Adventures in Arbovirus Virus Diagnostics

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Disclosures

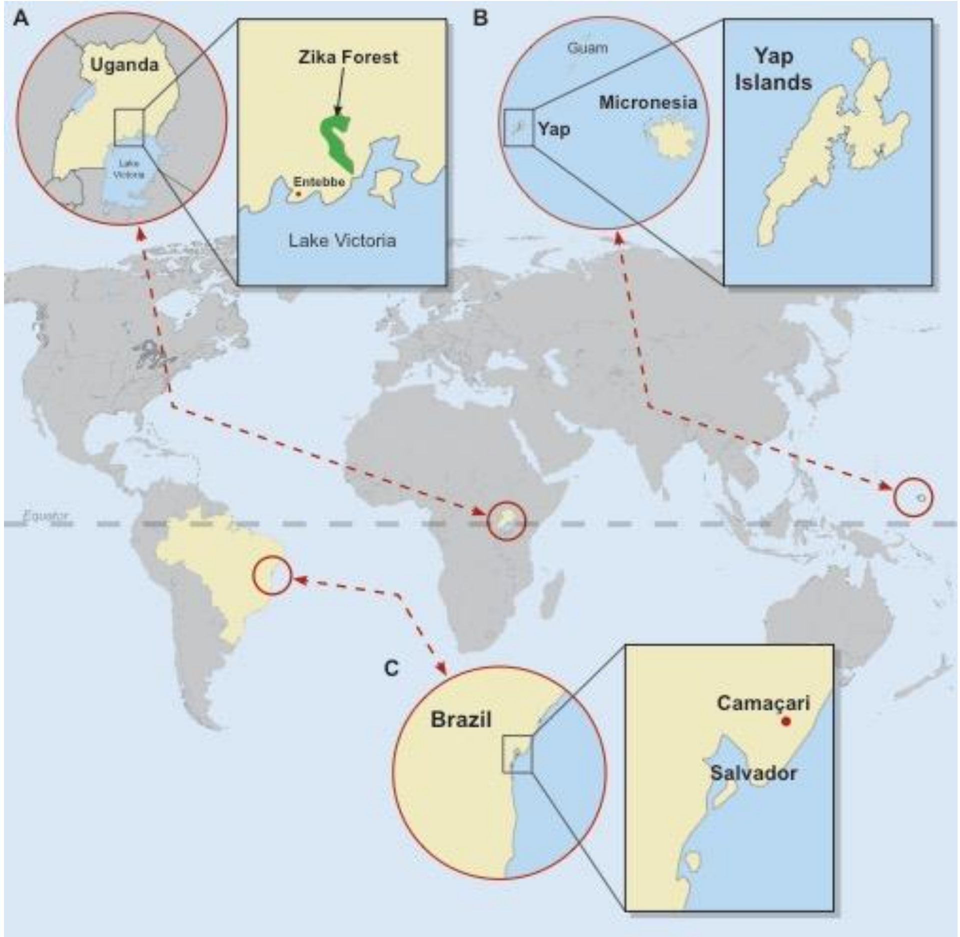
The Stanford Zika virus and chikungunya virus molecular assays presented here have been licensed by Bio-Rad.

The Stanford dengue virus molecular assays presented here have been licensed by Globavir and sub-licensed to Bio-Rad.

Consultant, Nirmidas, Inc.

Outline

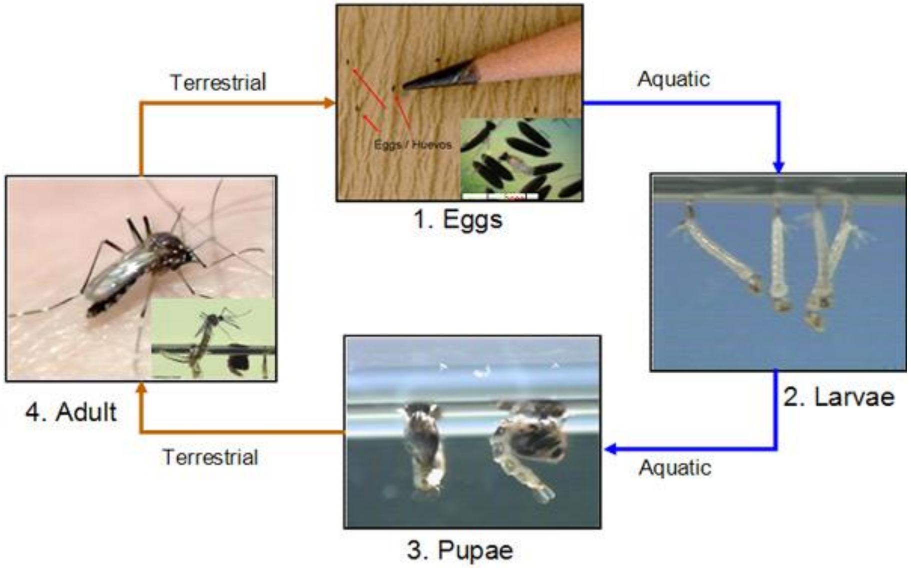
- Markers of Zika virus infection
- Nucleic Acid Amplification Testing (NAAT)
- Serologic Testing
- Future Directions



Waggoner and Pinsky, J Clin Microbiol 2016
Waggoner et al., Emerg Infect Dis 2016

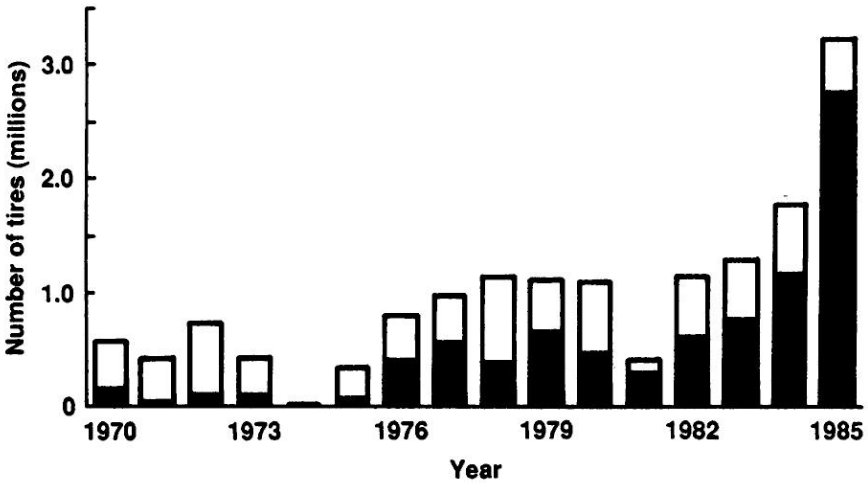
Aedes Mosquito Vectors

- Transmitted by *Aedes* species mosquitoes
  - *Ae. aegypti*
  - *Ae. albopictus*
- Live in urban/peri-domestic environments



Centers for Disease Control and Prevention

Introduction of *Aedes albopictus* to North America through the Used Tire Trade



Hawley *et al.*, Science 1987

Fig. 3. Yearly imports of used tires into the United States (1970–1985). Shaded areas show imports from Asian countries where *A. albopictus* is indigenous (*12*).

Reiter and Sprenger, J Am Mosq Control Assoc 1987

Craven *et al.,* J Am Mosq Control Assoc, 1988

Zika virus: A new TORCH infection?

- ZIKV linked to the development of neonatal microcephaly and neurologic abnormalities
- ZIKV identified in amniotic fluid and fetal tissues.

Johansson et al., NEJM 2016

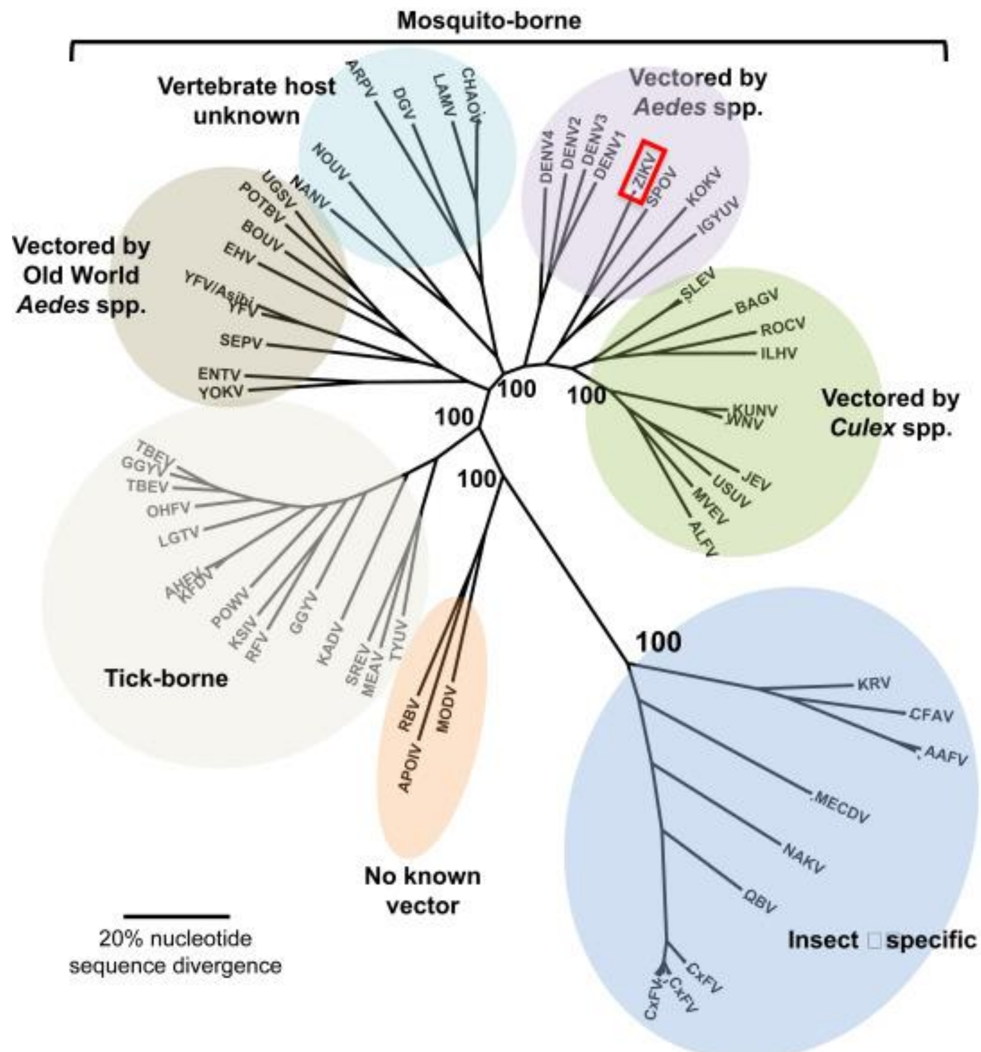
Brasil et al., NEJM 2016

Rasmussen et al., NEJM 2016

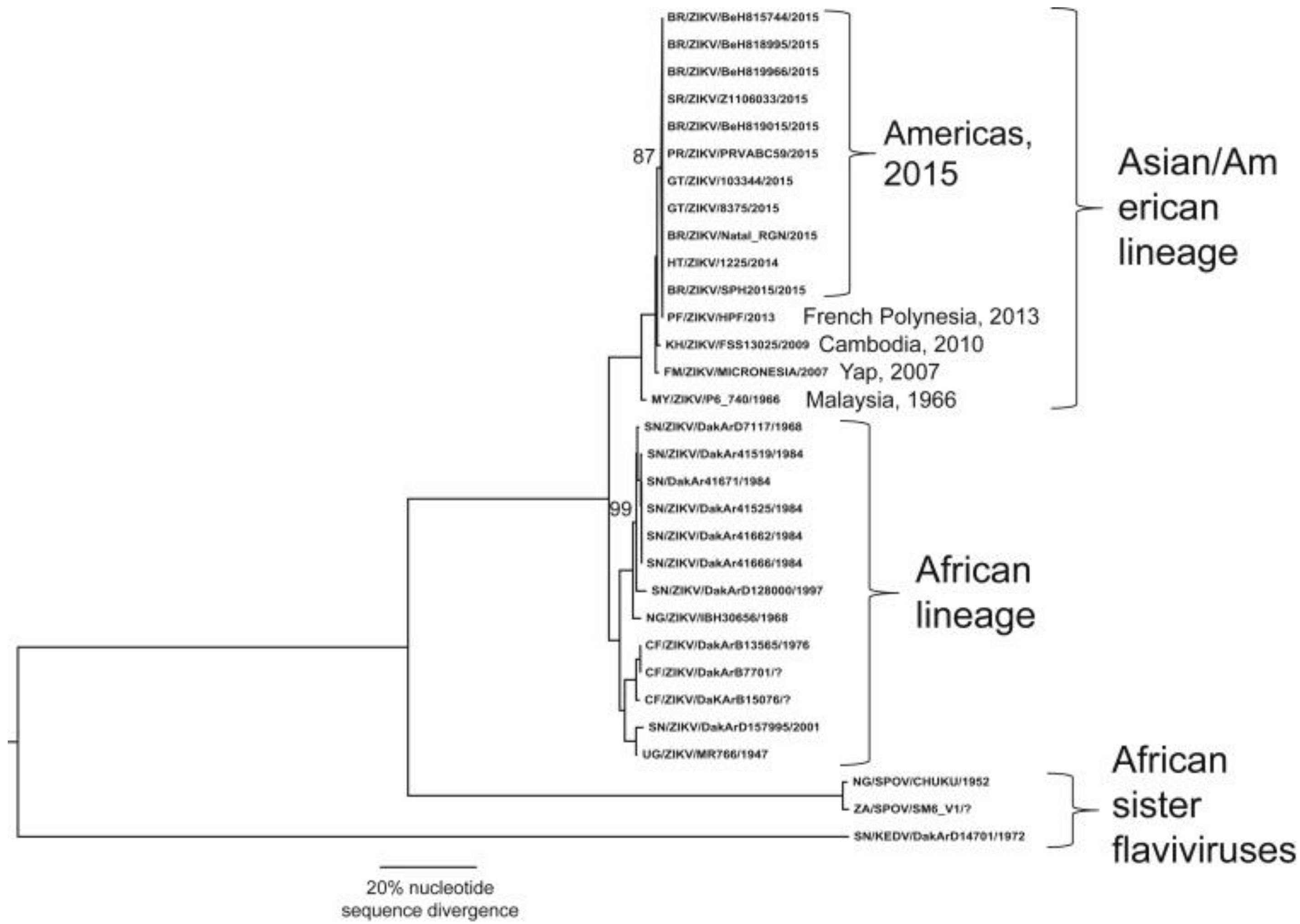
Reagan-Steiner et al., MMWR 2017

Zika Virus

- Family: *Flaviviridae*
- Genus: *Flavivirus*
- Species: Zika virus (ZIKV)
  - Antigenic complex: Spondweni
- Other mosquito-borne flaviviruses
  - – DENV, YFV, WNV, JEV



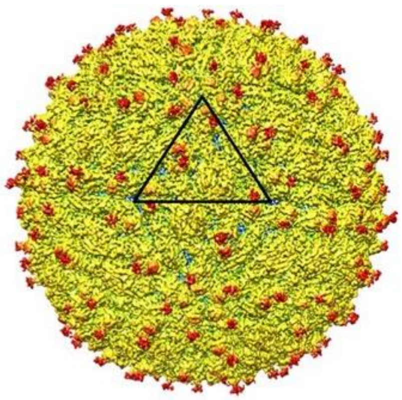
Zika virus Phylogeny: Asian and African Lineages

