ZIKA VIRUS DIAGNOSTICS: CURRENT TESTING APPROACHES AND CHALLENGES

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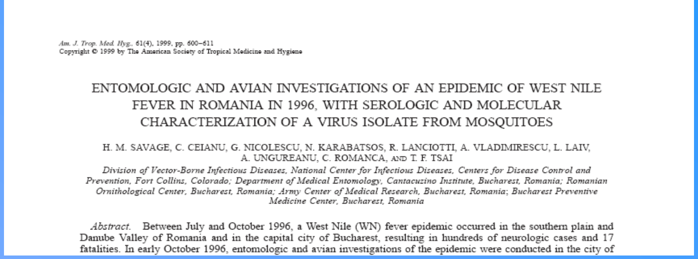
Global Arbovirus Movement

- West Nile virus: 1999 NYC



Am. J. Trop. Med. Hyg., 61(4), 1999, pp. 600–611

ENTOMOLOGIC AND AVIAN INVESTIGATIONS OF AN EPIDEMIC OF WEST NILE FEVER IN ROMANIA IN 1996, WITH SEROLOGIC AND MOLECULAR CHARACTERIZATION OF A VIRUS ISOLATE FROM MOSQUITOES

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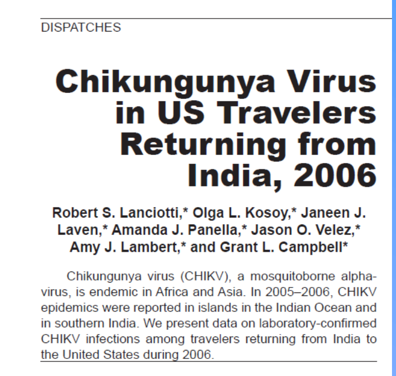
Abstract. Between July and October 1996, a West Nile (WN) fever epidemic occurred in the southern plain and Danube Valley of Romania and in the capital city of Bucharest, resulting in hundreds of neurologic cases and 17 fatalities. In early October 1996, entomologic and avian investigations of the epidemic were conducted in the city of

- Chikungunya 2005-2006: East Africa to India; then Western Hemisphere 2013



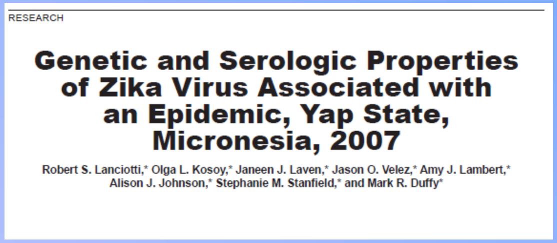
DISPATCHES

Chikungunya Virus in US Travelers Returning from India, 2006

Robert S. Lanciotti,* Olga L. Kosoy,* Janeen J. Laven,* Amanda J. Panella,* Jason O. Velez,* Amy J. Lambert,* and Grant L. Campbell*

Chikungunya virus (CHIKV), a mosquitoborne alphavirus, is endemic in Africa and Asia. In 2005–2006, CHIKV epidemics were reported in islands in the Indian Ocean and in southern India. We present data on laboratory-confirmed CHIKV infections among travelers returning from India to the United States during 2006.

- Zika Virus 2007: Micronesia; then Western Hemisphere 2015



RESEARCH

Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007

Robert S. Lanciotti,* Olga L. Kosoy,* Janeen J. Laven,* Jason O. Velez,* Amy J. Lambert,* Alison J. Johnson,* Stephanie M. Stanfield,* and Mark R. Duffy*

Zika Epidemic Yap State Federated States of Micronesia; 2007

- ≈75% residents infected (7,000 total residents)
- ≈ 19% reported symptoms
- Zika virus complete genome derived
- Serological diagnostics developed
- PCR diagnostics developed

RESEARCH

Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007

Robert S. Lanciotti,* Olga L. Kosoy,* Janeen J. Laven,* Jason O. Velez,* Amy J. Lambert,* Alison J. Johnson,* Stephanie M. Stanfield,* and Mark R. Duffy*

Zika virus (ZIKV) is a mosquito-borne flavivirus first isolated in Uganda from a sentinel monkey in 1947. Mosquito and sentinel animal surveillance studies have demonstrated that ZIKV is endemic to Africa and Southeast Asia, yet reported human cases are rare with <10 cases reported in the literature. In June 2007, an epidemic of fever and rash associated with ZIKV was detected in Yap State, Federated States of Micronesia. We report the genetic and serologic properties of the ZIKV associated with this epidemic.

