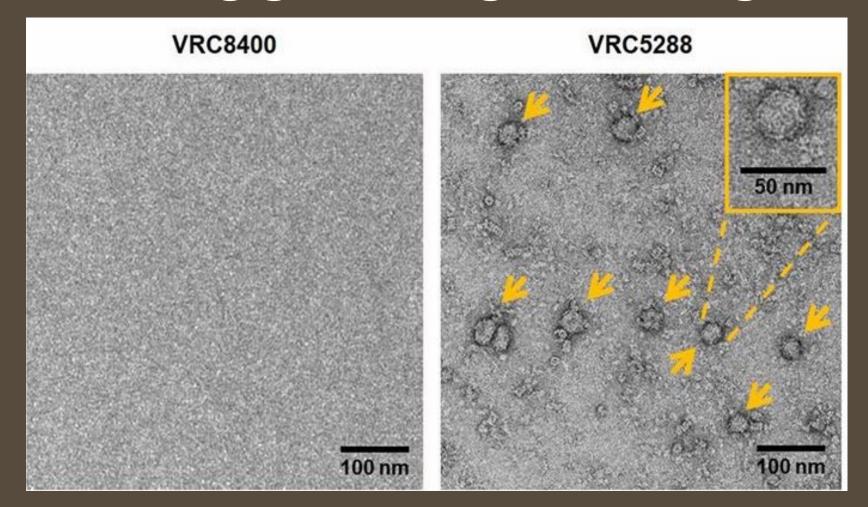


EFFICIENT SECRETION OF THE ANTIGEN FROM CELS

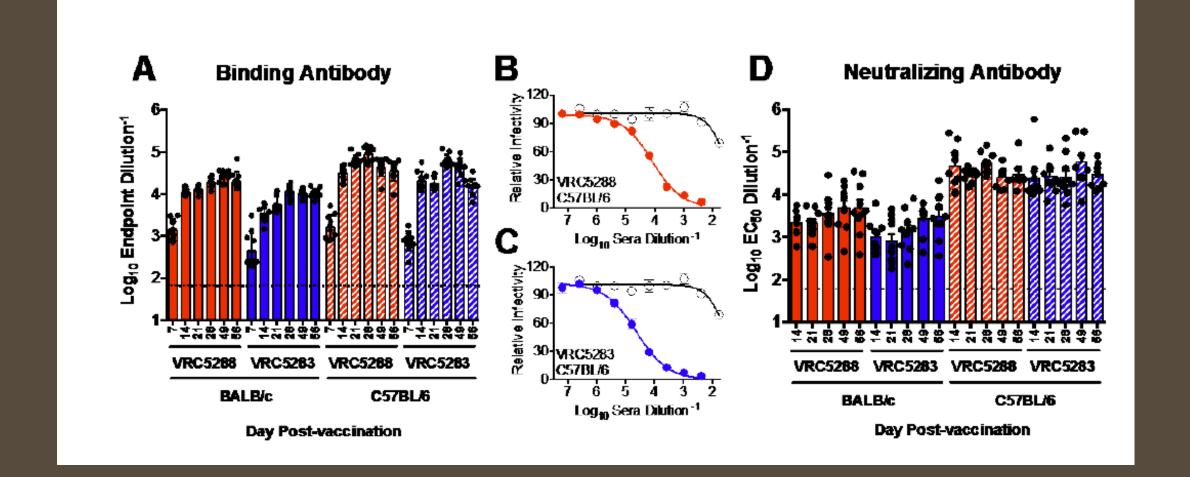




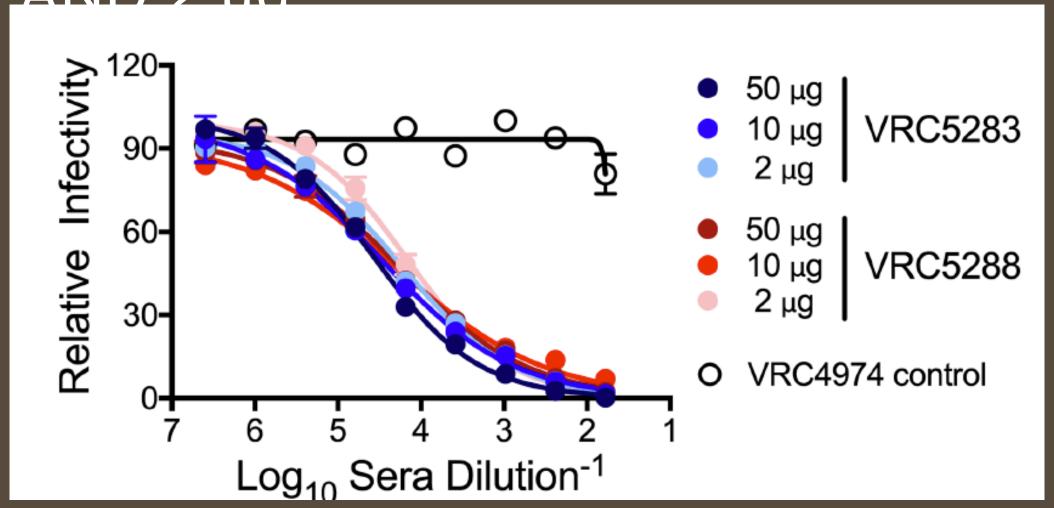
ELECTRON MICROSCOPY OF SECRETED PARTICLES SHOWS FLAVIVIRUS-LIKE SPHERES



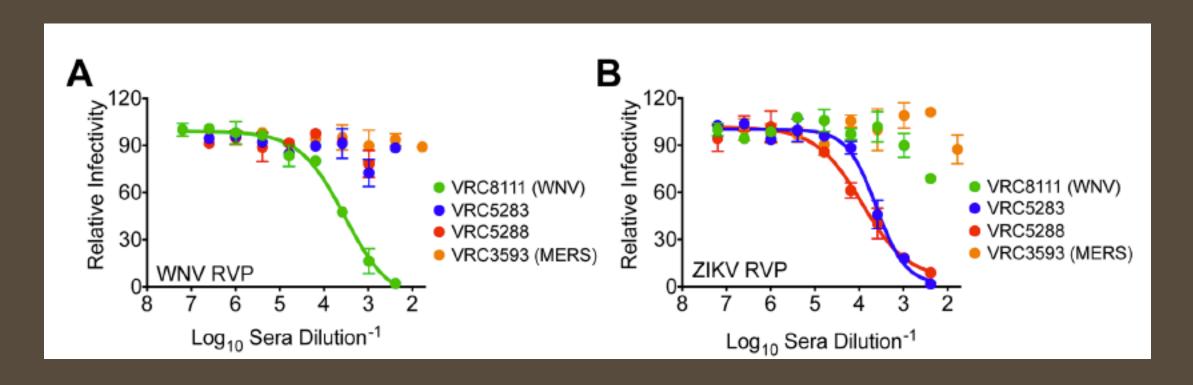
EFFICIENT SECRETION OF NEUTRALIZING ANTIBODIES AFTER VACCINATION



EFFICIENT VACCINATION THROUGH INJECTION OF 50, 10

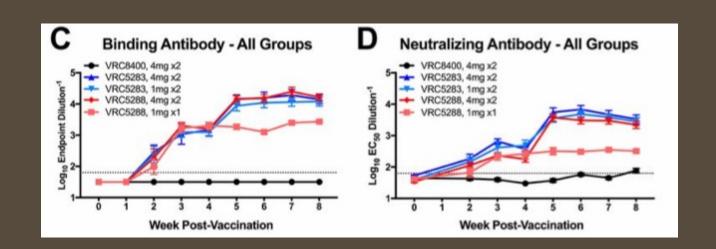


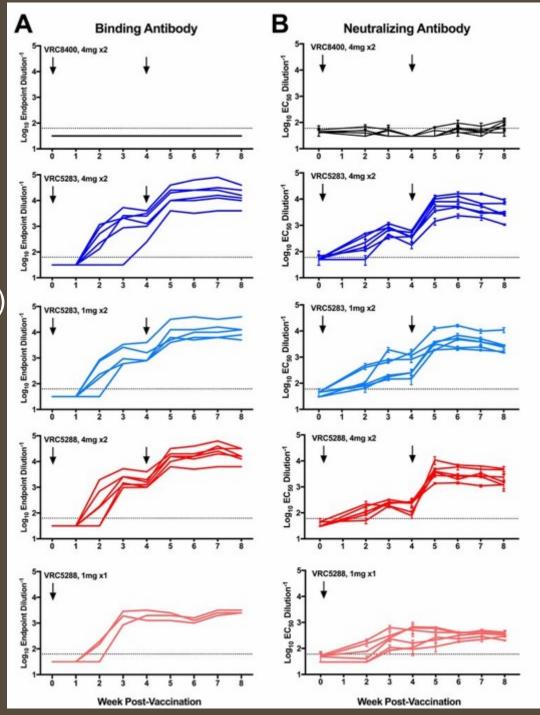
SIMILAR VACCINATION EFFICIACY OF ZIKA VACCINE WHEN COMPARED TO WNV VACCINE



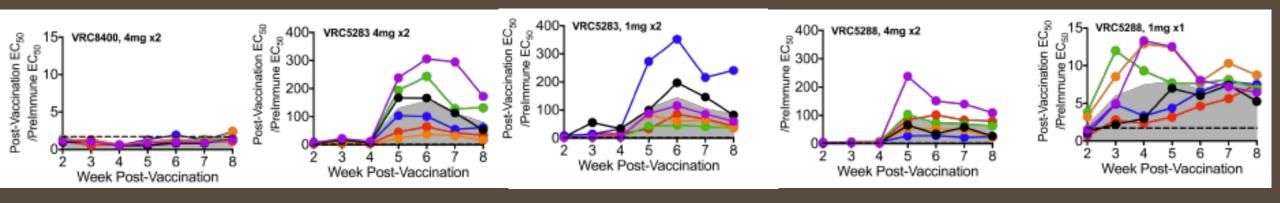
ASSESSING IMMUNOGENICITY IN NON-HUMAN PRIMATES

- treatment groups (n = 6 rhesus macaques per group)
 - 2 x 1 mg (VRC5283)
 - 2 x 4 mg (VRC5283+VRC5288)
 - 1 x 1 mg (VRC5288)