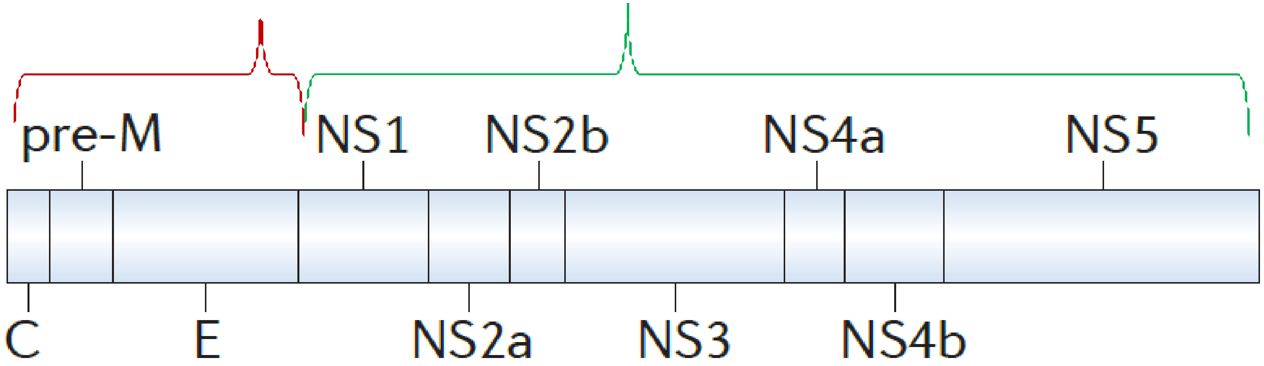


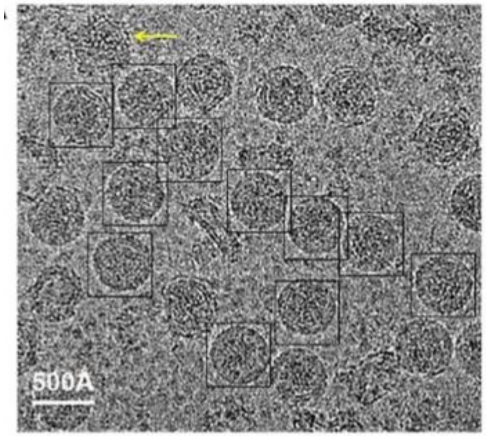
Weaver *et al., Antiviral Res 2016*

Zika Virology

- Enveloped virus, ~50 nm in diameter
- Positive-sense, single-stranded RNA genome
- ~10.8 kb in length
- Encoding 3 structural and 7 non-structural proteins







Zika virus infection presents with non-specific signs and symptoms

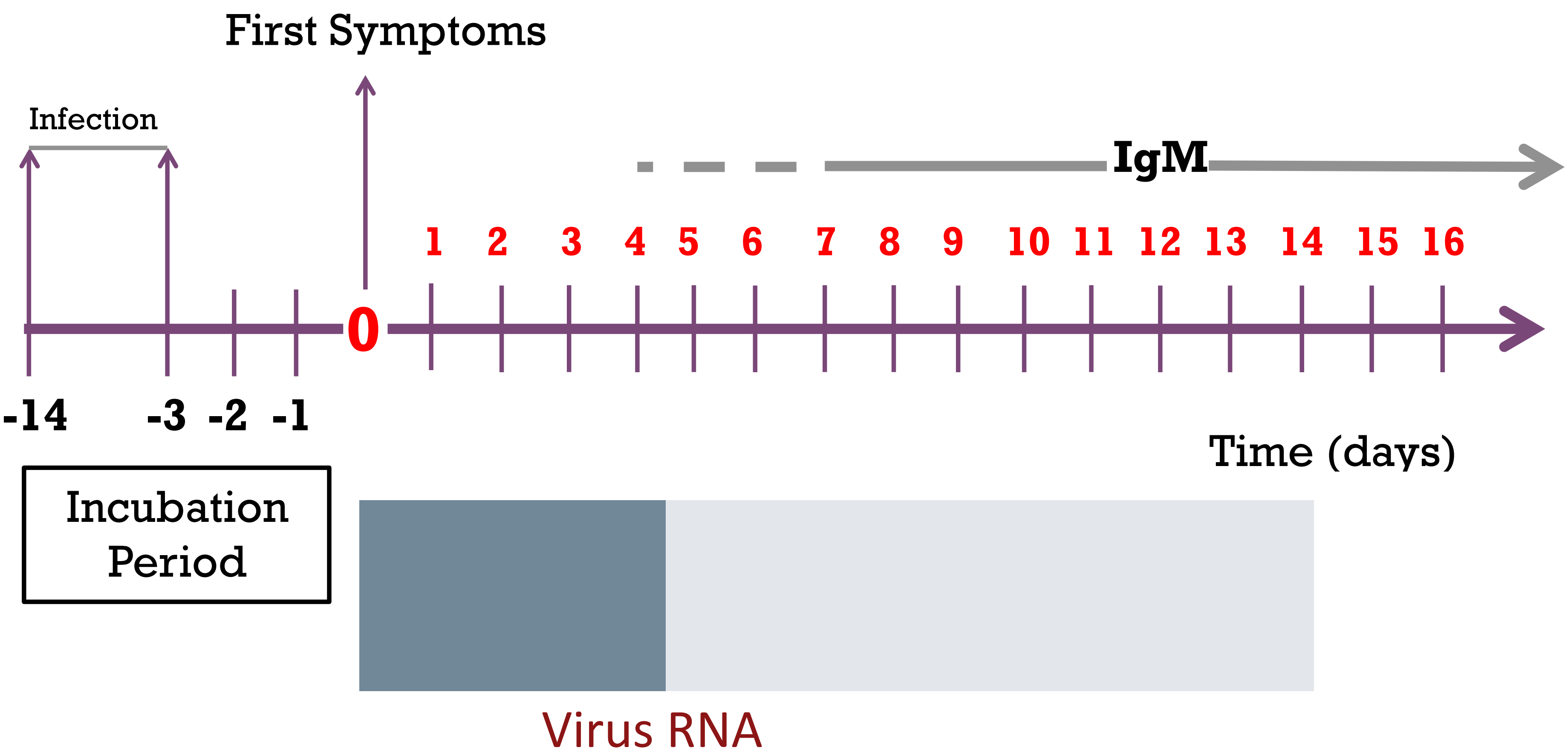
- ZIKV infection typically presents without localizing signs or symptoms.
  - Fever, muscle aches, rash, conjunctivitis

- Co-circulation of ZIKV, CHIKV, and DENV complicates clinical diagnosis and highlights the importance of diagnostics that can detect and differentiate these pathogens.

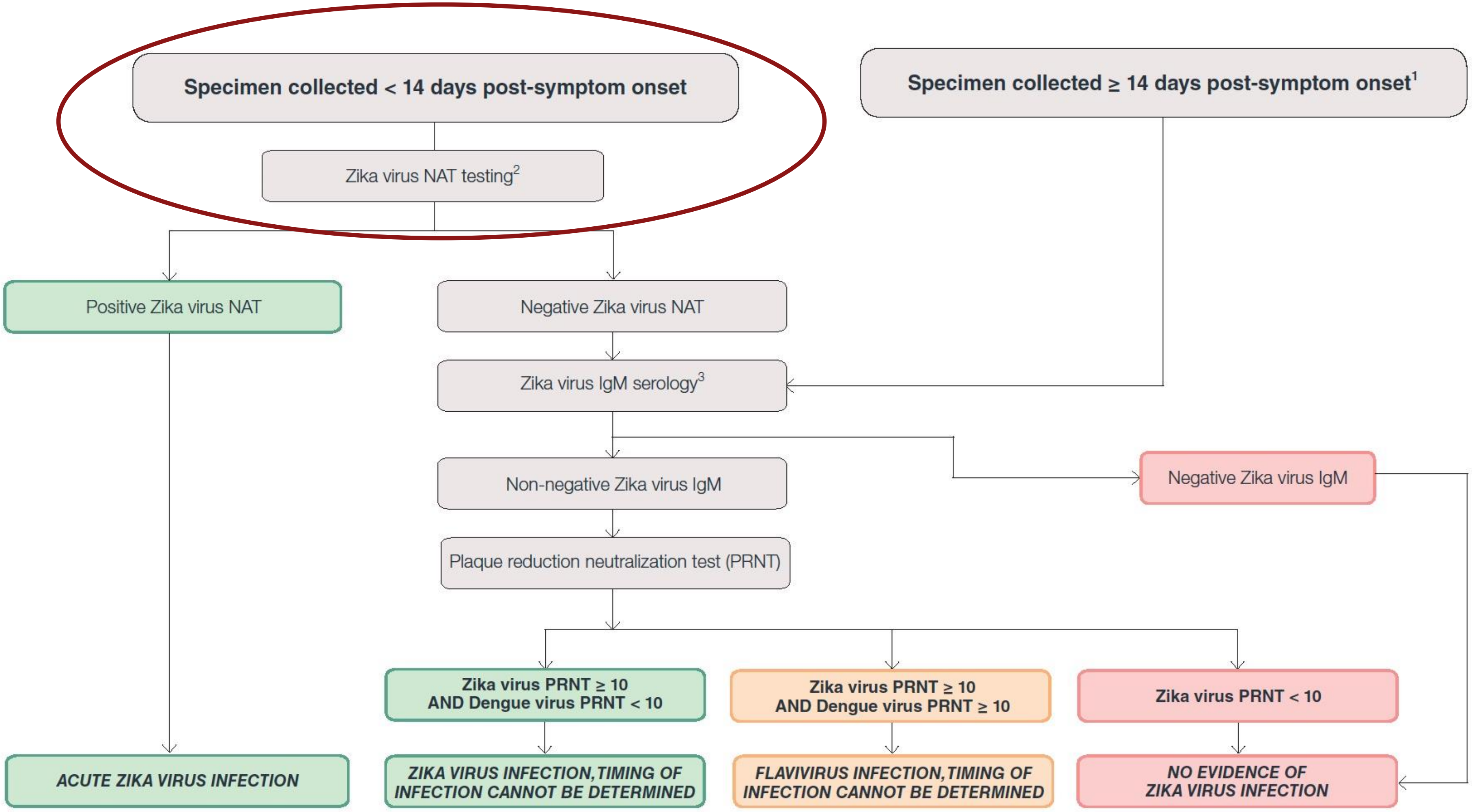
- Up to 80% of ZIKV infections may be asymptomatic or minimally symptomatic.

"BIGGEST DAMN VIRUS I'VE EVER SEEN!"

Zika Virus: Markers of Infection



CDC Testing Algorithm



CDC, August 27, 2017 https://www.cdc.gov/zika/pdfs/testing-algorithm-symptomatic-nonpregnant.pdf

Nucleic Acid Amplification Testing (NAAT): Serum

CDC ZIKV real-time RT-PCR (Lanciotti Assay)

- Assays based on sequence from the Yap Islands outbreak, 2007
- Targets the prM and E genes in 2 separate reactions
- Requires detection in both reactions with Ct values <38.5 to call Positive

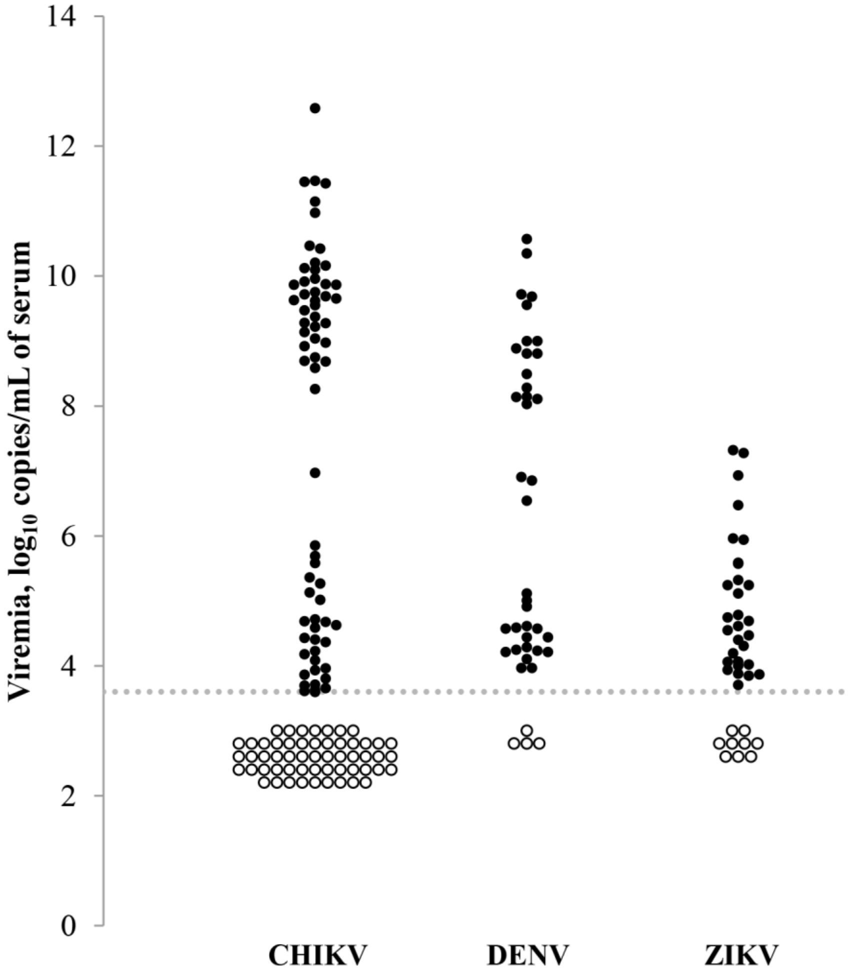
Result	Percent	#/Total
Positive	10.8%	17/157
Equivocal	6.4%	10/157
Negative	82.8%	130/157

- 88.2% (15/17) of positives were collected within the first 3 days of illness
- During the 2013-2014 French Polynesia outbreak a short duration of viremia was also observed

  Mean day of illness 3.3 days (SD = 1.8 days)

Lanciotti *et al., Emerg Infect Dis 2008*
Musso *et al. J Clin Virol 2015*

NAAT: Virus Load, Serum



Yap Islands, 2007

- CDC ZIKV rRT-PCR
- Mean ZIKV level: 4.40 $\log_{10}$ copies/mL (SD 0.94 $\log_{10}$ copies/mL)

Nicaragua, 2016

- ZCD multiplex rRT-PCR
- Mean ZIKV level: 4.7 $\log_{10}$ copies/mL of serum (SD, 0.97 $\log_{10}$ copies/mL)
- Significantly lower than mean viremia detected in DENV- and CHIKV-positive samples
  - DENV 5.84 $\log_{10}$ copies/mL, SD 1.84;p<0.001
  - CHIKV 6.42 $\log_{10}$ copies/mL, SD 2.72;p<0.001

Lanciotti *et al., Emerg Infect Dis 2008*
Waggoner et al., Clin Infect Dis 2016

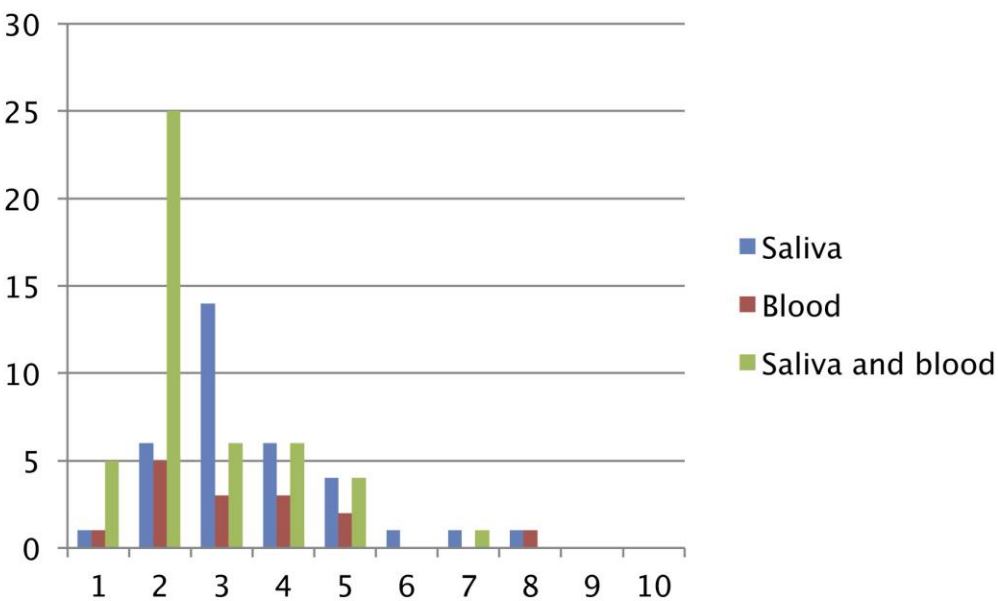
NAAT: Alternative Specimen Type
Saliva

<u>French Polynesia, 2013-14</u>

- CDC ZIKV rRT-PCR

  19.2% (35/182) Saliva +/Serum - vs.
  8.8% (16/182) Saliva -/Serum + (p=0.0117)