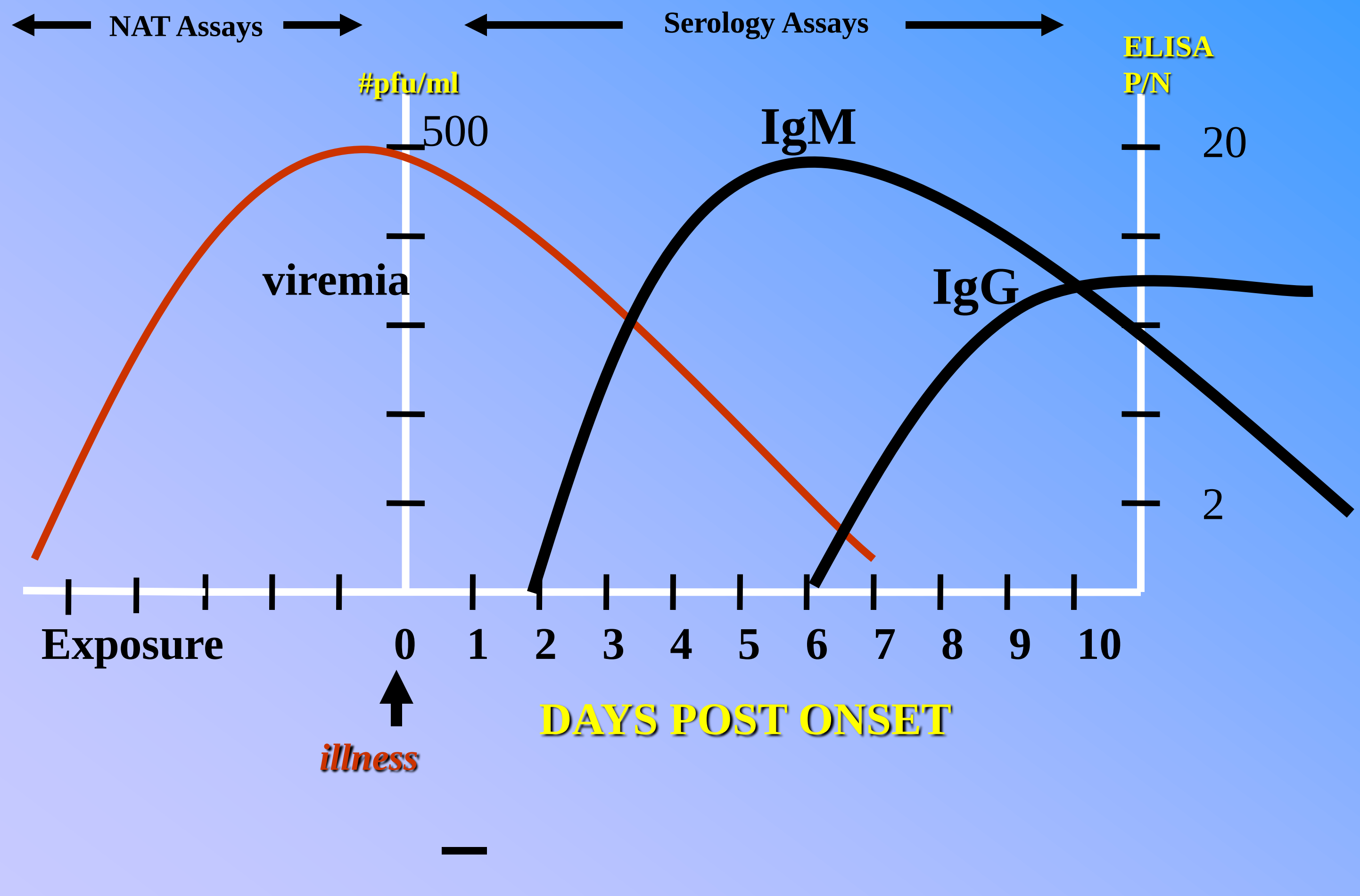
studies in the absence of epidemics (*6–8*). Additional serologic studies in the 1950s and 1960s detected ZIKV infections among humans in Egypt, Nigeria, Uganda, India, Malaysia, Indonesia, Pakistan, Thailand, North Vietnam, and the Philippines (*5*). These data strongly suggest widespread occurence of ZIKV from Africa to Southeast Asia west and north of the Wallace line.