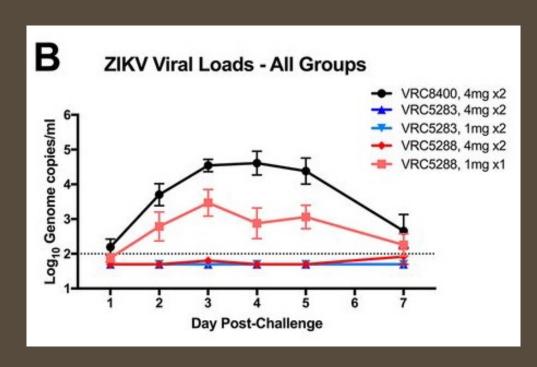


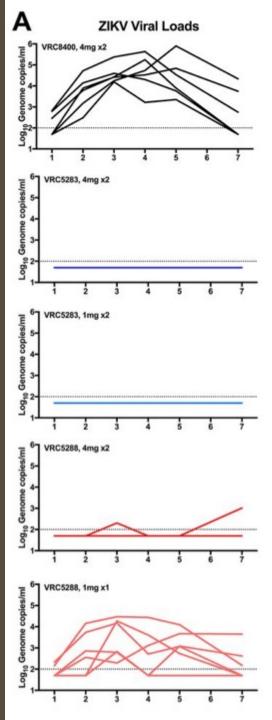


IMMUNOGENICITY OF DNA VACCINE IN NON-HUMAN PRIMATES

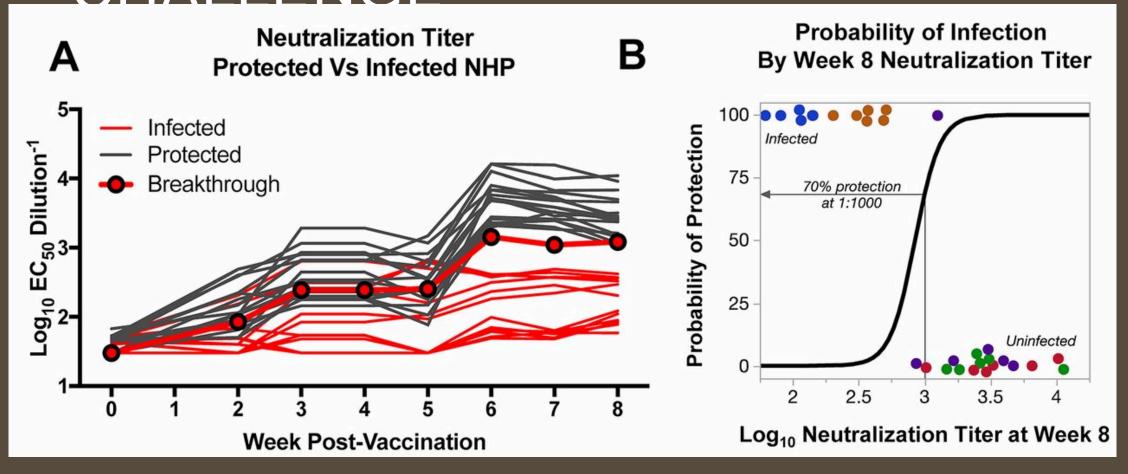


SUCCESSFUL ABLATION OF ZIKA VIREMIA THROUGH VACCINATION



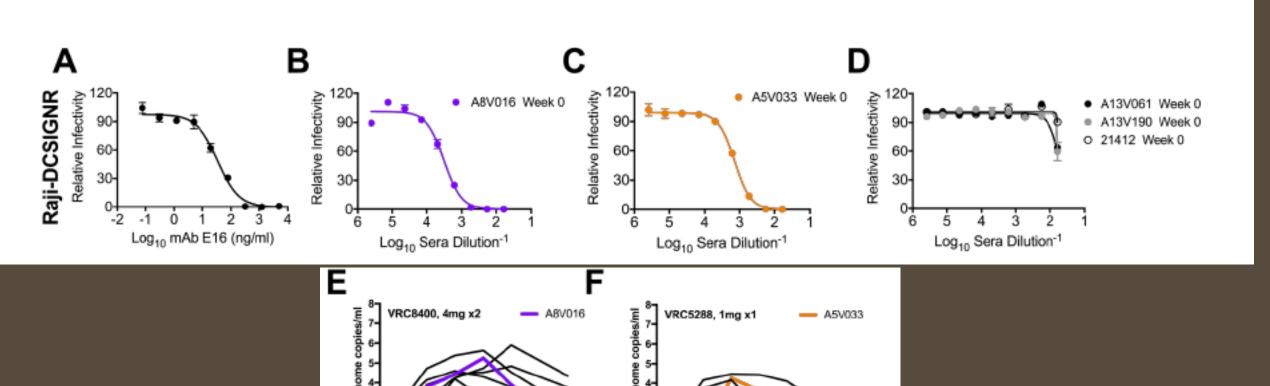


PROTECTION FROM ZIKV CHALLENGE CORRELATES WITH NAb TITERS PRESENT AT CHALLENGE



CAVEATS

o in Dengue virus (also a flavivirus), waning immunity was associated with a heightened risk for secondary infections and severe disease



Day Post-Challenge

Day Post-Challenge

https://clinicaltrials.gov/ct2/show/NCT02840487?term=NCT02840487&rank=1

CONCLUSIONS

- a Phase I trial with VRC5288 was initiated
 - > to test a variety of regimens and doses for safety and immunogenicity

Safety and Immunogenicity of a Zika Virus DNA Vaccine, VRC-ZKADNA085-00-VP, in Healthy Adults

This study is ongoing, but not recruiting participants.

Sponsor:

National Institute of Allergy and Infectious Diseases (NIAID)

Information provided by (Responsible Party):

National Institutes of Health Clinical Center (CC) (National Institute of Allergy and Infectious

Diseases (NIAID))

Estimated Enrollment: 120

Study Start Date: July 11, 2016 Estimated Study Completion Date: December 28, 2018

Estimated Primary Completion Date: December 29, 2017 (Final data collection date for primary outcome measure)

ClinicalTrials.gov Identifier: NCT02840487

First received: July 19, 2016 Last updated: April 20, 2017 Last verified: April 11, 2017 History of Changes



BIOMOLECULAR COMPONENTS

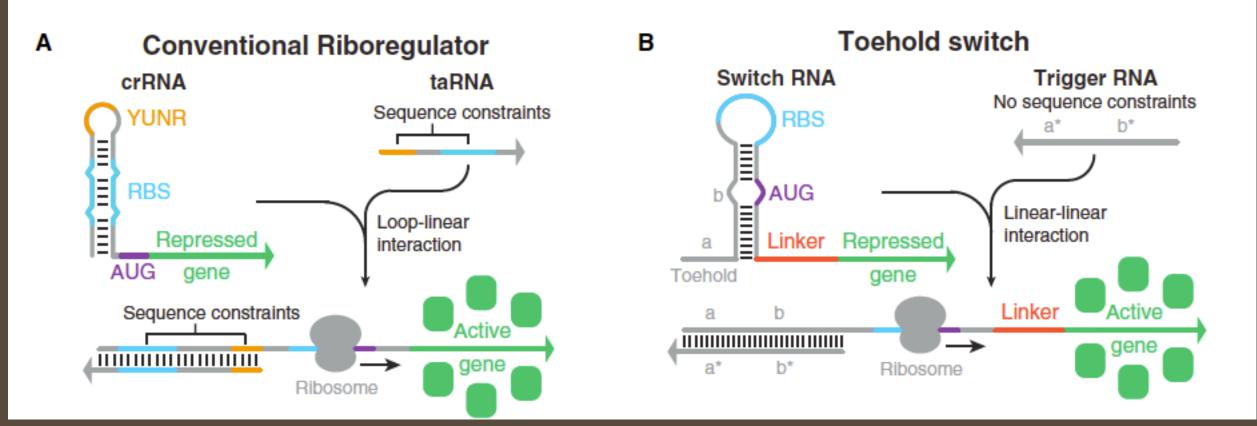
GOAL OF THE STUDY

• to create a cell-free, paper-based, low-cost RNA sensor for the Zika virus genome