- Saliva: Mean day of illness 3.5 days (SD = 1.5 days)



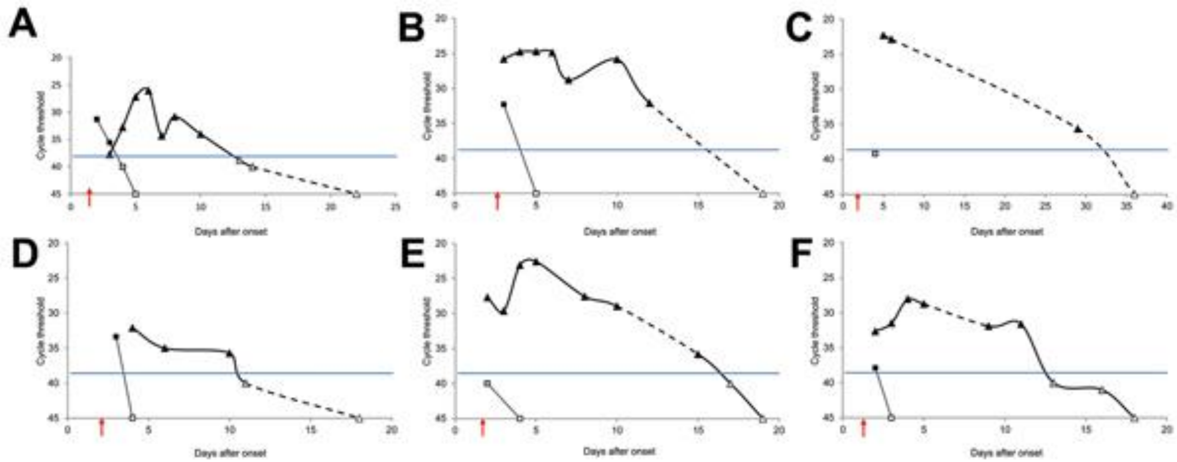
		Saliva		
		Positive	Negative	Total
Serum	Positive	52	16	68
	Negative	35	79	114
	Total	87	95	182

NAAT: Alternative Specimen Type
Urine

New Caledonia, 2014

- ZIKV RNA detectable in urine 7 or more days after it is no longer detectable in serum.

Gourinat *et al., Emerg Infect Dis 2015*



Brazil, 2015

- ZIKV RNA was more frequently detected in serum compared to urine
  - 13.6% (12/88) Urine +/Serum - vs. 29.5% (26/88) Urine -/Serum+

Brasil *et al., N Engl J Med 2016*

Florida, USA, 2016

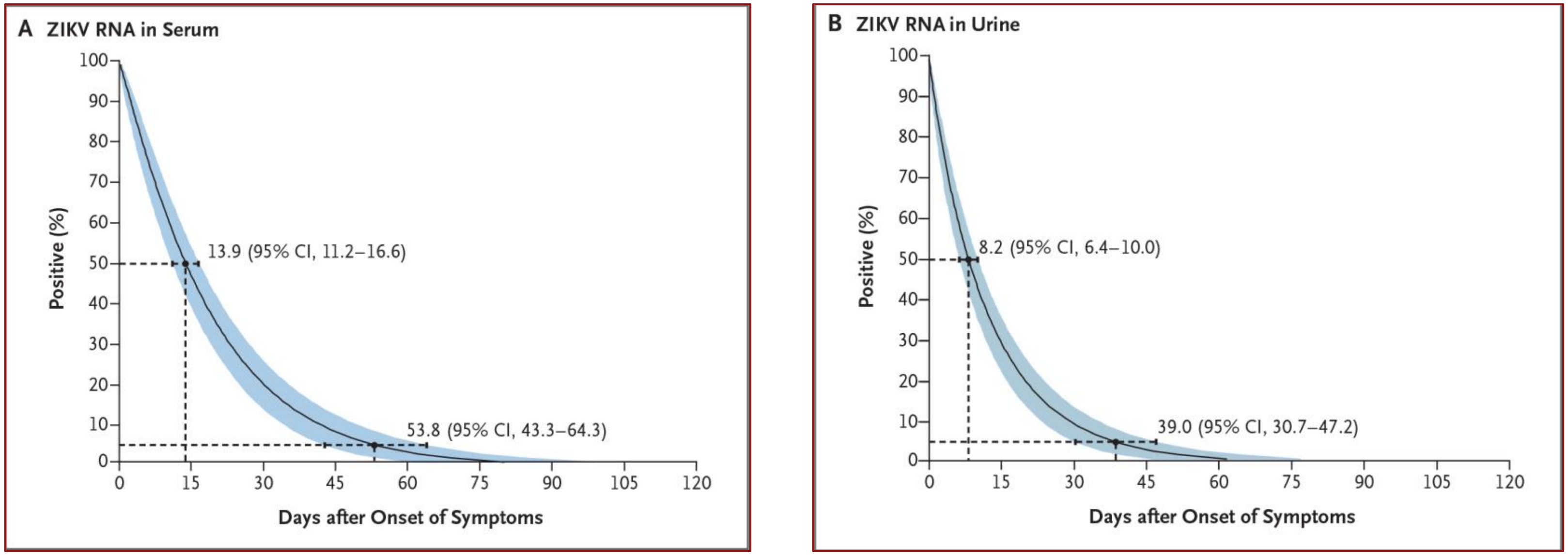
- ZIKV RNA was more frequently detected in urine compared to serum
  - 95% (52/55) Urine +/Serum - vs. 56% (31/55) Urine -/Serum+

Bingham *et al., MMWR 2016*

NAAT: Alternative Specimen Types
Urine

Puerto Rico, 2016-17

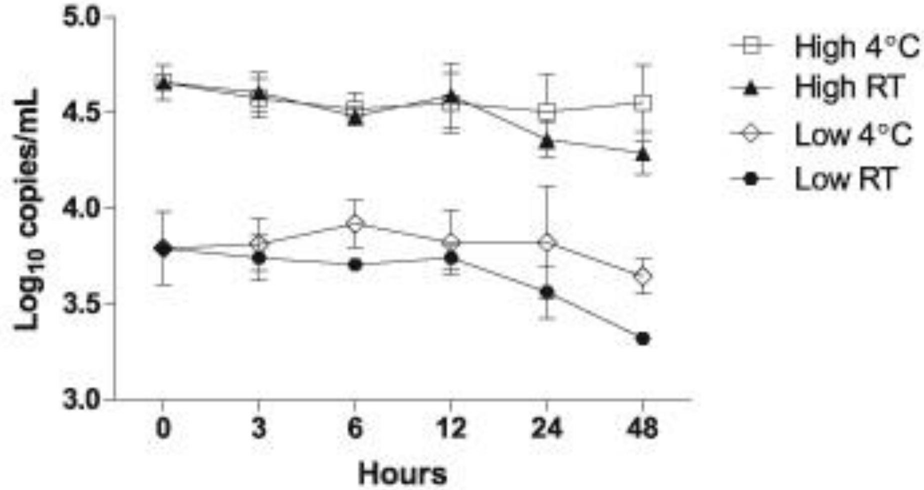
- Serum-median time until the loss of RNA detection was 14 days (95% CI, 11-17)
- Urine-median time until the loss of RNA detection was 8 days (95% CI, 6 to 10)



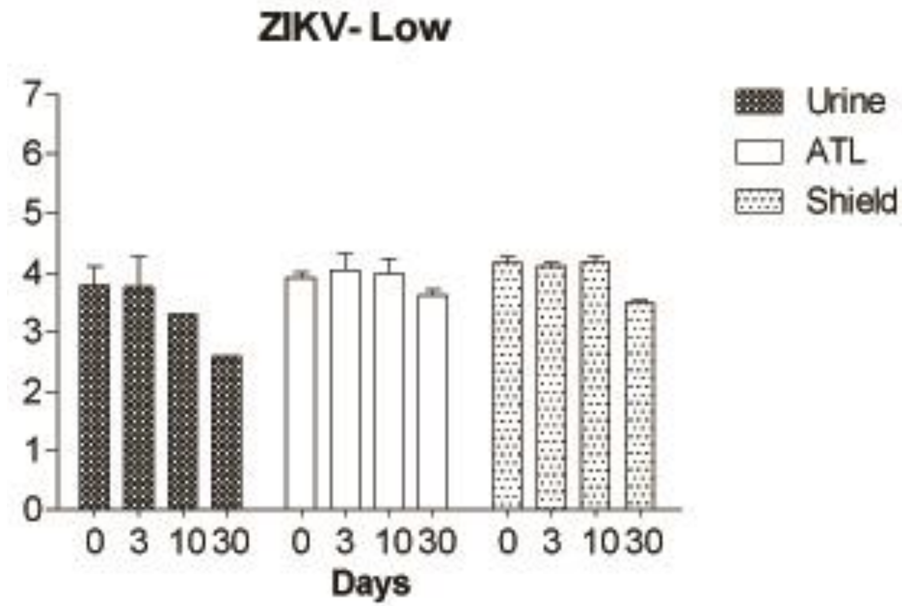
- 10.1% had detectable RNA in urine but not in serum, whereas 36.7% had RNA in serum but not urine.

Paz-Bailey *et al., N Engl J Med 2017*

ZIKV RNA is Unstable in Urine Stored at -80°C



Trend towards RNA instability for low ZIKV concentrations at room temperature for 48 hours



Significant instability for Low ZIKV concentrations at -80°C rescued by NA stabilizers

Tan *et al., J Virol Methods 2017*

NAAT: Alternative Specimen Types

- Amniotic Fluid (AF)

Oliveira Melo *et al., Ultrasound Obstet Gynecol 2016*
Calvet *et al., Lancet Infect Dis 2016*
Meany-Delman *et al. MMWR Morb Mortal Wkly Rep 2016*
Jouannic *et al., Lancet 2016*
Driggers *et al., N Engl J Med 2016*

- Cerebrospinal Fluid (CSF)

Cao-Lormeau *et al., Lancet 2016*
Waggoner *et al., submitted 2016*

- Semen

Musso *et al. ,Emerg Infect Dis 2015*
D'Ortenzio *et al., N Engl J Med 2016*
Mansuy *et al., Lancet Infect Dis 2016*
Atkinson *et al., Emerg Infect Dis 2016*
Paz-Bailey et al., N Engl J Med 2017

- Fetal Tissue

Driggers *et al., N Engl J Med 2016*
Mlakar *et al., N Engl J Med 2016*
Sarno *et al., PLoS Negl Trop Dis 2016*
Martines *et al., MMWR Morb Mortal Wkly Rep 2016*
Meany-Delman *et al. MMWR Morb Mortal Wkly Rep 2016*

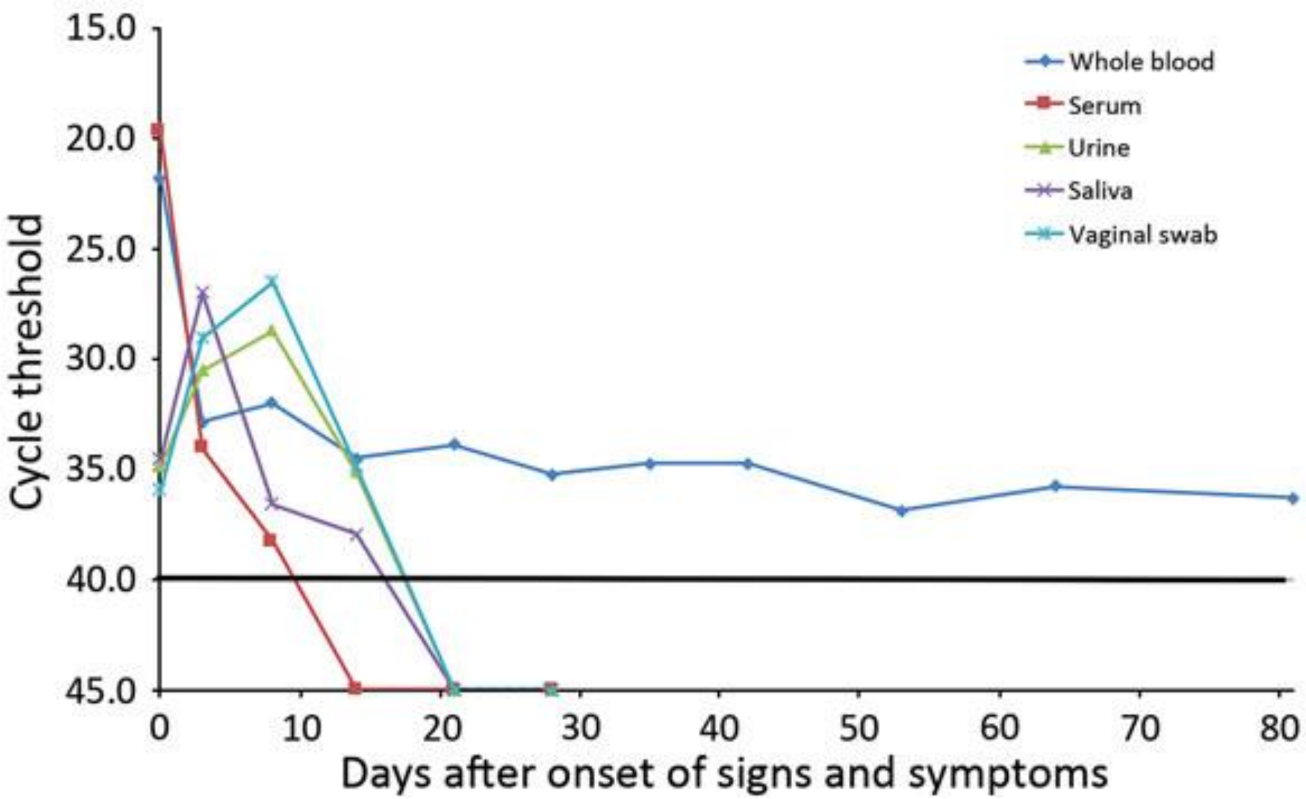
- Whole Blood

Murray *et al., Emerg Infect Dis 2017*
Mansuy *et al.,Emerg Infect Dis 2017*

NAAT: Alternative Specimen Type Whole Blood

Honduras, 2017

- ZIKV RNA detectable whole blood 80 days after onset of symptoms.