In 1977, ZIKV infection was confirmed among 7 patients in central Java, Indonesia, during an acute fever study (*9*). Data on these 7 ZIKV cases and several previously re-

Table 1. Clinical Characteristics of 31 Patients with Confirmed Zika Virus Disease on Yap Island during the Period from April through July 2007.

Sign or Symptom	No. of Patients (%)
Macular or papular rash	28 (90)
Fever*	20 (65)
Arthritis or arthralgia	20 (65)
Nonpurulent conjunctivitis	17 (55)
Myalgia	15 (48)
Headache	14 (45)
Retro-orbital pain	12 (39)
Edema	6 (19)
Vomiting	3 (10)

Human Viremia & Immune Response

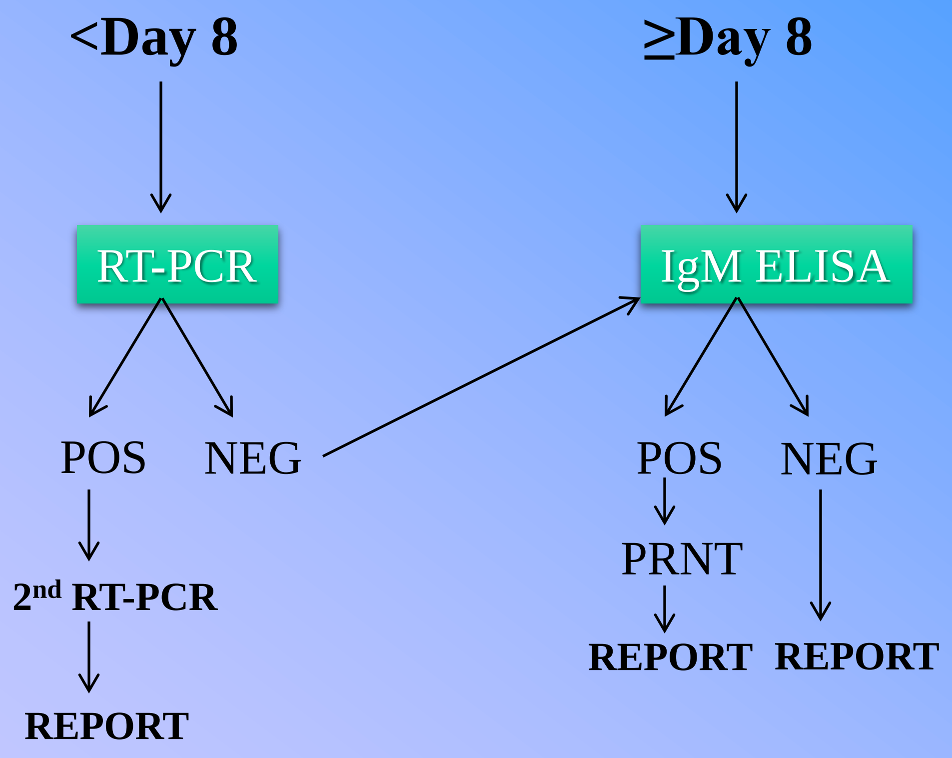


Zika Virus: Available Diagnostic Tests

- Nucleic acid testing (NAT)
  - Real-time RT-PCR

- Antibody testing
  - IgM ELISA
  - Plaque reduction neutralization test (PRNT)