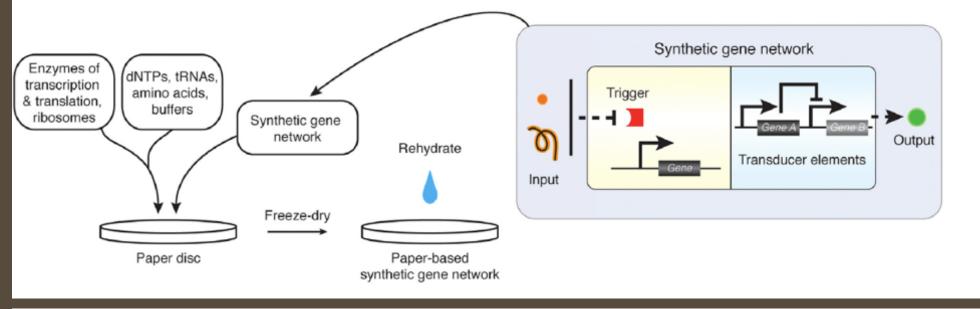
DETECTION OF ZIKA VIRUS

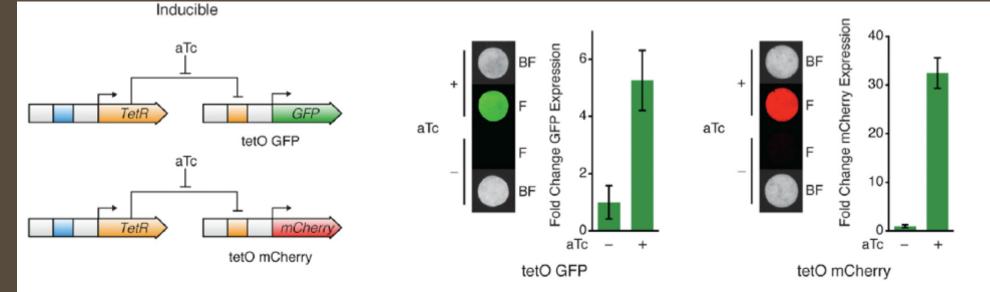
- serological approaches (e.g. antibodies) are flawed by cross-reactivity with infections through other flaviviruses
- nucleic acid based detection methods have to be employed to specifically detect the infectious agent, such as PCR or isothermal nucleic acid amplification
- however, NA-based detection methods are costly, need trained personnel and equipment