Murray *et al.*, *Emerg Infect Dis 2017*

Emergency Use Authorization (EUA) – Nucleic Acid Amplification Tests

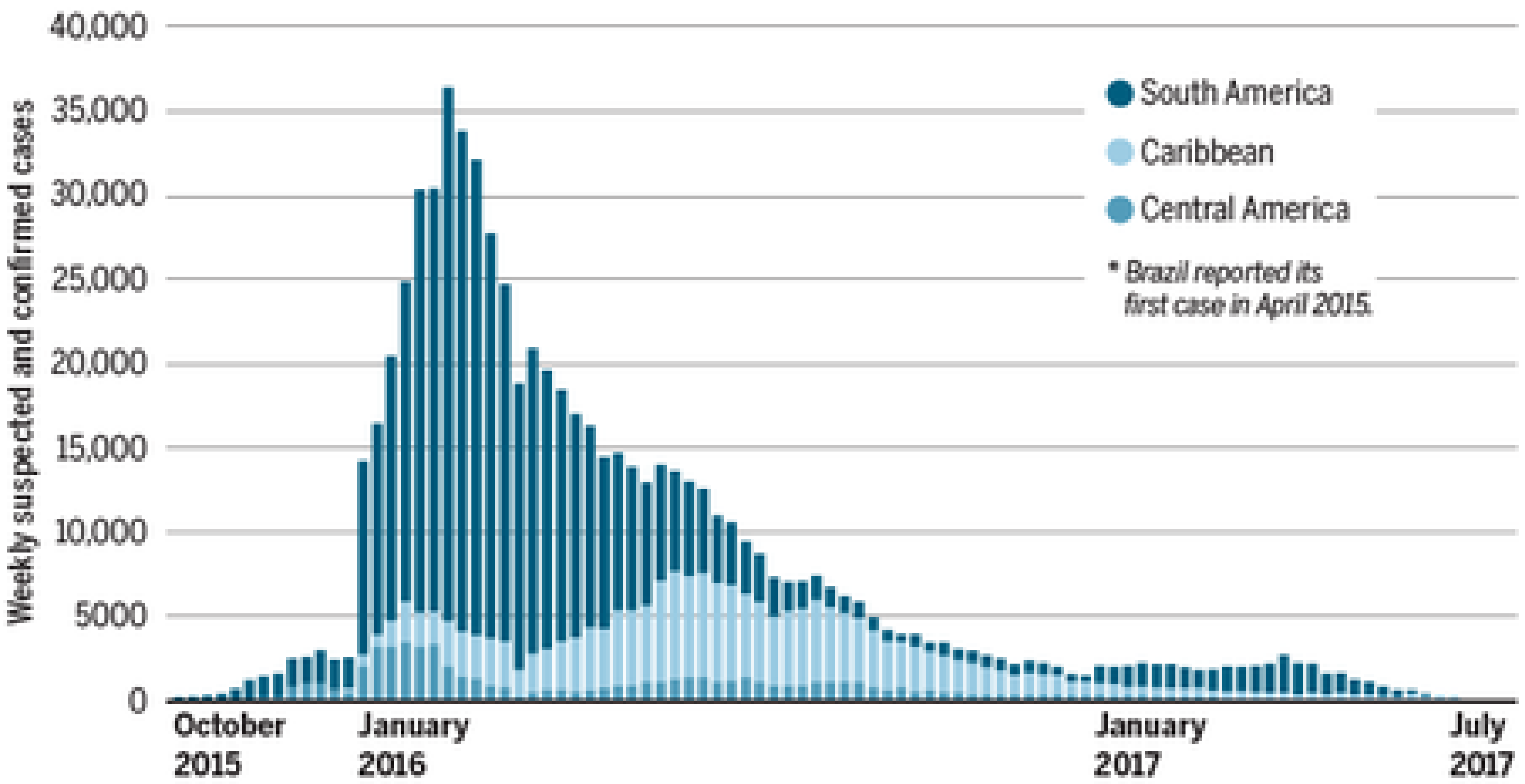
1. Trioplex real-time RT-PCR, CDC
2. Zika Virus RNA Qualitative RNA RT-PCR, Quest/Focus Diagnostics
3. RealStar® Zika Virus RT-PCR Kit, Altona Diagnostics
4. Aptima® Zika Virus Assay, Hologic Inc.
5. Viracor/Eurofins Zika virus real-time RT-PCR Test
6. VERSANT® Zika RNA 1.0 Assay (kPCR) Kit, Siemens Healthcare Diagnostics Inc.
7. xMAP® MultiFLEX™ Zika RNA Assay, Luminex Corporation
8. *Sentosa*® SA ZIKV RT-PCR Test, Vela Diagnostics USA, Inc.
9. Zika Virus Detection by RT-PCR Test, ARUP Laboratories
10. Abbott Zika Realtime, Abbott Molecular
11. Zika ELITe MGB Kit U.S., ELITech Group Inc. Molecular Diagnostics
12. Gene-RADAR® Zika Virus Test, Nanobiosym Diagnostics, Inc.
13. CII-ArboViroPlex rRT-PCR assay , Columbia University
14. TaqPath Zika Virus Kit, Thermo Fisher Scientific

As of October 18, 2017

And After!

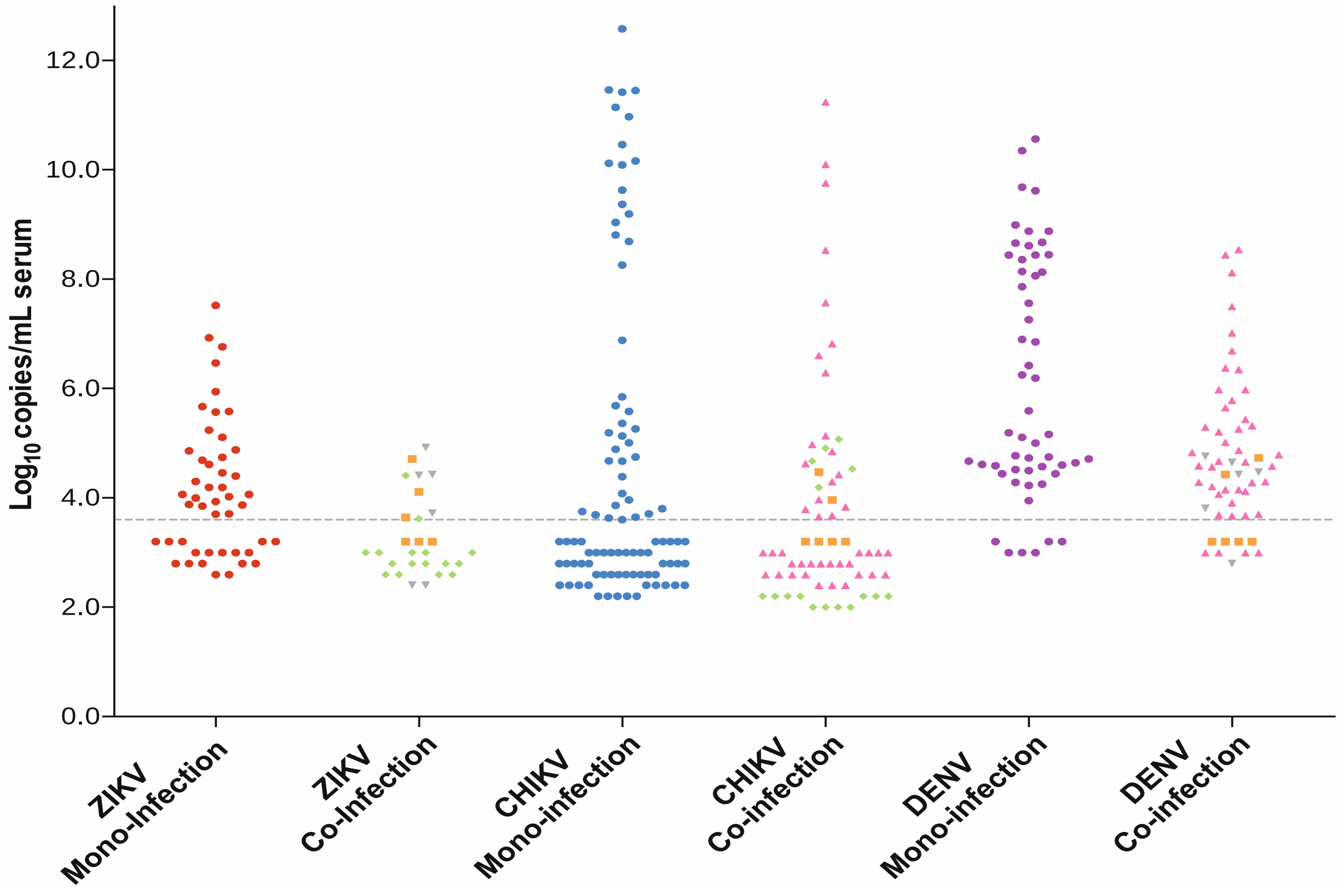
Gift from Stanford Pathology Residents- Sebastian Fernandez-Pol, Jana Broadhurst, and Morgan Ballard

Rapid Disappearance of Zika virus



Cohen, Science 2017

Co-Infections have lower levels of viremia than Mono-infections



Nicaragua, 2016

ZCD multiplex rRT-PCR

ZIKV Mono-infection: 4.70 $\log_{10}$ copies/mL of serum (SD, 0.97 $\log_{10}$ copies/mL)

ZIKV co-infection: 4.22 $\log_{10}$ copies/mL of serum (SD, 0.48 $\log_{10}$ copies/mL) p<0.018

Hospitalized cases had higher viremia than those who did not require hospitalization (7.1 vs 4.1 log10 copies/mL serum, respectively; p<0.001).

Waggoner et al., Emerg Infec Dis 2016

Waggoner et al., Clin Infect Dis 2016

Future Directions - NAATs

- Development and regulatory approval of additional multiplexed options for the molecular diagnosis of ZIKV, CHIKV, and DENV, as well as other pathogens in the differential, including Yellow Fever, Mayaro, etc…

Development of a Real-Time Reverse Transcription Polymerase Chain Reaction for O'nyong-nyong Virus and Evaluation with Clinical and Mosquito Specimens from Kenya

Jesse Waggoner,[1] Claire Jane Heath,[2] Bryson Ndenga,[3] Francis Mutuku,[4] Malaya K. Sahoo,[5] Alisha Mohamed-Hadley,[5] John Vulule,[3] Dunstan Mukoko,[6] A. Desiree LaBeaud,[2] and Benjamin A. Pinsky[5,7]*

[1]*Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia;* [2]*Division of Infectious Diseases, Department of Pediatrics, Stanford University School of Medicine, Stanford, California;* [3]*Kenya Medical Research Institute, Kisumu, Kenya;* [4]*Technical University of Mombasa, Mombasa, Kenya;* [5]*Department of Pathology, Stanford University School of Medicine, Stanford, California;* [6]*Ministry of Health, Nairobi, Kenya;* [7]*Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, California*

Molecular Diagnostics for Undifferentiated Systemic Febrile Illness (UFI)

Sensitive and Specific Multiplex UFI Diagnostics are critical for the care of individual patients as well as for efficient public health surveillance :

- Optimize patient triage, isolation, and supportive care
- Appropriately manage antimicrobial therapy, if available
- Tailor the public health response, if required
- Allow targeted use of Broad Range Pathogen Discovery techniques

The Express Tribune; Sept 6, 2011

The UFI Multiplex Assay Version 1.0

- Began with dengue virus, the most common mosquito-borne viral pathogen
- Malaria and Leptospira were added based on:
  - High disease burden
  - High levels of pathogen nucleic acid in blood
  - Worldwide distribution
  - The availability of antimicrobial therapy
- Now designed ~15 assays that can be interchangeably performed in multiplex

Component	Target	Channel
Pan-DENV	5'UTR-Core	Green
Pan-*Leptospira*	16S rRNA	Yellow
P. falciparum	Pfr364	Orange
Plasmodium species	18S rRNA	Red
Internal Control	RNAse P	Crimson

J Clin Microbiol 2014; 52(6):2011-8

Some of the Pathogens causing an Undifferentiated Systemic Febrile Illness

- Dengue
- Yellow Fever
- Zika
- West Nile

- Chikungunya
- O'nyong nyong
- Sindbis
- Mayaro
- Ross River

- Rift Valley Fever
- Hantavirus
- Oropouche

- Malaria
- Leptospira
- Rickettsia

- Rubella
- Measles
- HIV

- CMV
- EBV
- HHV-6
- Enteroviruses
- Respiratory Viruses

- Ebola
- Marburg
- Lassa

Syndromic Multiplex Panels Have Transformed Clinical Infectious Diseases Testing

- Respiratory
- Blood Culture
- Gastrointestinal
- Encephalitis/Meningitis

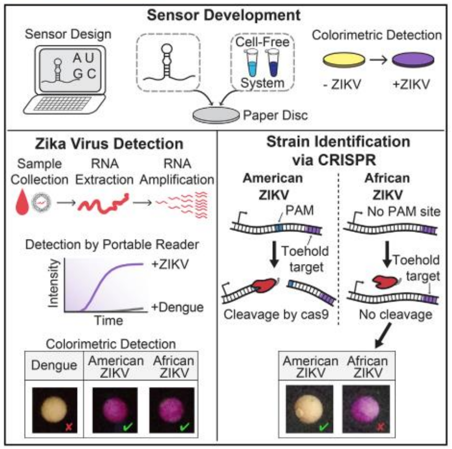
NAATs
for near-care or point-of-care testing

Cell

Resource

Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components

Graphical Abstract



Authors

Keith Pardee, Alexander A. Green, Melissa K. Takahashi, ..., David H. O'Connor, Lee Gehrke, James J. Collins

Correspondence

jimjc@mit.edu

In Brief

A diagnostic platform utilizing biomolecular sensors and CRISPR-based technology allows rapid, specific, and low-cost detection of the Zika virus at clinically relevant concentrations.

CRISPR TECHNOLOGY

Science AAAS

Nucleic acid detection with CRISPR-Cas13a/C2c2

Jonathan S. Gootenberg,[1,2,3,4,5]* Omar O. Abudayyeh,[1,2,3,4,6]* Jeong Wook Lee,[7] Patrick Essletzbichler,[1,2,3,4] Aaron J. Dy,[1,4,8] Julia Joung,[1,2,3,4] Vanessa Verdine,[1,2,3,4] Nina Donghia,[7] Nichole M. Daringer,[8] Catherine A. Freije,[1,9] Cameron Myhrvold,[1,9] Roby P. Bhattacharyya,[1] Jonathan Livny,[1] Aviv Regev,[1,10] Eugene V. Koonin,[11] Deborah T. Hung,[1] Pardis C. Sabeti,[1,9,12,13] James J. Collins,[1,4,6,7,8]† Feng Zhang[1,2,3,4]†

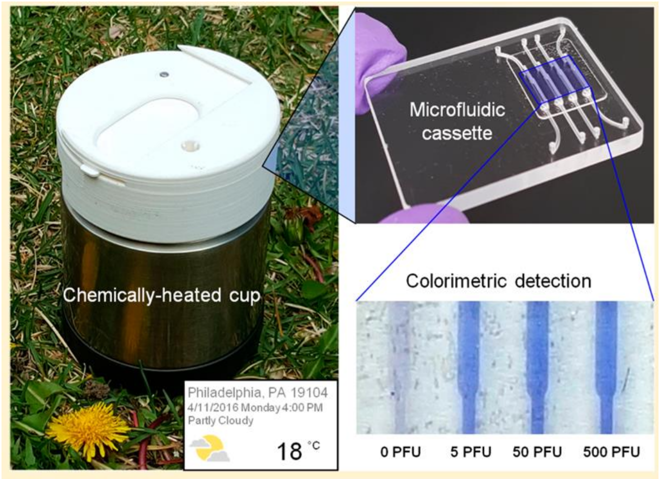
analytical chemistry

Article

pubs.acs.org/ac

Instrument-Free Point-of-Care Molecular Detection of Zika Virus

Jinzhao Song,† Michael G. Mauk,† Brent A. Hackett,‡ Sara Cherry,‡ Haim H. Bau,† and Changchun Liu*,†