General Arbovirus Testing Algorithm



Zika Testing by RT-PCR in Serum

Day Post-Onset	RT-PCR Zika	Est. copies/ml
104004(D3)	POS	1850
104014(D4)	POS	5800
103940(D2)	POS	4000
104121(D2)	POS	930
104139(D1)	POS	4320
104138(D1)	POS	1850
104218(D2)	POS	8570
104234(D0)	POS	92100
104004(D7)	POS	2000

94 PCR POS (approximately 2000 tested)

Positive Range: Day 0 to Day 7*

Viral Load: 200,000 to 500 copies/ml
(Ct average=35.4)

Average: 4000 copies/ml

62% of RT-PCR positive serum specimens are IgM positive

***onset dates are often estimates**

Real Time RT-PCR for Zika Virus Serum versus Urine

Sample	RT-PCR serum	RT-PCR urine
103922 (D4)	POS (1.5 x 10^4 copies/ml)	POS (5.7 x10^7 copies/ml)
104072 (D3)	NEG	POS (9.3 x 10^3 copies/ml)
109656 (D3)	POS (6.8 x 10^4 copies/ml)	NEG

28 total serum/urine pairs

- 9 (32%) positive both
- 15 (54%) positive urine/negative serum
- 4 (14%) positive serum/negative urine
- viremia higher in urine: 185,000 cp/ml (Ct 30.7)

CONCLUSION: Both sample types should be tested

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Zika Virus Testing Considerations: Lessons Learned from the First 80 Real-Time Reverse Transcription-PCR-Positive Cases Diagnosed in New York State

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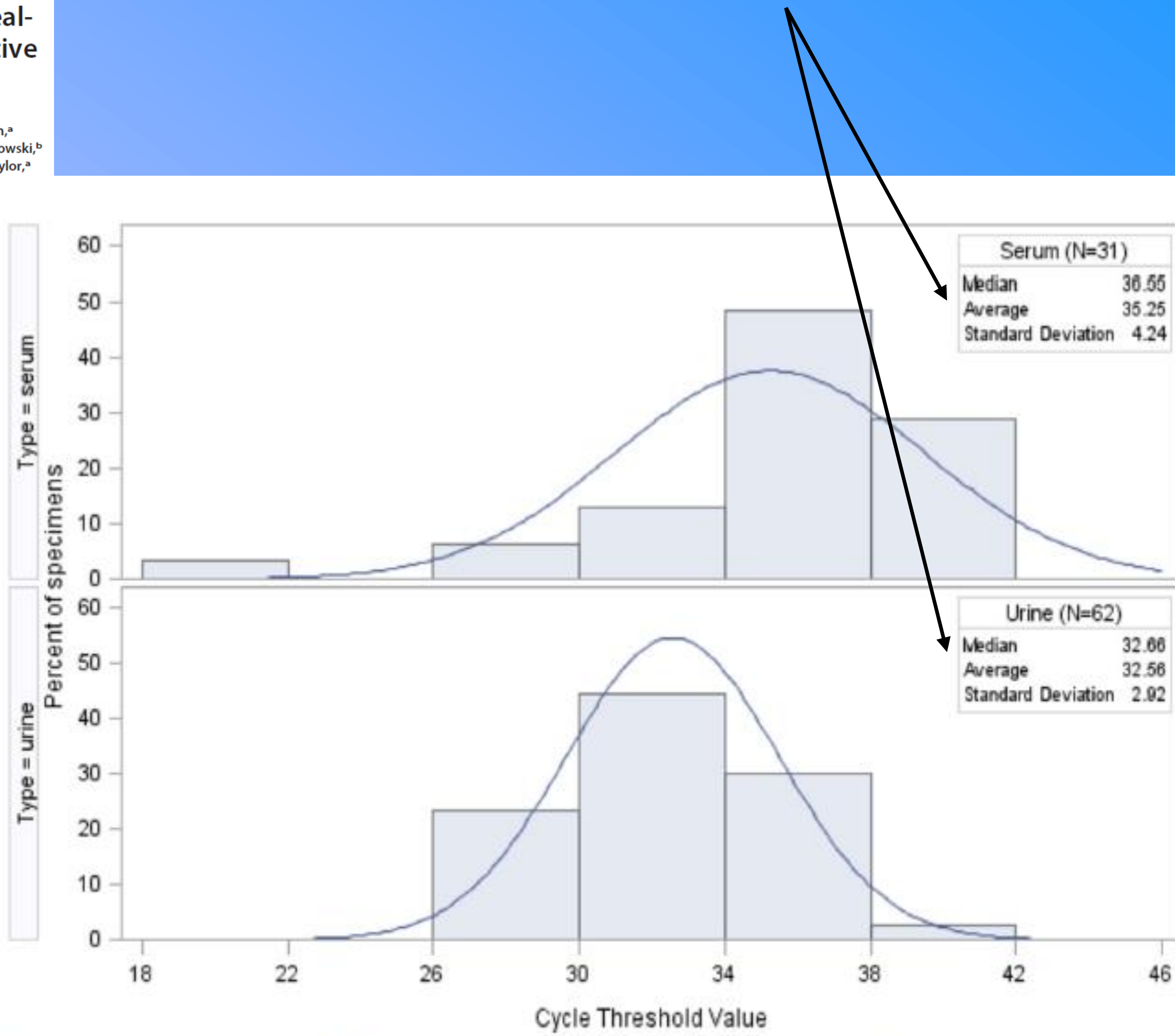

