



Zika Diagnosis: Challenges and Opportunities

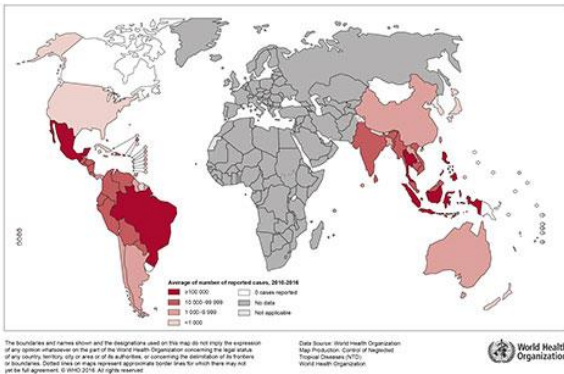
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Zika, dengue and chikungunya

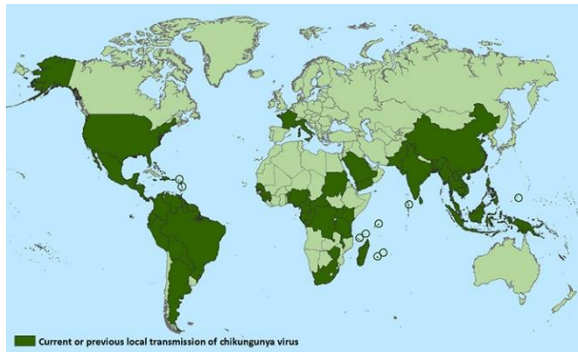


- Three similar diseases
- Transmitted by the same mosquitoes
- Similar geographical distribution (approx. 100 countries)
- 2 billion people at risk of infection every year
- Currently, there are no vaccines or therapeutics commercially available for Zika, dengue and chikungunya virus infections