TOEHOLD SWITCHES FOR CONTROL OF RNA TRANSLATION

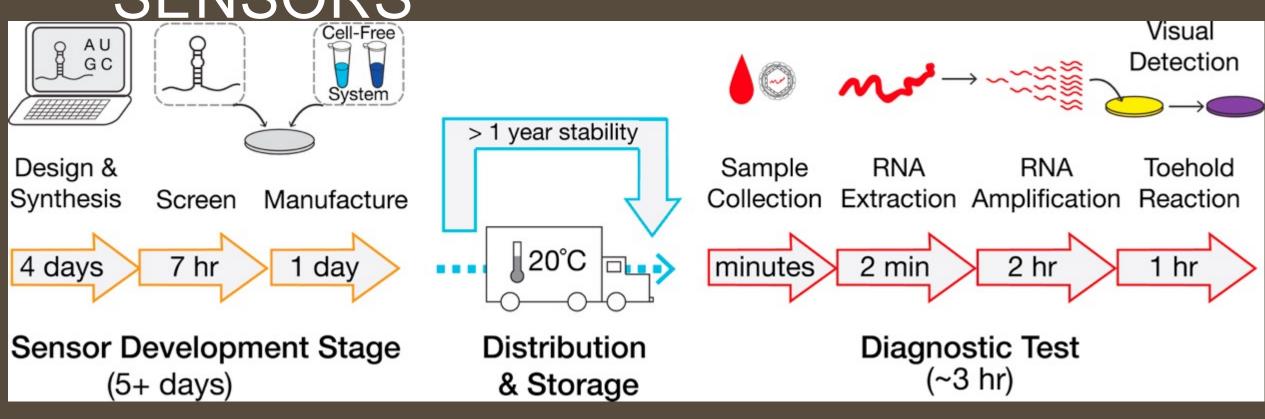


PAPER-BASED GENE NETWORKS

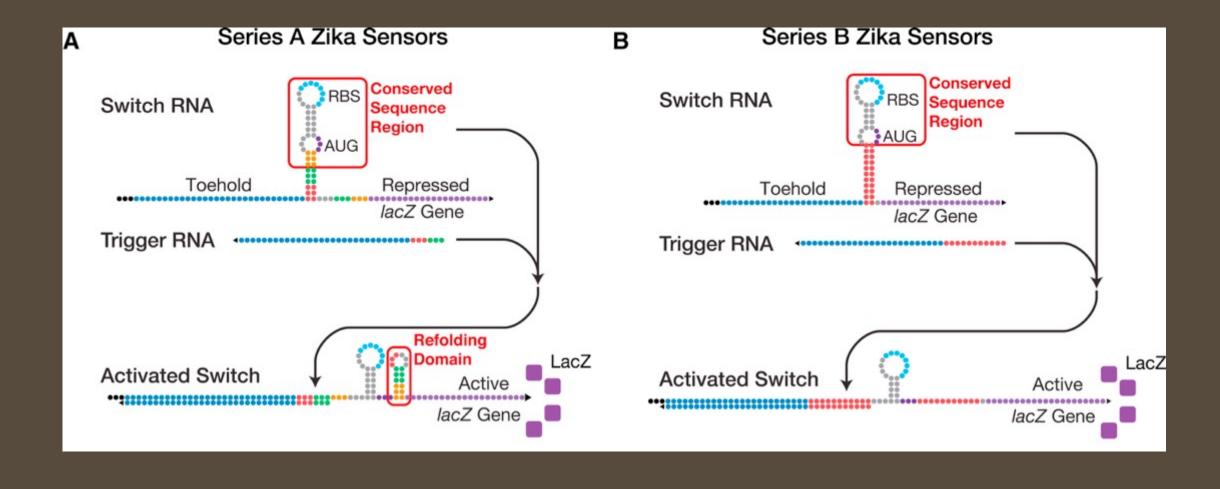




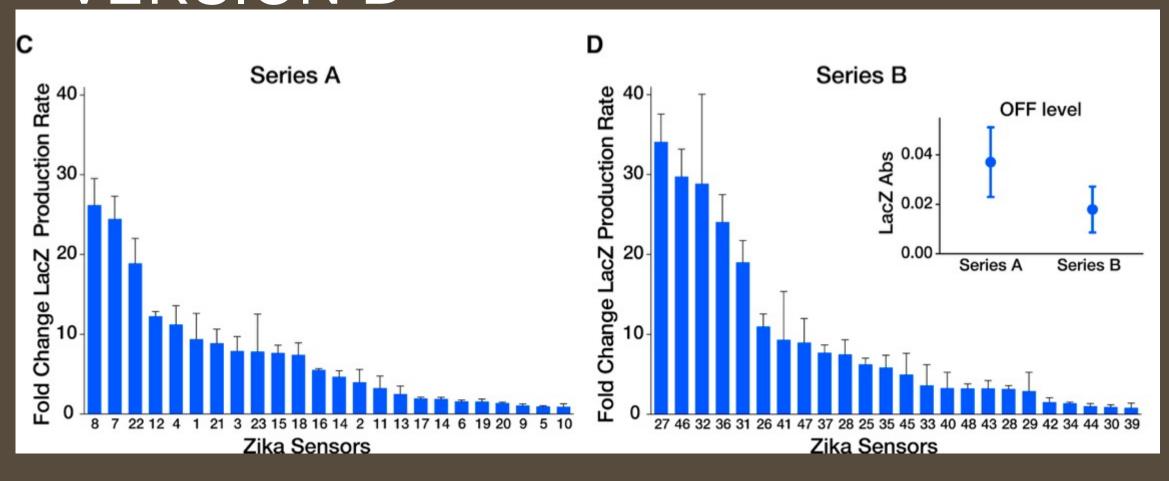
WORKFLOW FOR SYNTHESIS, VALIDATION AND APPLICATION OF ZIKA VIRUS TOEHOLD SENSORS



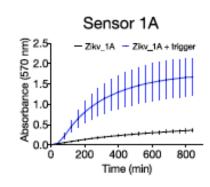
2 VERSIONS OF ZIKA VIRUS TOEHOLD SWITCHES

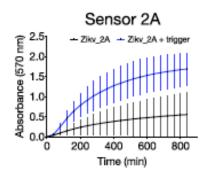


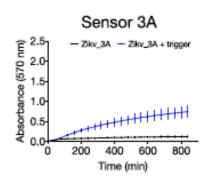
REDUCED lacZ LEAKAGE IN THE OFF STATE THROUGH LACK OF REFOLDING DOMAIN IN VERSION B

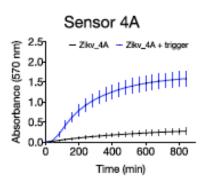


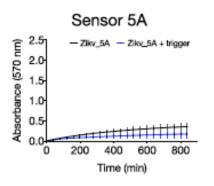
OVER 50% OF ALL SENSORS TESTED SHOWED A 5-FOLD INCREASE BY ADDITION OF TRIGGER+SENSOR