FIG 2 Distribution of cycle threshold values in positive specimens of serum (n = 31) and urine (n = 62) tested in the envelope rRT-PCR assay for Zika virus RNA.

Combimed RT-PCR & IgM Testing

135 acute serum specimens (day 0– day 7 post-onset)

- 13 (10%) positive by RT-PCR only
- 71 (52%) positive by IgM only
- 51 (38%) positive by both assays

Detection of Zika virus RNA in whole blood of imported Zika virus disease cases up to 2 months after symptom onset, Israel, December 2015 to April 2016

Y Lustig [1], E Mendelson [1,2], N Paran [3], S Melamed [3], E Schwartz [4]
1. Central Virology Laboratory, Ministry of Health, Tel-Hashomer, Israel

Zika virus RNA presence in serum, whole-blood and urine samples from six Israeli travellers symptomatic for Zika virus disease was examined. Whole-blood samples were positive for as late as 2 months (58 days) post-symptom onset, longer than for urine (26 days) and serum (3 days). These findings suggest the utility of whole blood in Zika infection diagnosis.

Patient number	Probable country of exposure	First set of samples				Second set of samples		
		Serology results (IgM/IgG)[a]	qRT-PCR			qRT-PCR		
			Serum result, days from symptom onset (pfu equivalent/ml)	Urine result, days from symptom onset (pfu equivalent/ml)	WB result, days from symptom onset (pfu equivalent/ml)	Serum result, days from symptom onset (pfu equivalent/ml)	Urine result, days from symptom onset (pfu equivalent/ml)	WB result, days from symptom onset (pfu equivalent/ml)
1	Colombia	ND	Pos 3, (496)	ND	ND	ND	ND	ND
2	Colombia	Pos/Pos	Neg, 5 (NA)	Pos, 5 (16)	Pos, 5 (88)	Neg, 120 (NA)	Neg, 120 (NA)	Neg, 120 (NA)
3	Colombia	Pos/Pos	Neg, 10 (NA)	Pos, 10 (12)	Pos, 34[b] (157)	Neg, 78 (NA)	Neg, 78 (NA)	Neg, 78 (NA)
4	Vietnam	Pos/Neg	Neg, 10 (NA)	Neg, 10 (NA)	Pos, 58[b] (88)	Neg, 79 (NA)	Neg, 79 (NA)	Neg, 79 (NA)
5	Dominican Republic	Pos/Pos	Neg, 26 (NA)	Pos, 26 (20)	Pos, 26 (47)	Neg, 46 (NA)	Neg, 46 (NA)	Pos, 46 (29)
6	Mexico	Neg/Neg	Neg, 26 (NA)	Neg, 26 (NA)	Pos, 26 (496)	Neg, 48 (NA)	Neg, 48 (NA)	Neg, 48 (NA)