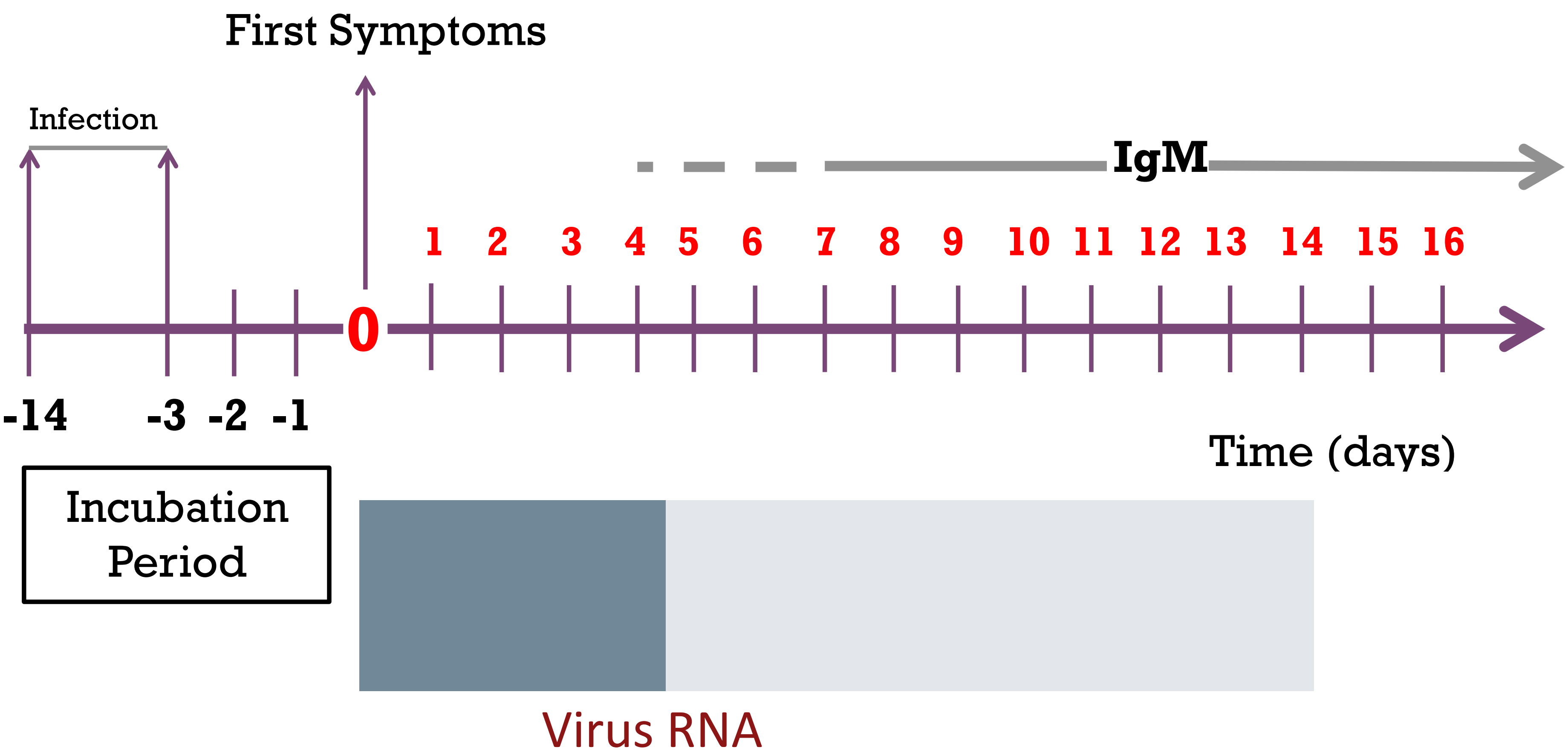
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

ZIKA VIRUS

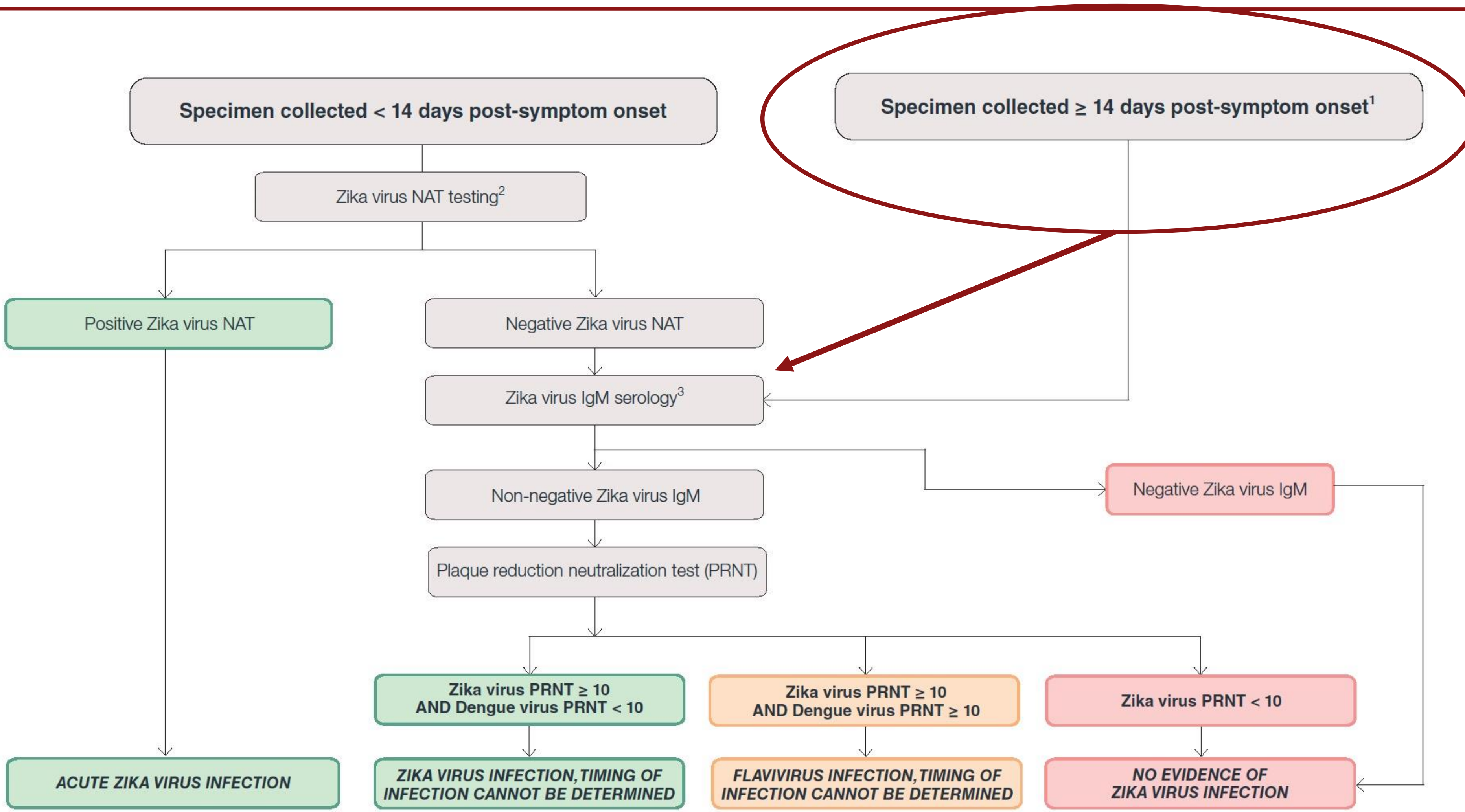
Rapid and specific detection of Asian- and African-lineage Zika viruses

Nunya Chotiwan,[1,2]* Connie D. Brewster,[1]* Tereza Magalhaes,[2,3] James Weger-Lucarelli,[1,2] Nisha K. Duggal,[4] Claudia Rückert,[1,2] Chilinh Nguyen,[1,2] Selene M. Garcia Luna,[1,2] Joseph R. Fauver,[1,2] Barb Andre,[1] Meg Gray,[1,2] William C. Black IV,[1,2] Rebekah C. Kading,[1,2] Gregory D. Ebel,[1,2] Guillermina Kuan,[5] Angel Balmaseda,[6] Thomas Jaenisch,[7,8] Ernesto T. A. Marques,[3,9] Aaron C. Brault,[4] Eva Harris,[10] Brian D. Foy,[1,2] Sandra L. Quackenbush,[1] Rushika Perera,[1,2] Joel Rovnak[1]†

Zika Virus: Markers of Infection

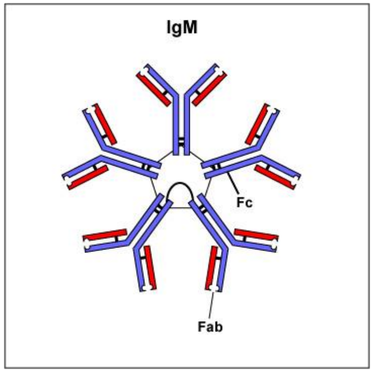


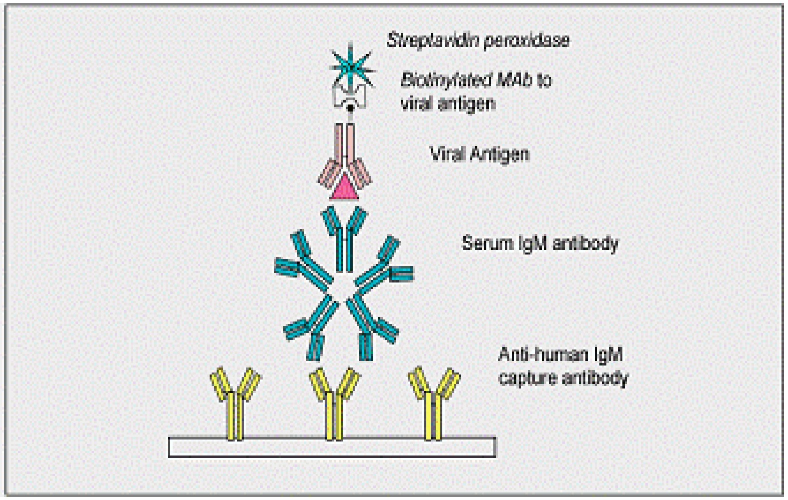
CDC Testing Algorithm



Zika Virus Diagnostics: Antibody Detection – IgM

- IgM Detected ≥4 days after the onset of illness and may persist for 2-12 weeks (or longer)
- Previous Infection or vaccination with other flaviviruses (*e.g.*, DENV, JEV, YFV) may result in false positive IgM results
- Emergency Use Authorization:
  - CDC ZIKV MAC ELISA
  - ZIKV Detect Capture IgM (InBios)
  - LIAISON® XL Zika Capture IgM Assay (DiaSorin Inc.)
  - DPP Zika IgM Assay System (ChemBio)
  - ADVIA Centaur Zika test (IgM, Siemens)
- InBios utilizes a Cross-Reacting Control Antigen (CCA) to generate an Immune Status Ratio (ISR = ZIKV Ag/CCA) in an attempt to improve specificity.





CDC MAC ELISA– Yap Island, 2007

Table 1. IgG and IgM testing with heterologous flaviviruses of patients infected with ZIKV, Yap State, Micronesia, 2007*

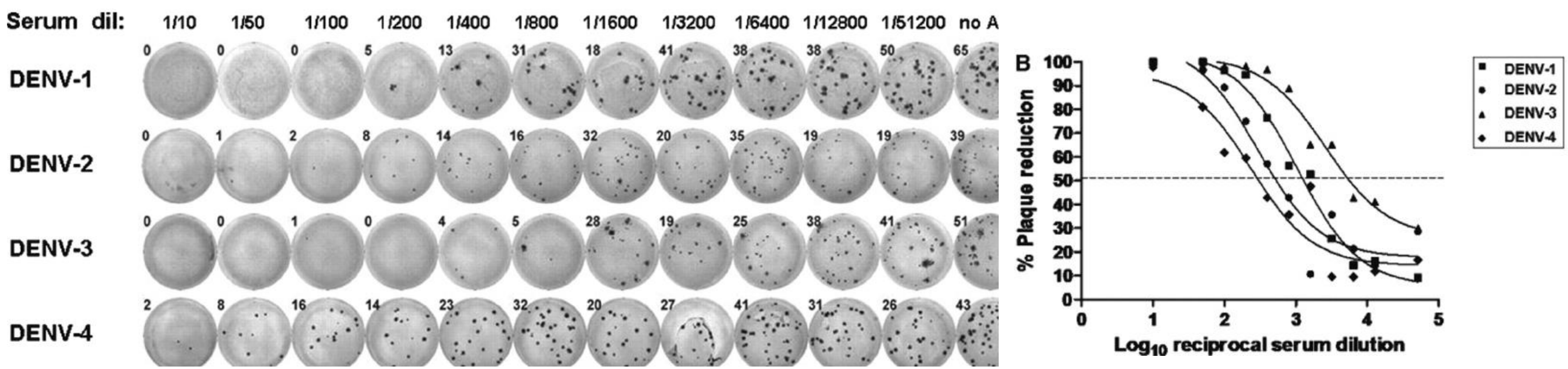
		IgG	IgM					
Patient	Days after onset	ZIKV	ZIKV	DENV	YFV	JEV	MVEV	WNV
Primary flavivirus ZIKV								
822a	5	1.5	23.2	1.3	1.4	1.7	1.1	–
822b	10	1.2	39.5	1.2	1.0	2.4	1.2	–
822c	24	3.3	13.1	2.7	0.63	1.8	1.3	–
830a	2	1.1	1.3	4.4	0.48	4.4	2.9	–
830b	21	1.8	16.3	1.9	0.63	1.3	1.6	–
849a	3	1.5	4.5	0.92	0.95	1.2	0.66	–
849b	18	3.0	18.2	2.2	1.0	2.7	1.5	–
862a	6	1.9	25.4	1.7	1.1	1.8	1.0	–
862b	20	2.6	15.4	2	1.1	2.3	1.1	Eq
Secondary flavivirus ZIKV (probable)								
817a	1	5.9	1.4	1.7	0.8	1.7	0.7	–
817b	19	5.7	8.1	5.1	2.1	1.7	1.0	–
833a	1	3.4	1.7	3.7	1.0	2.8	1.3	–
833b	19	8.2	3.1	2.3	0.9	2.5	1.3	–
844a	2	3.8	3.8	6.8	2.0	21.5	0.7	–
844b	16	8.5	12.7	14.9	7.0	42.9	1.6	–
955a	1	5.0	1.8	3.7	1.0	3.4	2.4	Eq
955b	14	26.6	10.9	3.4	0.8	1.7	4.0	Eq
968a	1	4.0	1.7	1.3	0.6	1.2	1.2	–
968b	3	12.3	20.4	2.9	0.8	0.9	2.0	–
839a	3	1	0.92	3.4	0.7	2.7	2.1	–
839b	20	4.9	17.2	2.2	2.1	1.9	1.8	–
847a	5	0.9	0.94	4.1	4.1	2.3	1.3	–
847b	8	14.1	21.5	1.4	3.3	1.1	2.6	–

*Ig, immunoglobulin; ZIKV, Zika virus; DENV, dengue virus type 1–4 mixture; YFV, yellow fever virus; JEV, Japanese encephalitis virus; MVEV, Murray Valley encephalitis virus; WNV, West Nile virus; –, negative. Eq, result in equivocal range of the assay. IgG and IgM testing was conducted by ELISA except for WNV, which was tested by microsphere assay; ELISA values are patient optical densities divided by negative control optical densities; <2, negative; 2–3 equivocal; >3 positive.

Lanciotti *et al., Emerg Infect Dis 2008*

Zika Virus Diagnostics: Antibody Detection – PRNT

- Plaque Reduction Neutralization Testing (PRNT)
  - PRNT90 may help distinguish ZIKV infection from infection with DENV or other flaviviruses
  - Criteria for ZIKV Positive
    - Titer ≥4-fold compared to other flaviviruses
    - ≥4-fold increase in acute and convalescent sera



PRNT– Yap Island, 2007

Table 2. Neutralization testing with heterologous flaviviruses of patients infected with ZIKV, Yap State, Micronesia, 2007*

Patient	Days after onset	PRNT90 titer: ZIKV	DENV1	DENV2	DENV3	DENV4	JEV	YFV	WNV	SLEV	MVEV
Primary flavivirus ZIKV											
822a	5	320	<10	<10	<10	<10	<10	<10	<10	<10	<10
822b	10	2,560	10	10	10	10	<10	<10	<10	<10	<10
822c	24	5,120	10	10	10	10	<10	<10	<10	<10	<10
830a	2	<10	<10	NT‡	NT	NT	NT	NT	NT	NT	NT
830b	21	2,560	<10	<10	<10	<10	<10	<10	<10	<10	<10
849a	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
849b	18	10,240	<10	<10	<10	<10	<10	20	<10	<10	<10
862a	6	320	<10	<10	<10	<10	<10	<10	<10	<10	<10
862b	20	2,560	10	10	<10	<10	<10	<10	<10	10	<10
Secondary flavivirus ZIKV (probable)											
817a	1	80	80	160	320	160	<10	<10	<10	40	40
817b	19	10,240	2,560	20,480	5,120	5,120	20	320	160	1,280	640
833a	1	160	320	80	40	20	<10	<10	<10	<10	<10
833b	19	81,920	20,480	5,120	5,120	1,280	<10	<10	80	320	320
844a	2	20	1,280	640	320	160	<10	<10	5	20	20
844b	16	10,240	40,980	10,240	5,120	1,280	5	<10	160	640	640
955a	1	40	1,280	640	160	320	<10	<10	<10	20	20
955b	14	163,840	81,920	20,480	10,240	5,120	10	<10	640	2,560	1,280
968a	1	80	320	320	80	40	<10	<10	<10	40	20
968b	3	10,240	640	640	160	160	<10	<10	10	40	20
839a	3	<10	<10	10	<10	<10	<10	40	<10	<10	<10
839b	20	10,240	40	320	80	80	<10	640	40	80	80
847a	5	<10	<10	<10	<10	<10	<10	640	<10	<10	<10
847b	8	2,560	40	320	160	40	<10	1,280	80	320	320

*$PRNT_{90}$ titer, 90% plaque reduction neutralization test titer; ZIKV, Zika virus; DENV, dengue virus; JEV, Japanese encephalitis virus; YFV, yellow fever virus; WNV, West Nile virus; SLEV, St. Louis encephalitis virus; MVEV, Murray Valley encephalitis virus; NT, not tested (sample depleted).