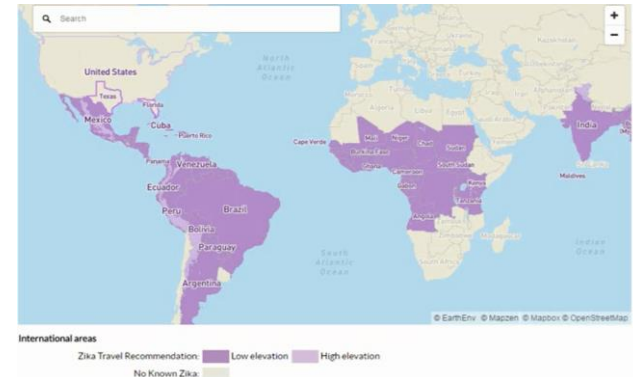
Dengue, 2016



Chikungunya, 2015



Zika, 2017



Zika, dengue and chikungunya

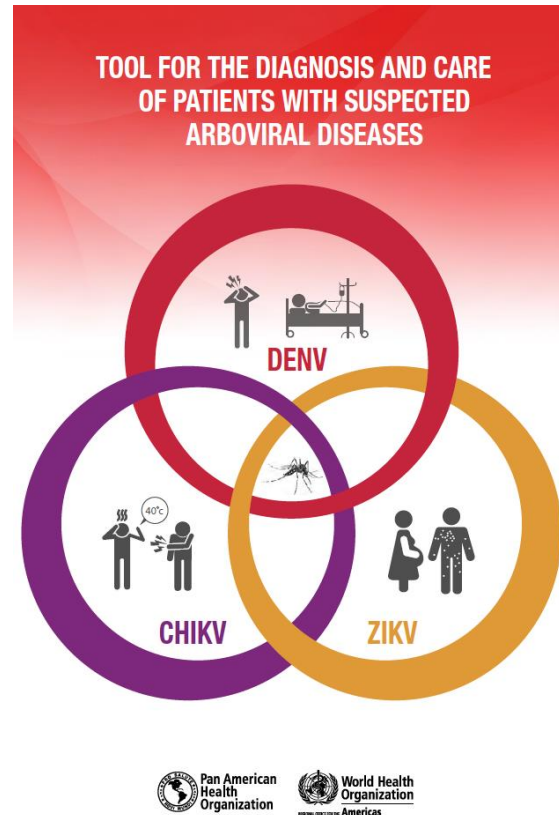
Common Symptoms

- **Febrile Illness**
- **Rash**
- **Body aches**

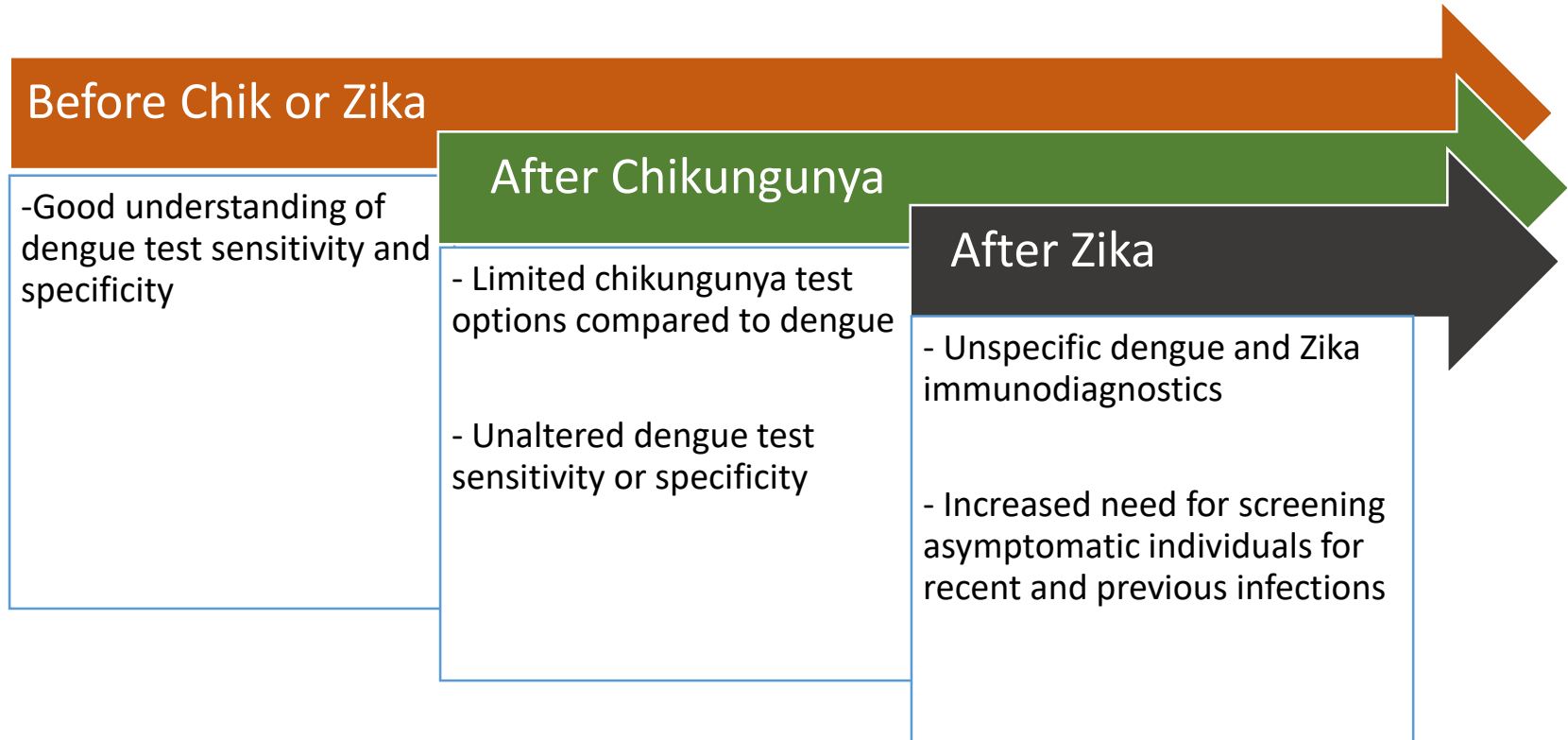
Specific symptoms/risks

- **Dengue: hemorrhagic disease**
- **Chikungunya: severe arthritis**
- **Zika: Guillain Barre' syndrome and birth defects**