Summary & Future Directions for NAT Testing

- RT-PCR testing has limited utility for testing patient population in USA (travelers)
- Serum & urine testing should both be performed
- RT-PCR negative samples should be tested by IgM ELISA
- Whole blood testing?

Zika: Available NAT Tests

- Abbott RealTime Zika assay (Abbott Molecular)
- Zika ELITe MGB® Kit (ELITechGroup Inc.)
- Trioplex Real-time RT-PCR Assay (CDC)
- Zika Virus Detection by RT-PCR Test (ARUP Laboratories)
- *Sentosa*® SA ZIKV RT-PCR Test (Vela Diagnostics USA, Inc.)
- LightMix® Zika rRT-PCR Test (Roche Molecular Systems, Inc.)
- xMAP® MultiFLEX™ Zika RNA Assay (Luminex Corporation)
- VERSANT® Zika RNA 1.0 Assay (kPCR) Kit (Siemens Healthcare Diagnostics Inc .)
- Zika Virus Real-time RT-PCR Test (Viracor-IBT Laboratories, Inc.)
- Aptima® Zika Virus Assay (Hologic, Inc.)
- RealStar® Zika Virus RT-PCR Kit U.S. (Altona Diagnostics)
- Zika Virus RNA Qualitative Real-Time RT-PCR (Focus Diagnostics)

  - Blood Screening NAT: <5 copies/ml
  - Diagnostic NAT: 35-400 copies/ml

Flavivirus Serology: Welcome to the Hall of Mirrors

Karl Johnson: "Flaviviruses are an antigenic hall of mirrors."

JOURNAL OF VIROLOGY, Jan. 2011, p. 410–421 Vol. 85, No. 1
0022-538X/11/$12.00 doi:10.1128/JVI.01826-10

An In-Depth Analysis of Original Antigenic Sin in Dengue Virus Infection[▽]

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"Dengue seems to be an extreme example of original antigenic sin, where the secondary response is entirely constructed from antibody that cross-reacts with previously encountered virus."

Am. J. Trop. Med. Hyg., 32(1), 1983, pp. 154–156

ORIGINAL ANTIGENIC SIN IN DENGUE

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Abstract. Sequential blood samples were obtained from eight Thai children before, during and 3–5 months after hospitalization for dengue shock syndrome. All patients experienced a secondary-type antibody response as evidenced by hemagglutination-inhibition antibody responses in acute and convalescent sera. Dengue 2 viruses were recovered from two patients. In their pre-illness blood sample, all children had monotypic neutralizing antibodies; five to dengue 1, two to dengue 3 and one to dengue 4. The highest neutralizing antibody titers in acute phase and late convalescent sera were to the initial infecting virus type. This report documents for sequential dengue infections the existence of an original antigenic sin antibody response. It may be possible to apply this phenomenon to identify initial dengue serotype infection in individuals experiencing secondary dengue infections, thus helping to clarify the antecedents to dengue shock syndrome.

The Doctrine of Original Antigenic Sin was described and named by Francis and co-workers in the influenza hemagglutination-inhibition (HI) antibody test system.[1–3] Infection in previously im-

yong, Thailand (population 52,329). Between January and April 1980, finger-tip blood samples were collected on filter paper strips from 4,136 children, ages <1 through 14 years. During 1980,

"Highest PRNT titers after the second infections correspond
to the serotypes in the first infections"

Zika Virus Serology Testing: The Problems

- **Cross-Reactivity**. Humoral immune response generates antibodies (IgM & IgG) that predominately react with cross-reactive epitopes on the E glycoprotein.