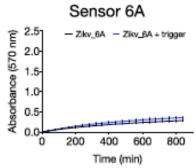




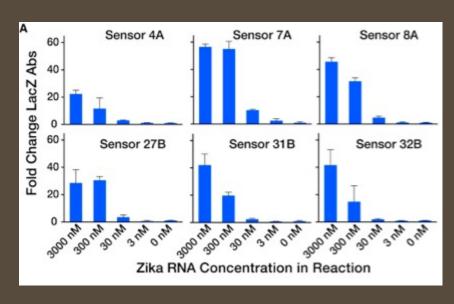


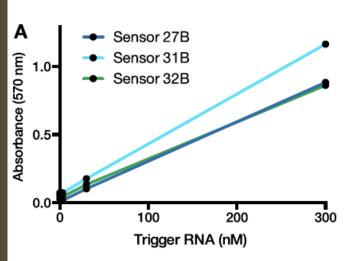


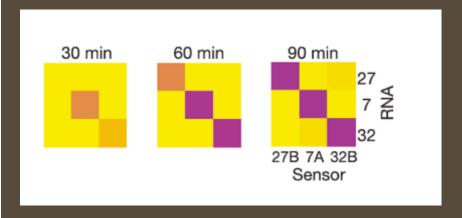




SENSORS ARE ACTIVATED FROM c(RNA)=30 nM ON AND THE RESPONSE IS LINEAR AND ORTHOGONAL







30 nM IS NOT ENOUGH

Concentrations of Zika virus in body fluids:

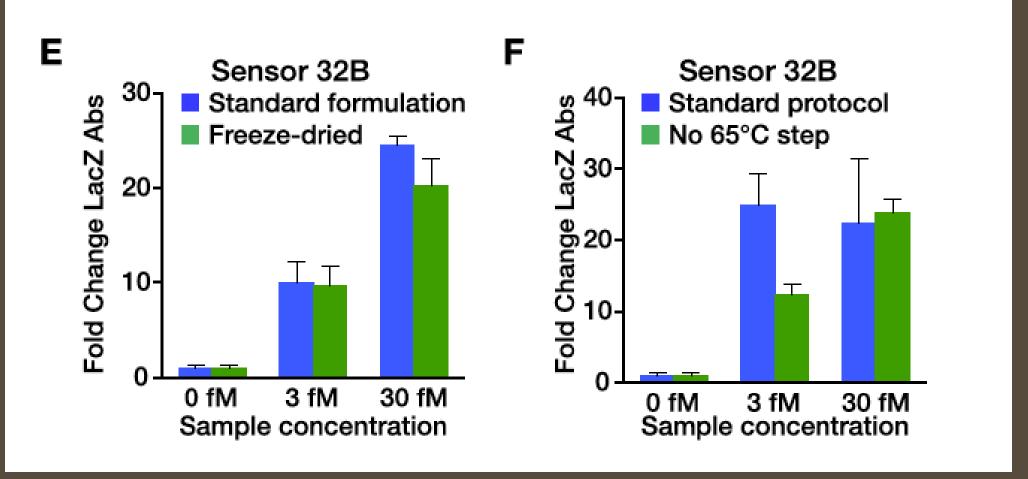
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o urine (human) – 365 fM (Gourinat et al., Emerg Infect Dis 2015)
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- o saliva (human) 4.9 fM (Barzon et al., Euro Surveill 2016)
- serum (non-human primate) 4.1 fM (Zika Experimental Science Team.
 2016)
- o serum (human) 1.2 fM (Lanciotti et al., Emerg Infect Dis 2008)