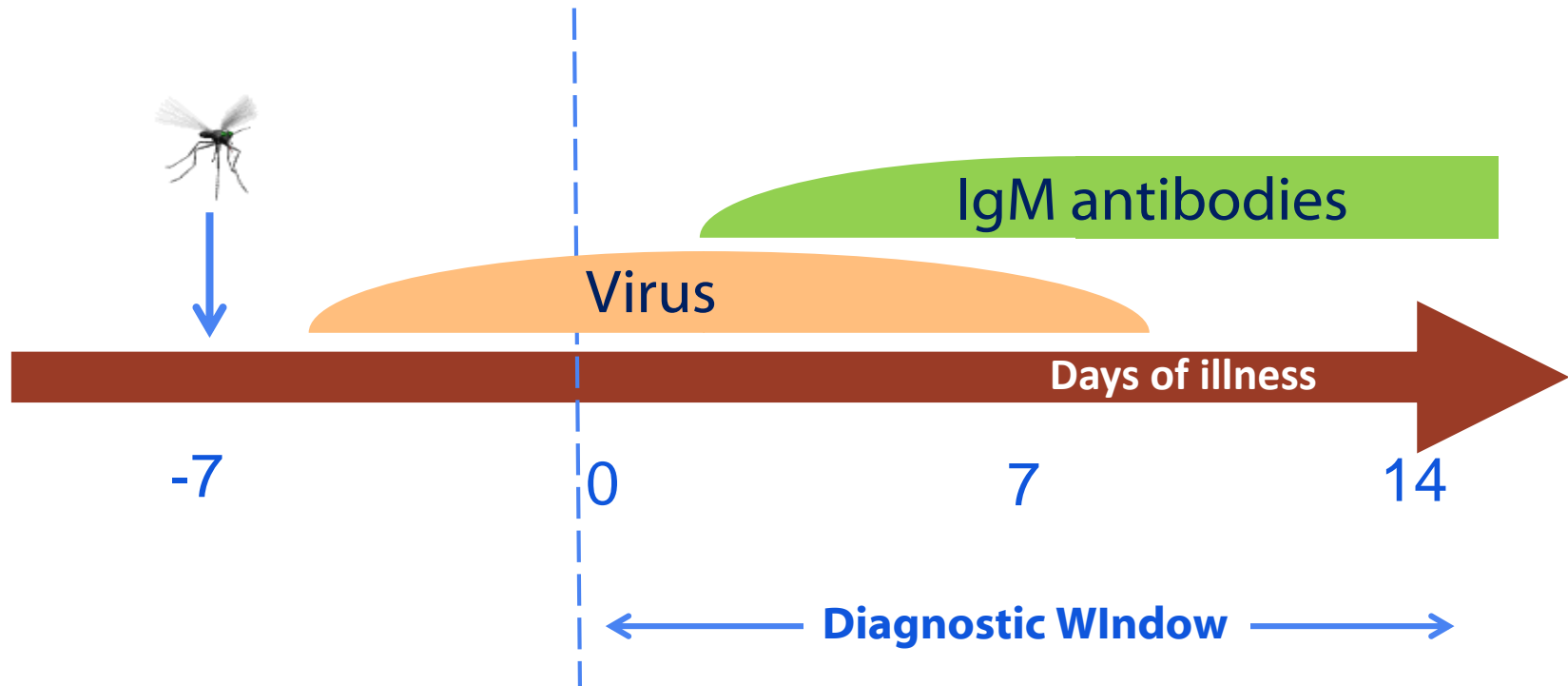
Integrated diagnosis and care of dengue, chikungunya and Zika cases