Lanciotti *et al., Emerg Infect Dis 2008*

CDC MAC ELISA

Table 5: Data for Sera Submitted to CDC Ft. Collins for Testing 2015-present

		PRNT Results			
		Zika	flavivirus	dengue	negative
Zika MAC-ELISA	positive	45	16	23	9
	equivocal	1	0	9	13
	negative	0	0	6	39

Positive Percent agreement: 97.8% (45/46) (95% CI: 88.7% 99.6%)
Negative percent agreement: 45/99 = 45.5% (95% CI: 36.0% -55.3%)

PRNT is unable to resolve 17.2% (16/93) of Zika MAC ELISA positive specimens

ZIKV Detect Capture IgM, InBios

			CDC MAC-ELISA / PRNT / PCR			
			Zika +	Flavivirus +	Negative	Total
InBios ZIKV *Detect™* IgM Capture ELISA	**Presumptive Zika Positive** (Zika ISR ≥ 1.70)		37	15	0	*52*
	NCA Analysis	Zika/NCA ≥ 1.70 CCA/NCA ≥ 1.70	0	4	4	*8*†
		Zika/NCA ≥ 1.70 CCA/NCA < 1.70	0	0	0	*0*
		Zika/NCA < 1.70 CCA/NCA ≥ 1.70	0	0	4	*4*†
	Negative		0	0	98	*98**
	Total		*37*	*19*	*106*	*162*

Positive Percent Agreement for Zika	100% (37/37, 90.6% - 100%)
Positive Percent Agreement for Zika *AND* Flavivirus	100% (56/56, 93.6% - 100%)
Negative Percent Agreement	92.5% (98/106, 85.8% - 96.1%)

Independent Comparison of the CDC and InBios MAC ELISAs

- Mayo Medical Laboratories: CDC and InBios MAC-ELISAs performed comparably to each other
  - positive agreement 87.5%-93.1%
  - negative agreement range 95.7%-98.5%
- Euroimmun IgM ELISA demonstrated poor positive percent agreement
  - 17.9%-42.9%
- Of 19 prospectively collected samples with non-negative IgM results, **only 1** was confirmed ZIKV antibody positive by PRNT
  - 1 ZIKV
  - 1 DENV
  - 8 unidentified flavivirus
  - 9 negative

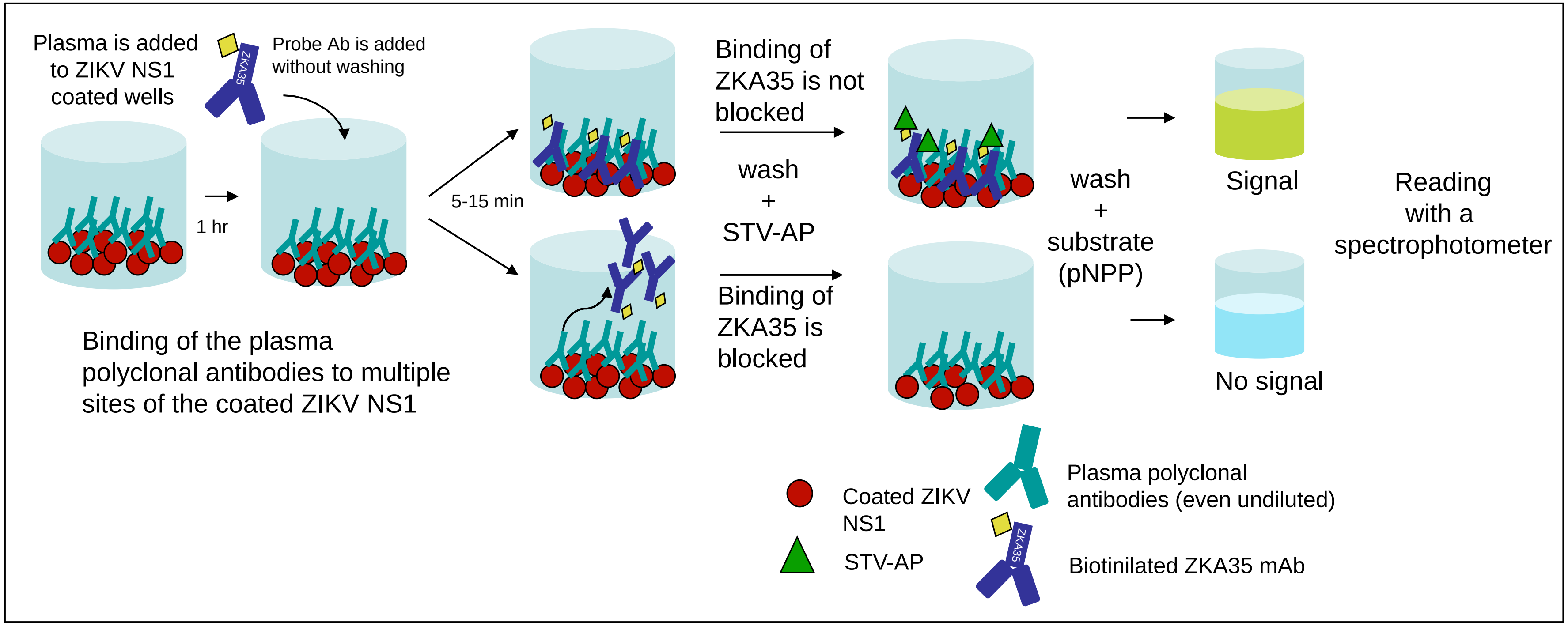
Granger *et al., J Clin Microbiol 2017*

Future Directions - Serology

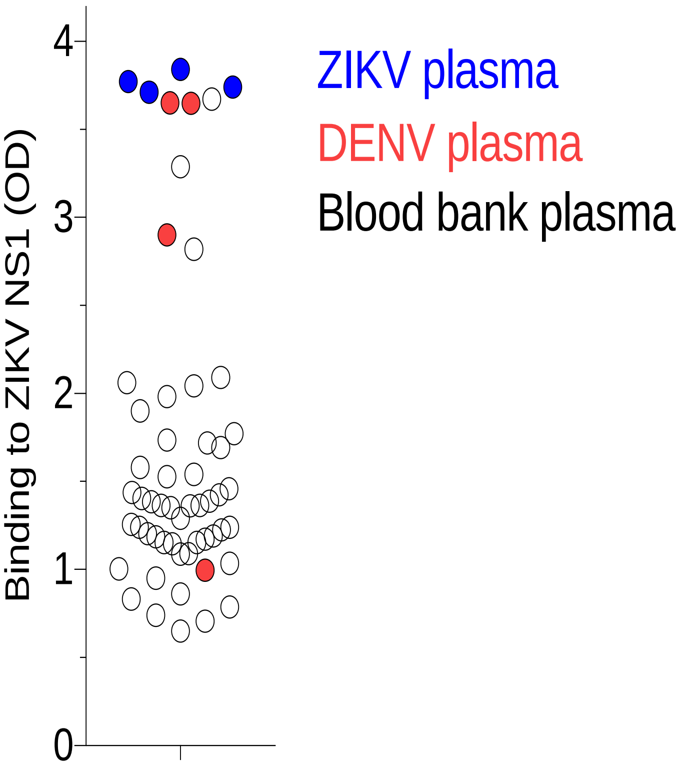
- Widespread availability of well-characterized antibody tests for diagnostic and public health laboratories
- Improvement in ZIKV antibody test specificity
  - Identification of ZIKV-specific epitopes
  - Identification and deletion of ZIKV-DENV common epitopes
- Alternatives to PRNT
  - ZIKV Western Blot
  - Multiplex Flavivirus IgG/IgG Avidity
- Development of specific ZIKV IgG assays, NS1 antigen assays, and multiplexed assays with ZIKV, CHIKV, and DENV
- Development of antigen and antibody assays for near-care or point-of-care testing.

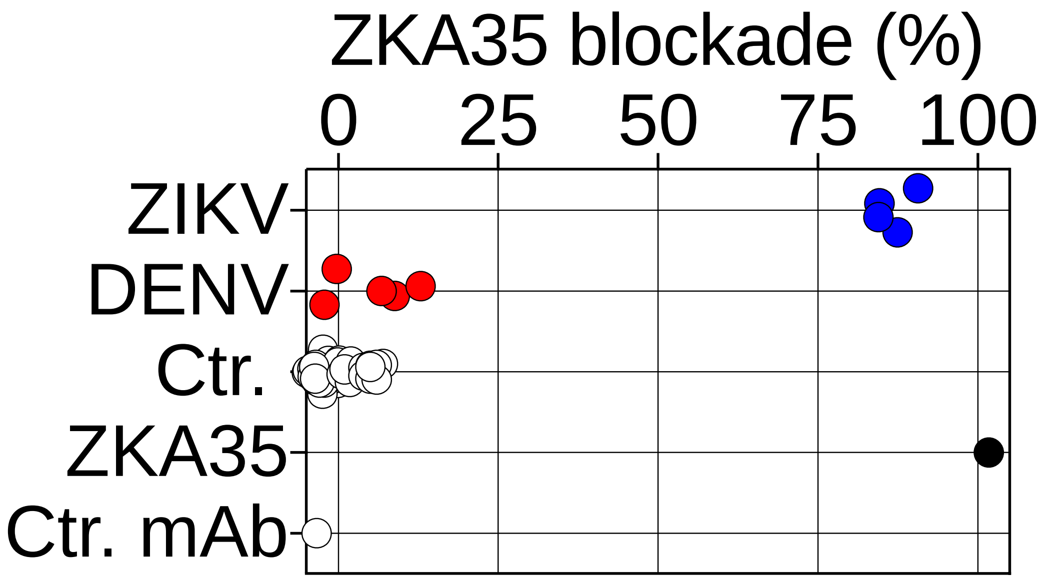
ZIKV NS1 Blockade of Binding Assay

A ZIKV NS1-specific monoclonal antibody (ZKA35) was isolated from a ZIKV-infected, DENV-naïve donor and used as a probe in a blockade of binding assay.



ZIKV NS1 Blockade of Binding Assay





Balmaseda *et al. PNAS* 2017

Stettler *et al. Science* 2016

Ancient Nanotechnology meets an Emerging Infectious Disease

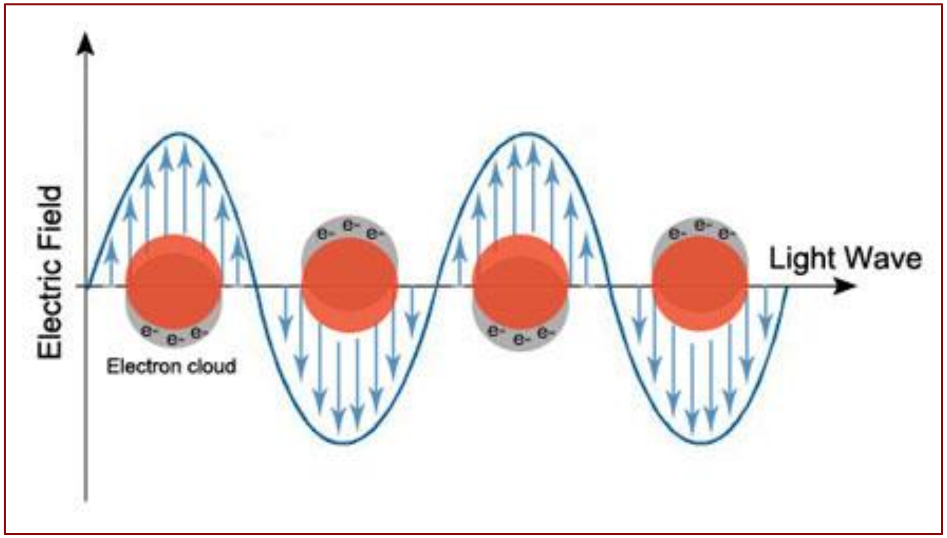
Gold (Au) nanoparticles have been used in art for centuries due to their brilliant color.

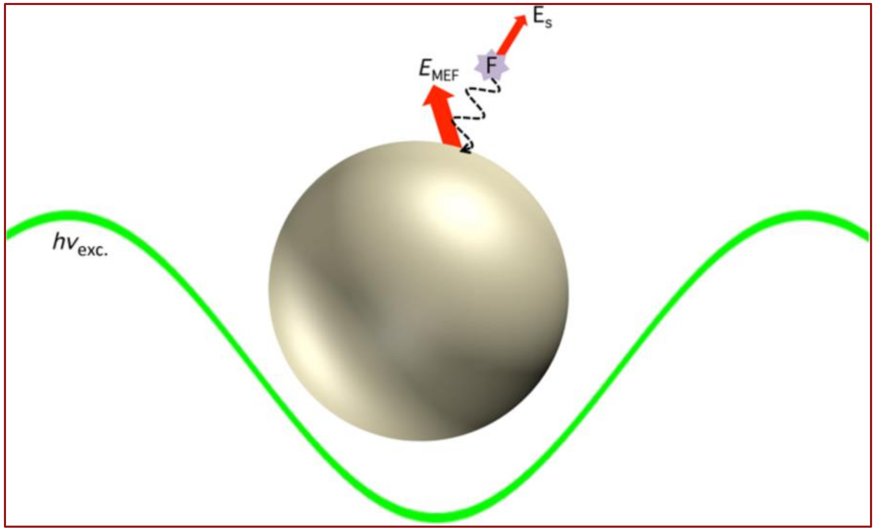
The Lycurgus Cup, 4th Century - The British Museum

Au nanoparticles have been used for decades in rapid antigen and antibody assays

Gold Nanoparticles undergo Plasmonic Resonance

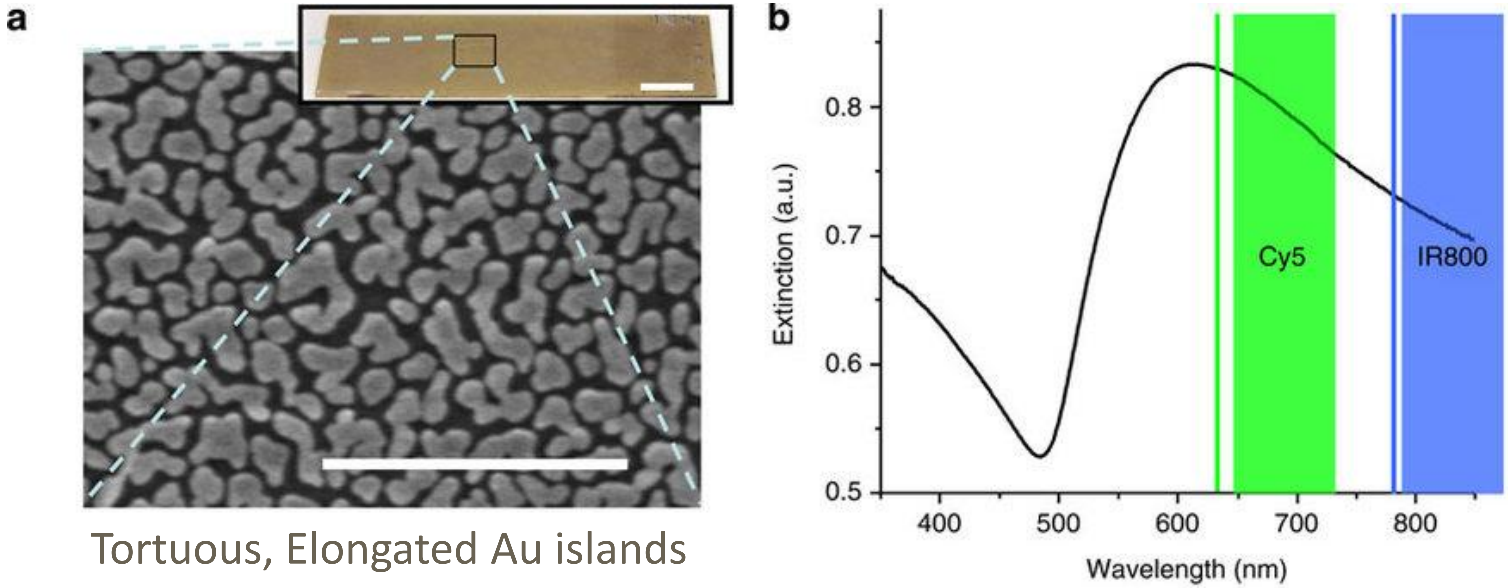
- The field of plasmonics represents the study of the interaction between light and conduction electrons of a metal.
- Plasmonic resonance is the collective oscillation of free electrons.
- Metal enhanced fluorescence can be used for analyte detection.