GAINING SENSITIVITY THROUGH IMPLEMENTATION OF NUCLEIC ACID SEQUENCE BASED AMPLIFICATION

Zika RNA Toehold Reaction Reverse Transcription NASBA (2 hr, 41°C) T7 RNA Transcription RNase H Degradation dsDNA Synthesis Abs Water 60 Sensor 32B Sensor 27B Serum Fold Change LacZ 3 PM 300 11 3011 311 OW 30 pm SPM 311 OW 300 m 3011 10⁻¹⁵ M 10⁻¹² M 10⁻¹² M 10⁻¹⁵ M

NASBA REACTION KIT CAN BE FREEZE DRIED AND IS FUNCTIONAL IN THE ABSENCE OF INITIAL 65°C HEATING STEP



MOVING TOWARD A FIELD-READY DIAGNOSTIC PLATFORM

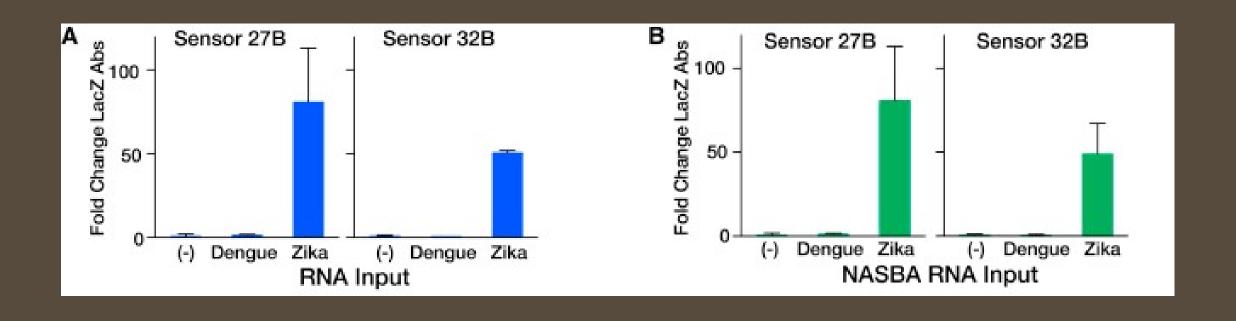
- 1) testing sensor specificity against related viruses that share clinical symptoms, partial homology, and geographic range with Zika virus
- 2) building a second-generation portable, battery-powered reader to provide lab-quality results in low-resource environments
- 3) developing a low-cost and tractable method for viral RNA extraction.

DENGUE AND ZIKA SHARE AROUND 50% SEQUENCE HOMOLOGY.

51 ATCCAGCCAG AGAATCTGGA GTACCGGATA ATGCTGTCAG TTCA---TGG Dengue27 51 GTTCAATATG AAAACCTAAA ATATTCAGTG ATAGTCACTG TCCACACTGG 101 CTCCCAGCAC AGTGGGATGA TCGTTAATGA CACAGGACAT GAAACTGATG Dengue27 101 GGACCAGCAC CAGGTGGGAA ACGAGAGT-- - ACAGAACAT GGAACAATTC 151 AGAATAGAGC GAAAGTTGAG ATAACGCCCA ATTCACCAAG AGCCGAA 201 ACCCTGGGGG GGTTTGGAAG CCTAGGACTT GATTGTGAAC CGAGGACAGG Zika27 CAGCTGACCG ACTACGGAGC CCTCACATTG GACTGCTCAC CTAGAACAGG 251 CCTTGACTTT TCAGATTTGT ATTACTTGAC TATGAATAAC AAGCACTGGC 301 TGGTTCACAA GGAGTGGTTC CACGACATTC CATTACCTTG GCACGCTGGG Dengue27 301 Zika32 AGCTGGAGTG TTGTTTGGTA TGGGCAAAGG GATGCCATTC TACGCATGGC GGCAGCTATAT TGATGGGAC TTGACAAGGG ATGGCCAATA TCGAAGATGG ACTTTGGAGT CCCGCTGCTA ATGATAGGTT GCTACTCACA ATTAACACCC ACATAGGAGT TCCACTTCTC GCCTTAGGGT GCTATTCCCA GGTGAACCCA CTGACCCTAA TAGTGGCCAT CATTTTGCTC GTGGCGCACT ACATG-TAC TTGACACTGA CAGCGGCGGT GTTGATGTTA GTGGCTCATT ATGCCATAA 151 TGATCCCAGG GCTGCAGGCA GCAGCTGCGC GTGCTGCCCA GAAGAGAAC Dengue32 151 TGGACC- AGG ACTGCAAGCA AAGGCCACTA GAGAAGCTCA AAAAAGGACA 201 GCAGCTGGCA TCATGAAGAA CCCTGTTGTG GATGGAATAG TGGTGACTGA 251 CATTGACACA ATGACAATTG ACCCCCAAGT GGAGAAAAAG ATGGGACAGG 251 CTTGGATCCT GTGGTTTATG ATACAAAATT TGAAAAACAG CTAGGCCAAA 301 --- TGCTACT CATAGCAGTA GCCGTCTCCA GCGCCATA 338 Dengue32 301 TAATGTTACT GATA-CTTTG TACATCAC-A GATCCTC- 338

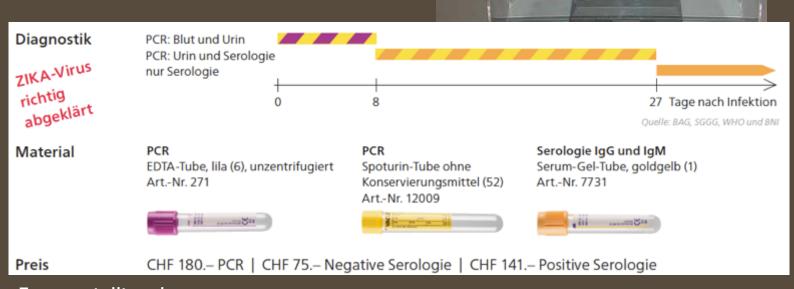
Red boxes: trigger RNA Blue boxes: NASBA sequences