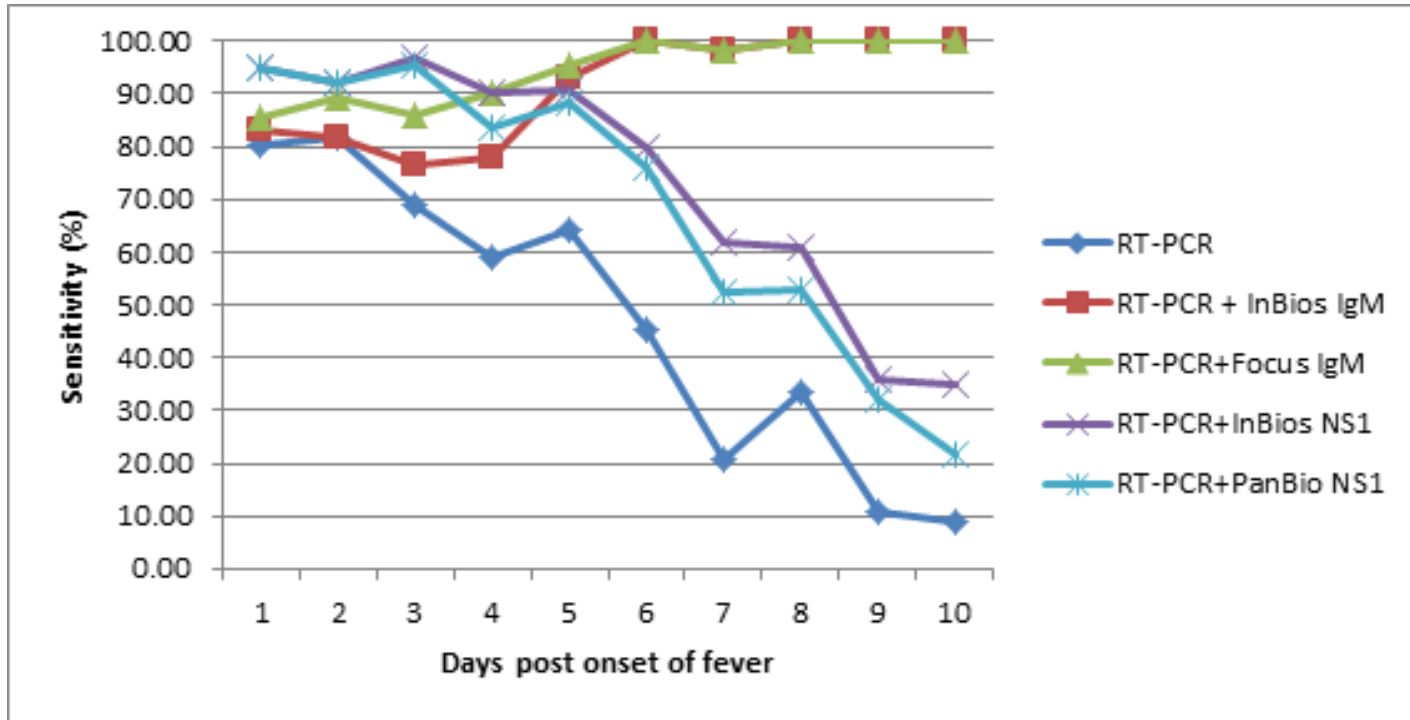
The changing landscape of arbovirus diagnostics