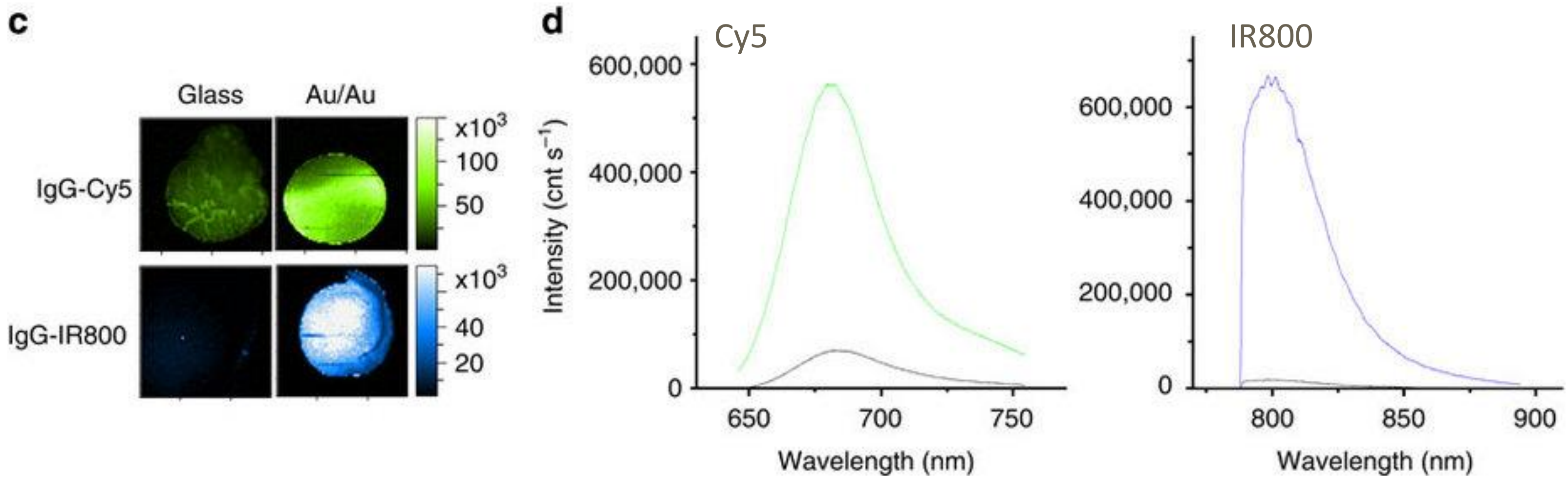


Strobbia et al., Opt. Eng. 2015

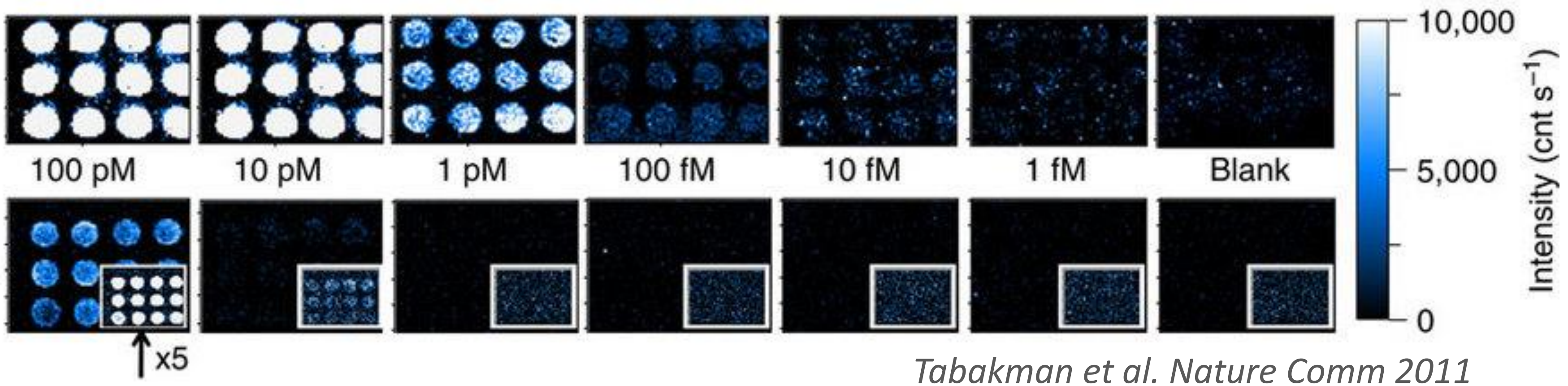
Near-Infrared Fluorescence Enhanced (NIR-FE) Imaging on Plasmonic Gold



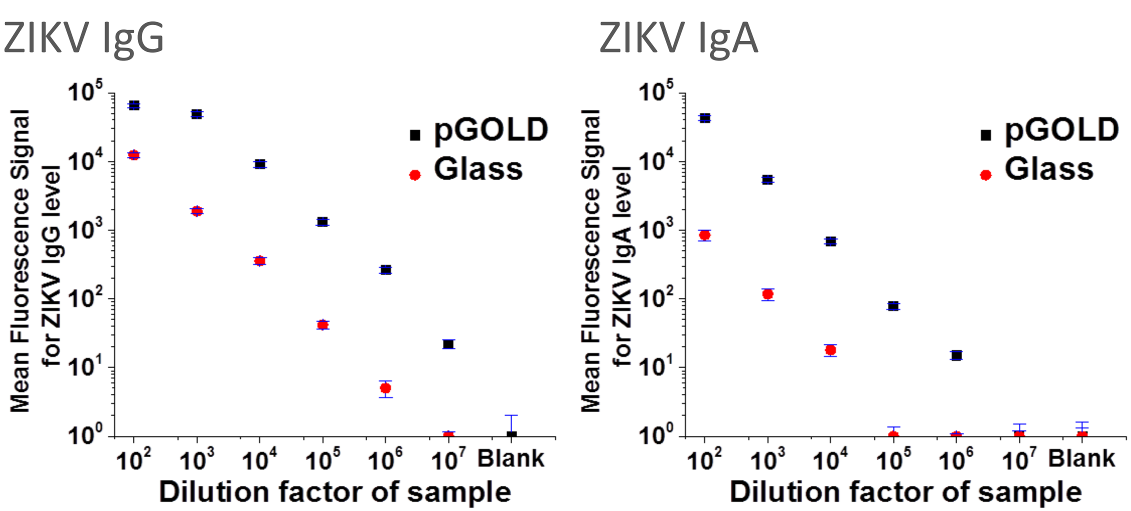
Tortuous, Elongated Au islands



Near-Infrared Fluorescence Enhanced (NIR-FE) Imaging on Plasmonic Gold continued…

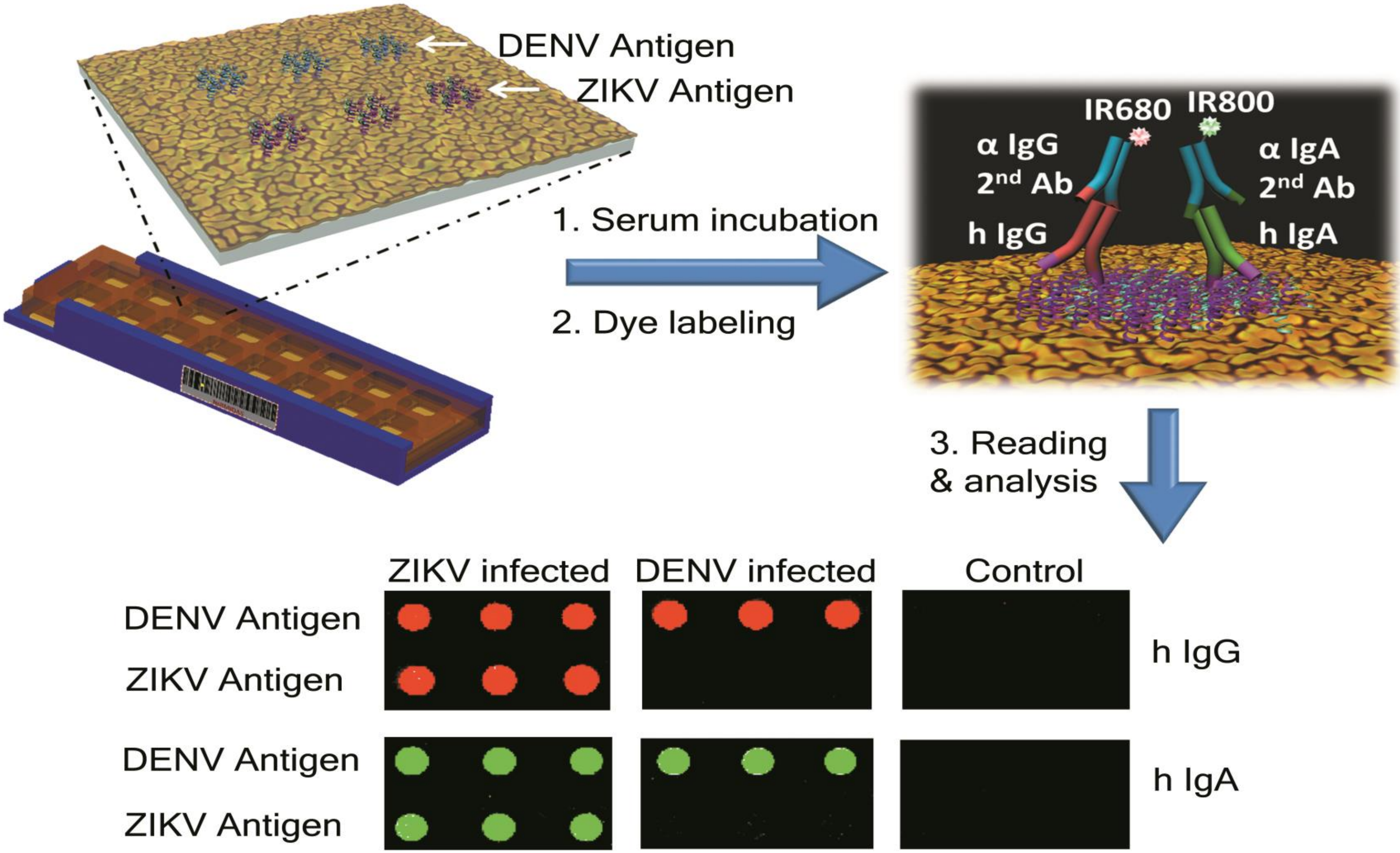


Tabakman et al. Nature Comm 2011



Zhang and Pinsky et al. Nature Med 2017

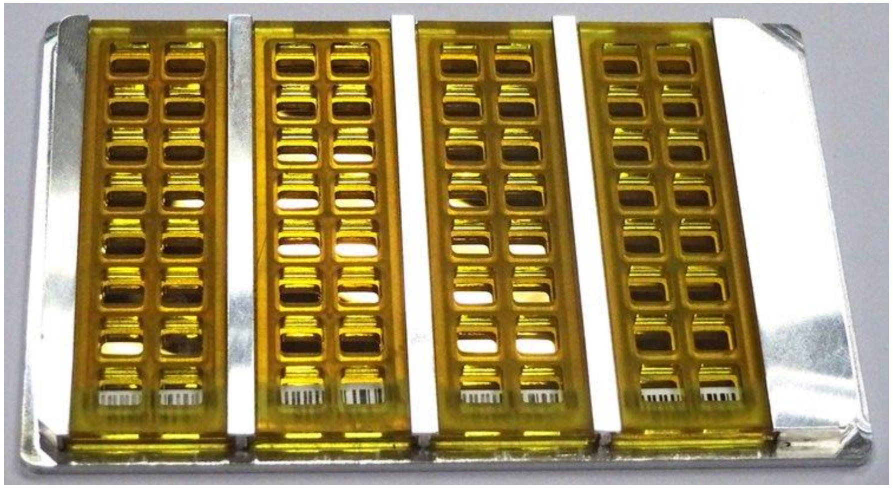
Nanoscale Plasmonic-Gold (pGOLD) Platform for Antibody Detection



Zhang and Pinsky et al. Nature Med 2017

ZIKV/DENV pGOLD Tests

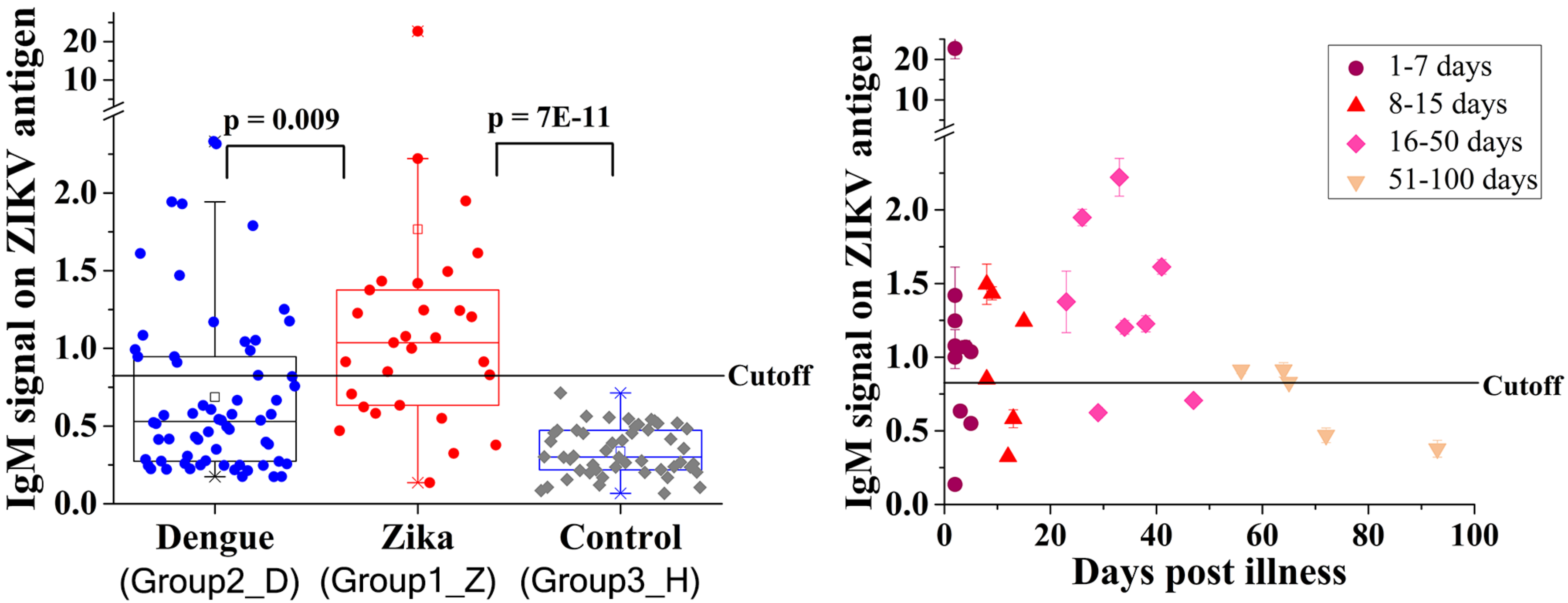
- Antigens:
  - ZIKV NS1
  - DENV-2 whole virus
  - Up to 12 antigens per well
- Specimen Volume: <5 µL serum
- 16 wells per slide
- ~2 hour turnaround time