DENGUE RNA AND NASBA-AMPLIFIED DENGUE RNA DOES NOT ACTIVATE TOEHOLD SENSORS



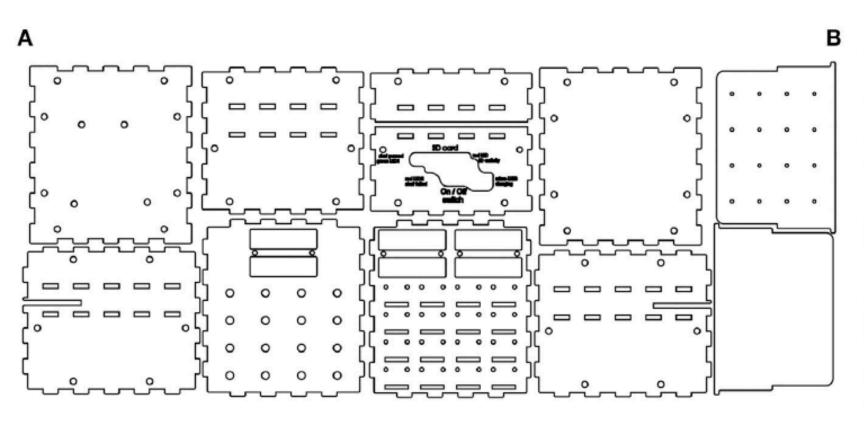
BUILDING AN ELECTRONIC READER

- readily available consumer components
- low-cost companion technology
- open-source software
- laser-cut acrylic housing
- Li-lon battery, rechargable
- 4 GB on-board storage
- total cost < 250\$



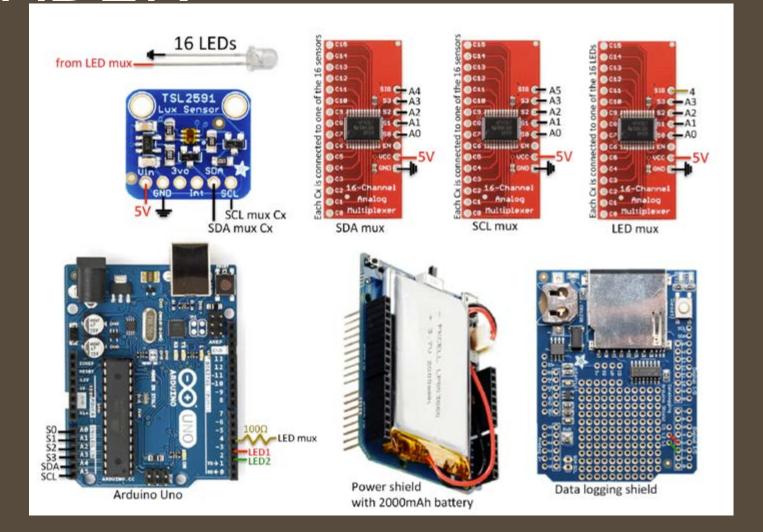
From: viollier.ch

PORTABLE ELECTRONIC READER

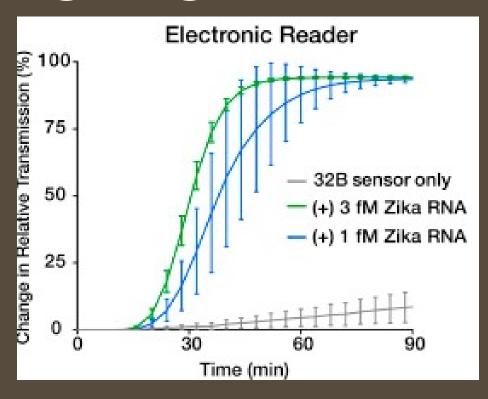




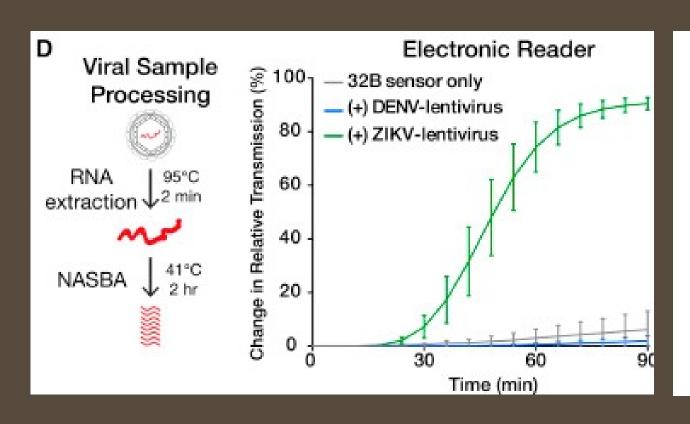
PORTABLE ELECTRONIC READER

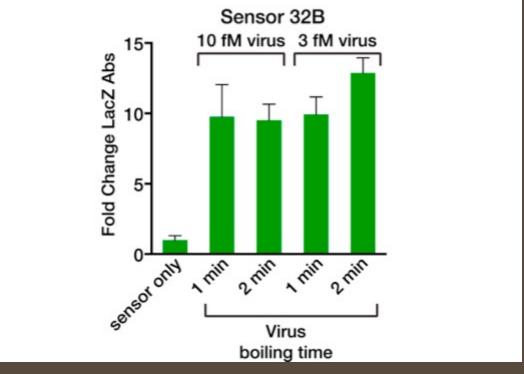


DETECTION OF CLINICALLY RELEVANT RNA CONCENTRATIONS AFTER A FEW MINUTES



INCORPORATION OF A VIRUS EXTRACTION PROTOCOL





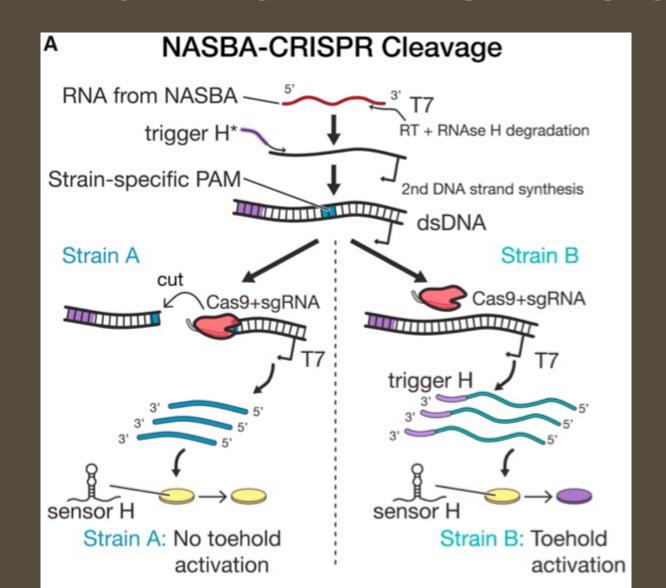
NASBA-CRISPR BASED CLEAVAGE (NASBACC) ASSAY

 development of a CRISPR-based cleavage assay that can distinguish different strains on a single-base resolution