- **Original Antigenic Sin**. In a second flavivirus infection, the antibodies generated are primarily directed against the E protein of the primary flavirus infection

- **Antibody Dependent Enhancement.** Non-neutralizing antibodies from the first dengue infection bind to a heterotypic dengue virus and ***enhance*** infection of cells via Fc receptors.

Zika Virus Serology: Available Tests

- IgM Capture ELISA (envelope protein)
  - CDC IgM capture ELISA
  - ZIKV Detect™ IgM Capture ELISA (InBios)

- Plaque Reduction Neutralization Test

Data From Zika Epidemic 2015-2016
Primary Zika Infection

Patient ID (Day Post-Onset)	IgM ELISA Zika	IgM ELISA DEN	PRNT-90% Zika	PRNT-90% DEN-1	PRNT-90% DEN-2
726(D17)	POS	NEG	1:10,240	NEG	NEG
698 (D6)	POS	POS	1:20480	NEG	NEG

In a primary Zika infection:
28% samples cross-react in the Zika IgM ELISA (n≈250)

CONCLUSION: Primary Zika infections cross-react in the IgM ELISA yet can be diagnosed accurately by utilizing the PRNT.

Data From Zika Epidemic 2015-2016
Secondary Infection

Patient ID (Day Post-Onset)	IgM ELISA Zika	IgM ELISA DEN	PRNT-90% Zika	PRNT-90% DEN-1	PRNT-90% DEN-2
675(D5)**	POS	NEG	1:160	1:5120	1:80
004(D7)**	POS	POS	1:160	1:5120	1:320

** Zika PCR POS

In a secondary flavivirus (Zika) infection:
53% samples cross-react in the Zika IgM ELISA (n≈500)

CONCLUSION: In secondary flavivirus infections, IgM and PRNT testing is unable to determine the recently infecting virus.

Data From Zika Yap Epidemic 2007

Primary

Secondary

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

Patient	Days after onset	PRNT$_{90}$ 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).

Testing of Neonates

Patient ID (Age, Days)	IgM ELISA Zika	IgM ELISA DEN	PRNT-90% Zika	PRNT-90% DEN-1	PRNT-90% DEN-2
053 (1)	NEG	NEG	1:1280	NEG	NEG
182 (9)	NEG	NEG	1:2560	NEG	NEG
934 (20)	NEG	NEG	1:1280	1:1280	1:80

Rare Exceptions

Patient ID (Day Post-Onset)	IgM ELISA Zika	IgM ELISA DEN	PRNT-90% Zika	PRNT-90% DEN-1	PRNT-90% DEN-2
109(D2)	POS	NEG	NEG	1:80	1:320

- IgM positive to Zika/IgM negative to dengue & PRNT positive to dengue only: 8/567 (1.4%).

How Often Does the PRNT Identify Zika or Dengue Infection?

LOCATION	% IDENTIFIED ZIKA or DEN
USA	44%
USVI	29%
American Samoa	16%
Puerto Rico	15%

CONCLUSION: Identifying the flavivirus infection in flavivirus endemic areas by IgM/PRNT testing is difficult.

Diagnosis of Zika Virus Infection by Serology

- IF: Zika virus is the first infection by a flavivirus:
  - IgM ELISA is fairly specific for Zika
  - Plaque reduction neutralization test shows ≥4-fold higher titer to Zika

- IF: Zika virus is <u>the second</u> or subsequent infection:
  - IgM ELISA is not specific; cross-reactivity with other flaviviruses
  - PRNT shows high titers to many flaviviruses
  - Definitive diagnosis is not possible

Future Directions for Zika Serology Testing

- IgM ELISA-envelope protein
  - Virus specific domain III
  - Cross-reactive epitope removal
  - Removal of cross-reactive antibodies

- IgM ELISA-NS1 protein

- Microsphere Immunoassay
  - Quantitative measure of Ab binding to various antigens should improve specificity


THANKS ! !
HERO
ITSO