- Slides read on MidaScan-IR
  - Dual channel (700 nm and 800 nm) near-infrared (NIR) confocal microscope scanner

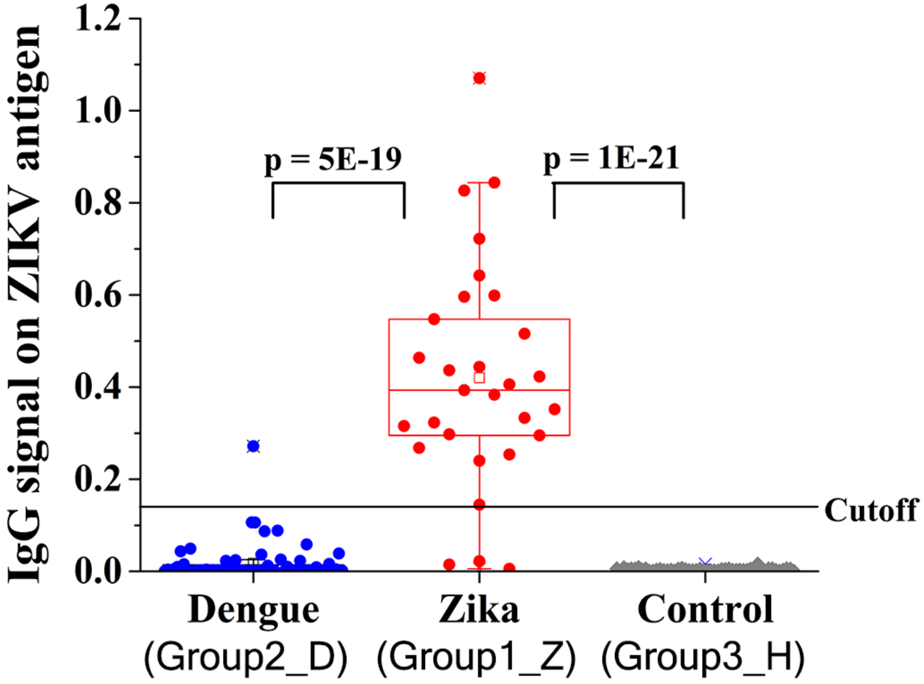
Images Courtesy of Meijie Tang, Nirmidas Biotech, Inc.

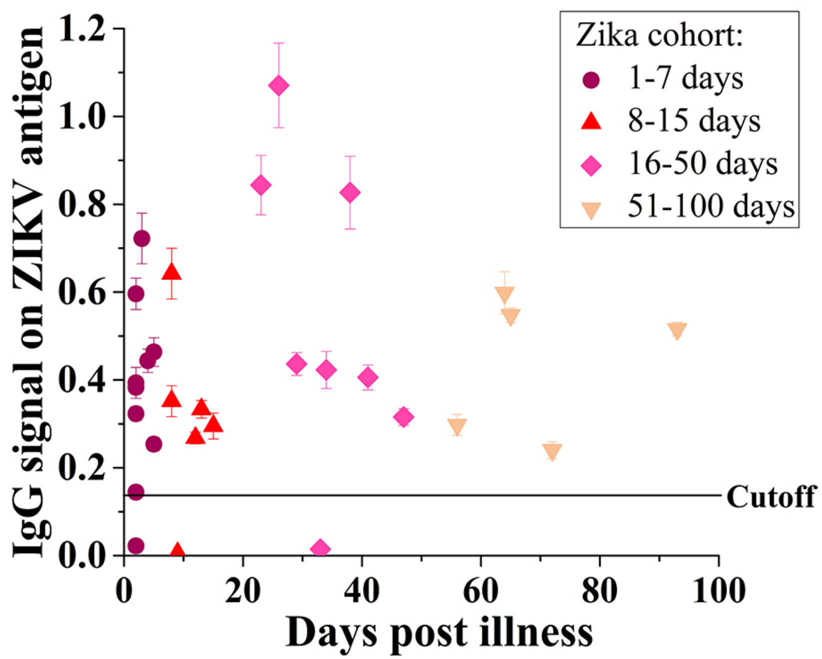
pGOLD IgM Assays Do Not Distinguish Between ZIKV and DENV



Zhang and Pinsky et al., Nature Med 2017

pGOLD ZIKV IgG distinguishes ZIKV from DENV infection





Zhang and Pinsky et al. Nature Med 2017

Diagnosis of the TORCH Infections

Toxoplasma

Others (Syphilis, Parvovirus B19, HBV, HIV)

Rubella

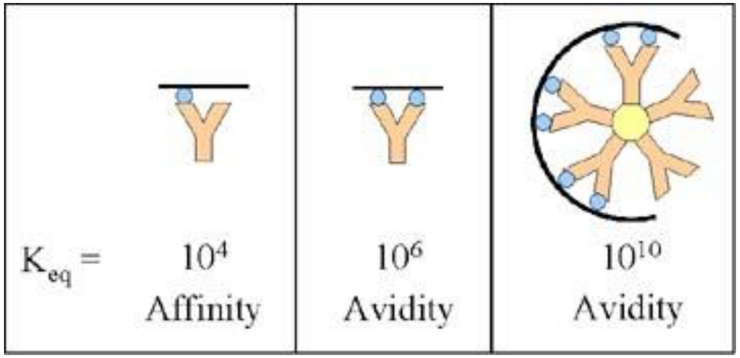
Cytomegalovirus

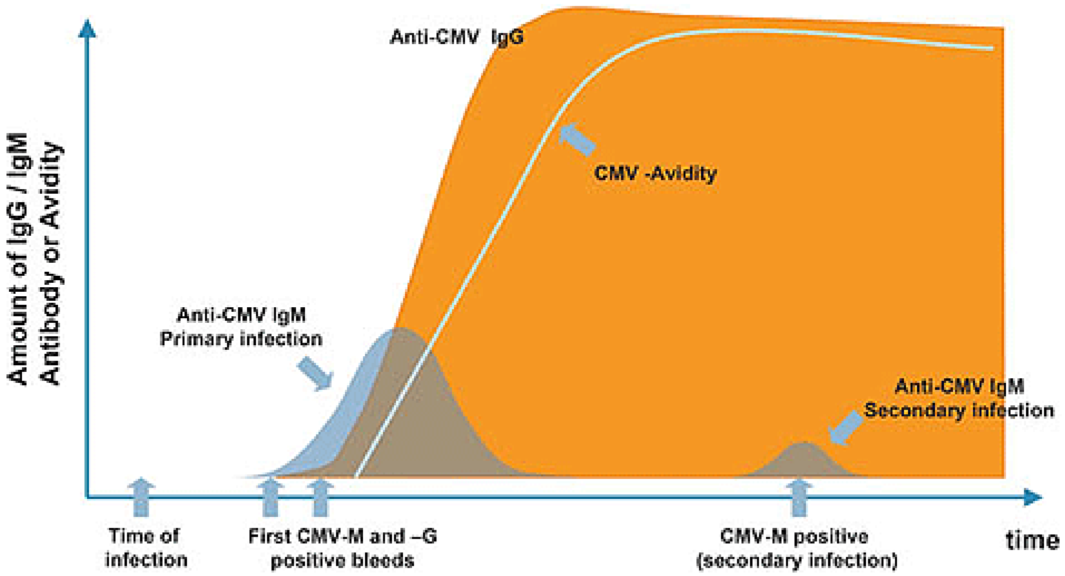
Herpes Simplex Virus

Maternal Evaluation of the Timing of Exposure

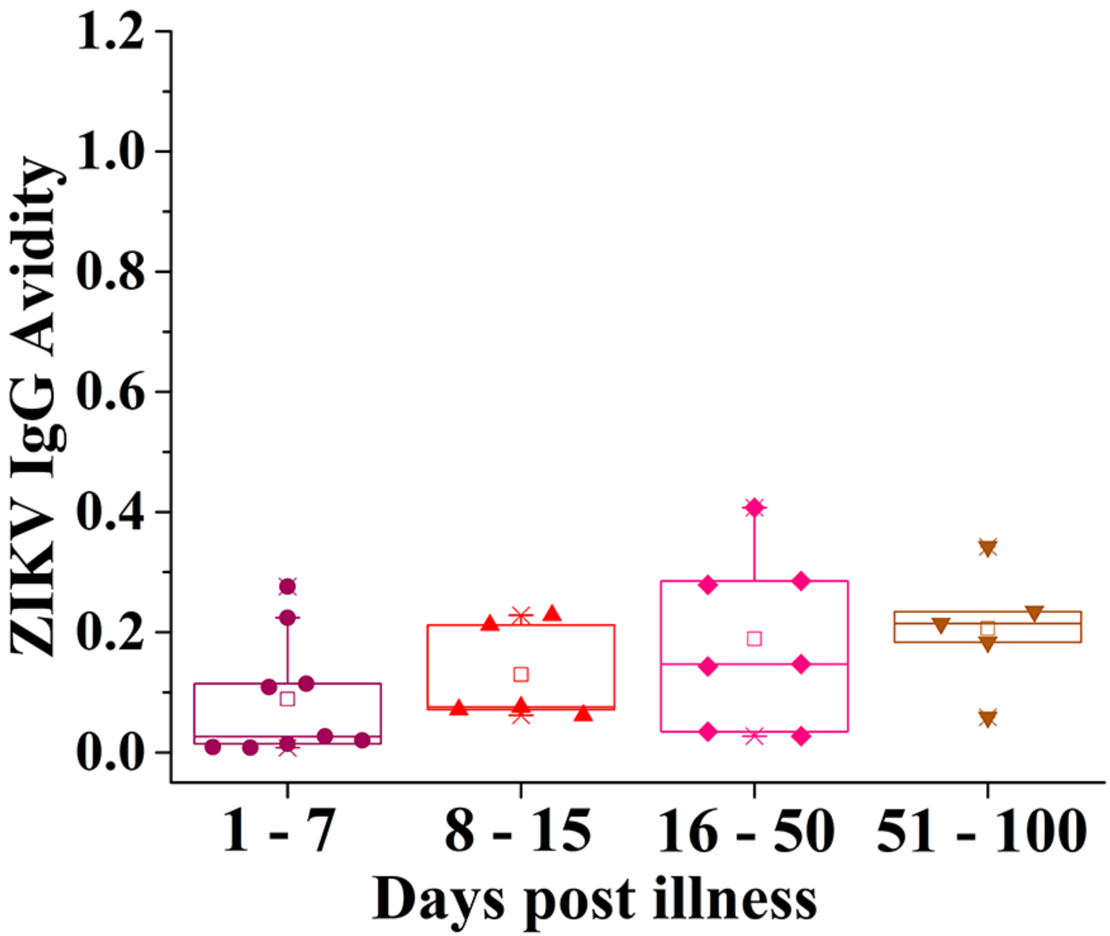
IgG Avidity can be used to estimate the Timing of Exposure

- Avidity takes into account the presence of multiple Ag binding sites per Ab
- As B-cells undergo affinity maturation, antibody avidity increases
- Anticipate using ZIKV IgG + avidity to rule out exposure during pregnancy

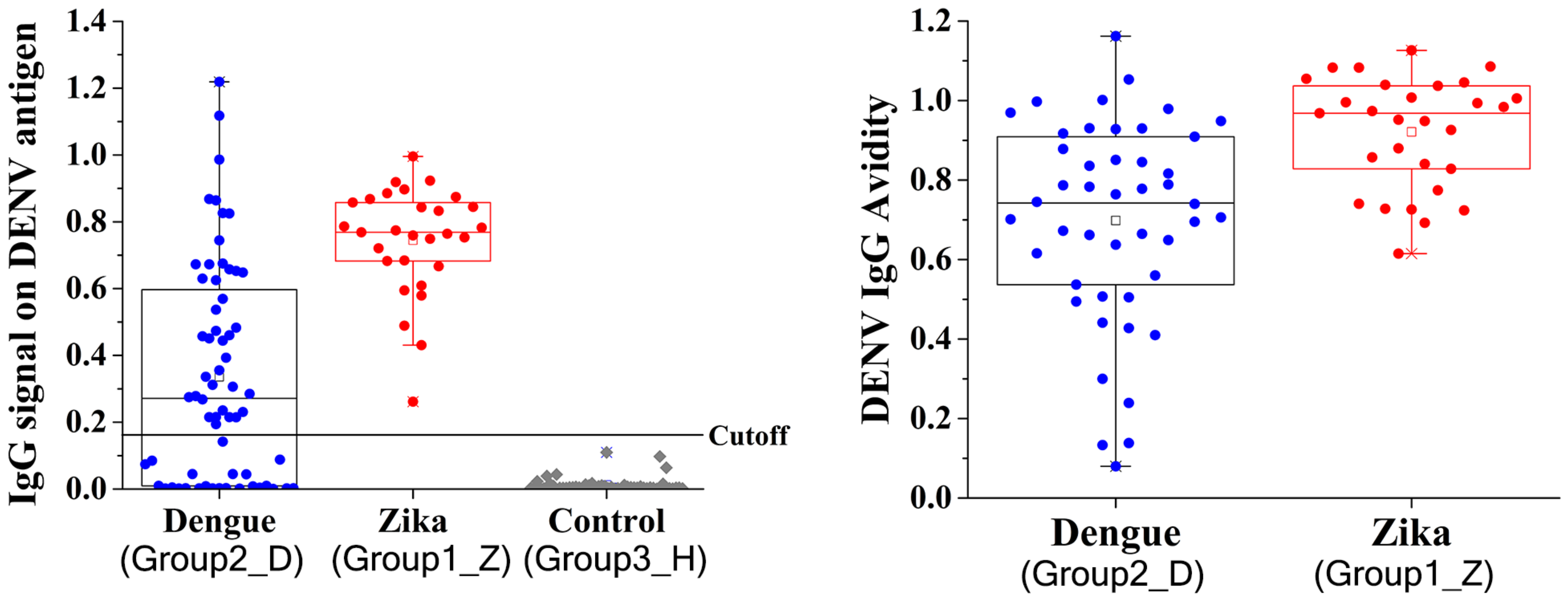




pGOLD ZIKV IgG Avidity is consistent with recent infection



DENV pGOLD Avidity Detection reveals ZIKV infected patients were DENV experienced



Summary – pGOLD ZIKV/DENV

- Multiplexed assay on nanostructured plasmonic gold to detect and distinguish IgG antibodies from patients infected with ZIKV and DENV.

- IgG Avidity may be useful to determine the timing of exposure, particularly in pregnancy.

- Potential for multiplexing
  - Detection of a panel of flavivirus/alphavirus antibodies
    - ZIKV/CHIKV/DENV/YFV/JEV/WNV/TBEVMAYV
  - Development of a 1st trimester maternal screening panel
    - IgG/IgG Avidity/ (IgM):ZIKV, Toxoplasma, CMV, (DENV)
    - IgG: Rubella, *T. pallidum*, Varicella
    - Antigen: HBV surface Ag, ZIKV Ag, (DENV Ag)

Government Accountability Office Report on the Zika Virus Response

Emerging Infectious Diseases: Actions Needed to Address the Challenges of Responding to Zika Virus Disease Outbreaks.

GAO-17-445, May 23

Report: http://www.gao.gov/products/GAO-17-445

Highlights: http://www.gao.gov/assets/690/684836.pdf

Podcast: http://www.gao.gov/multimedia/podcasts/684715

TESTIMONIES

Emerging Infectious Diseases: Actions Needed to Ensure Improved Response to Zika Virus Disease Outbreaks.

GAO-17-612T, May 23

Testimony: http://www.gao.gov/products/GAO-17-612T

Highlights: http://www.gao.gov/assets/690/684839.pdf

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Thank You!

Questions? Comments?