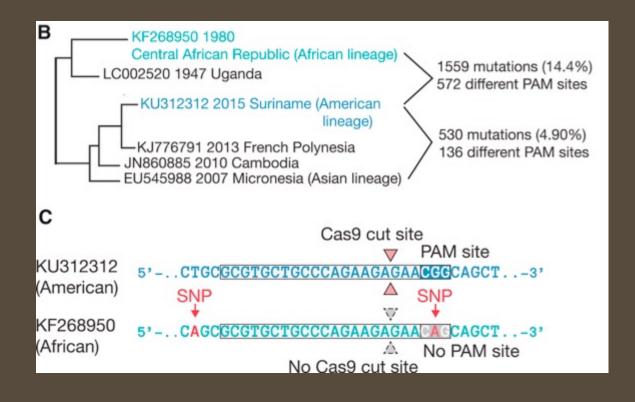
Each mutation has a 23% probability (44/192) of creating a new PAM site, a 23% probability (44/192) of destroying and existing PAM site, and a 2% probability (4/192) of inverting the orientation of an existing PAM site.

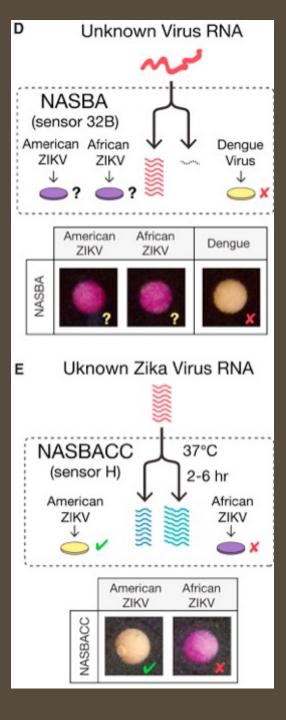
Overall, any given point mutations has a 48% probability (92/192) of disrupting an existing PAM site.

WORKFLOW OF NASBACC

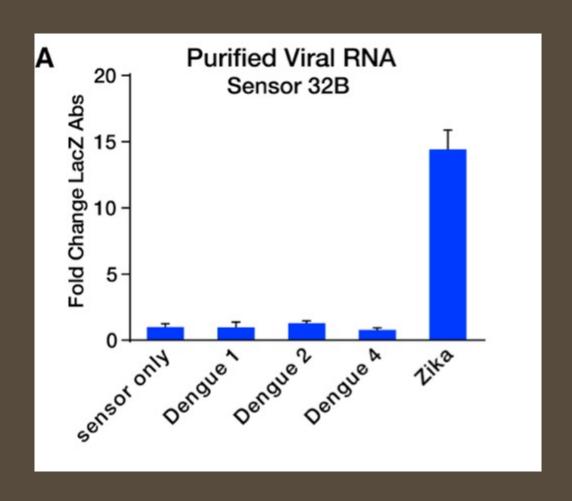


DETECTION OF DIFFERENT STRAINS USING NASBACC

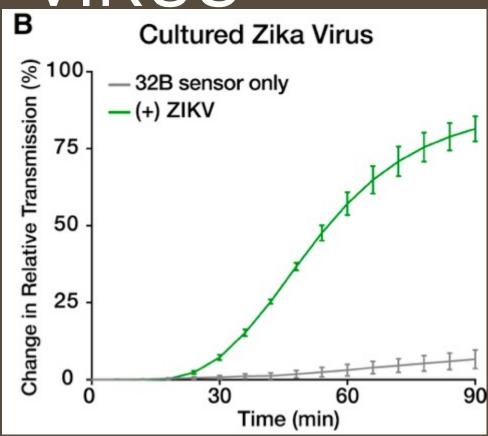




VALIDATION OF DIAGNOSTIC WORKFLOW ON PURIFIED ZIKA VIRUS RNA

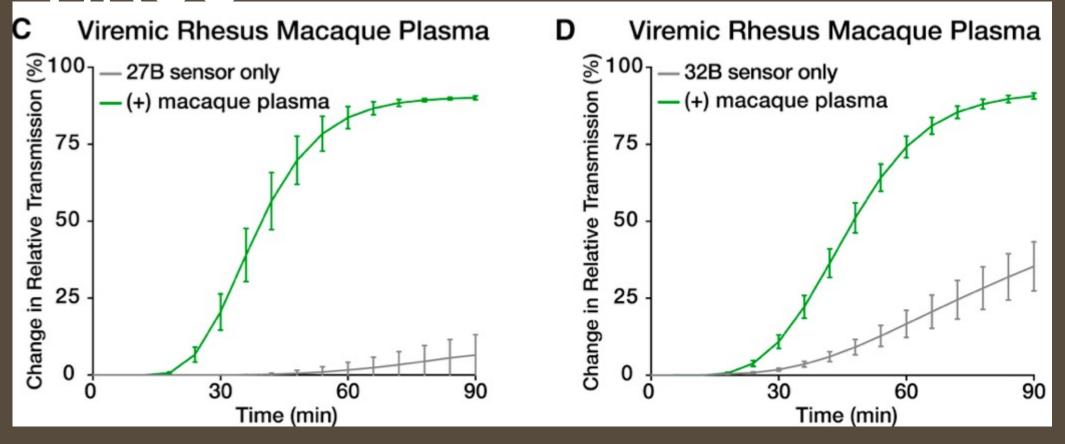


VALIDATION OF DIAGNOSTIC WORKFLOW ON LIVE ZIKA VIRUS



Active Zika virus was cultured in the laboratory and spiked into human serum (7%) at a final concentration of 10 fM, to mimic a clinical sample

VALIDATION OF DIAGNOSTIC WORKFLOW ON LIVE ZIKA VIRUS



CONCLUSIONS

PROs

 cheap, reliable detection method for fast (and strain-specific detection) of several Zika, but not Dengue strains

CONTRAS

- sensitivity is on the edge of what is described in the literature (3 fM, one report suggests 1.8 fM RNA viral load in one human)
- test is just established with blood, but not with saliva, urine etc. (maybe more readily accessible in the field)

Thank you