Diagnostic markers in serum during symptomatic infections



Sensitivity of Dengue Diagnostic Tests



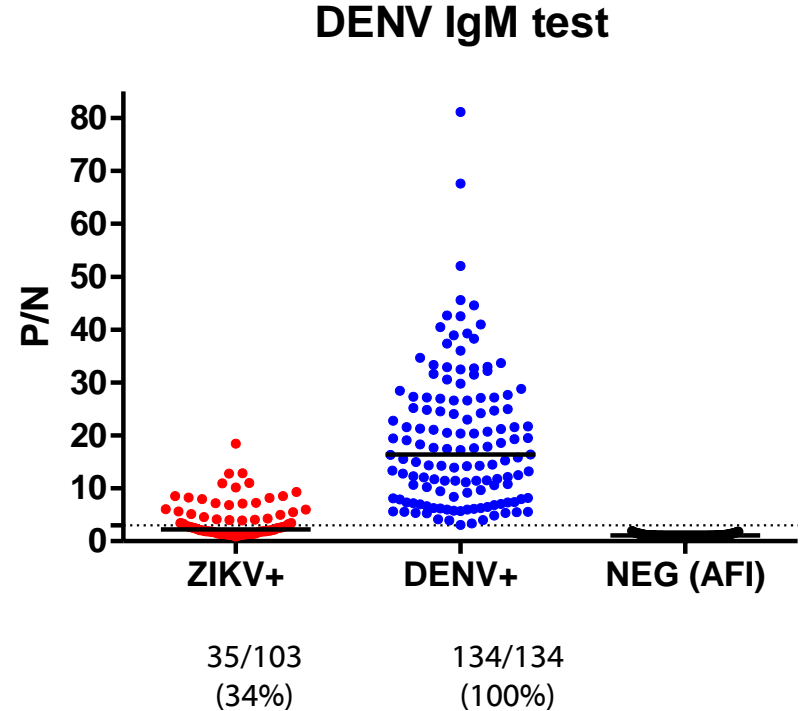
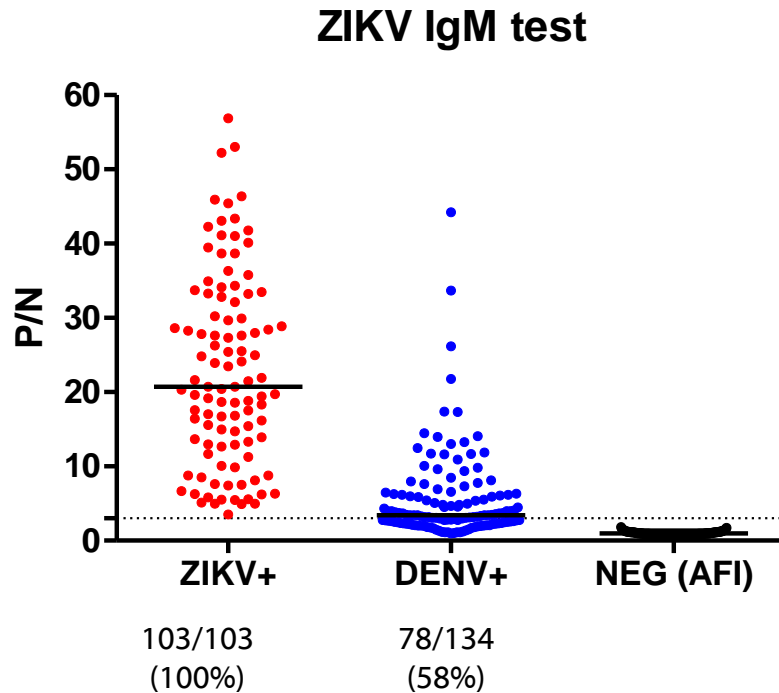
Hunsperger et al, 2016

New Diagnostic Challenges



- Flavivirus serological cross-reaction
 - IgM and IgG cross reactivity, particularly in secondary flavivirus infections and in co-endemic areas
- Large proportion of asymptomatic infections
 - Need for detection of IgG antibodies to determine immune status (previous exposure) due to ZIKV and DENV infections.
 - Screening of pregnant women or women of reproductive age
 - Screening of potential dengue vaccine recipients

Traditional MAC-ELISAs Cannot Differentiate ZIKV vs DENV Infections



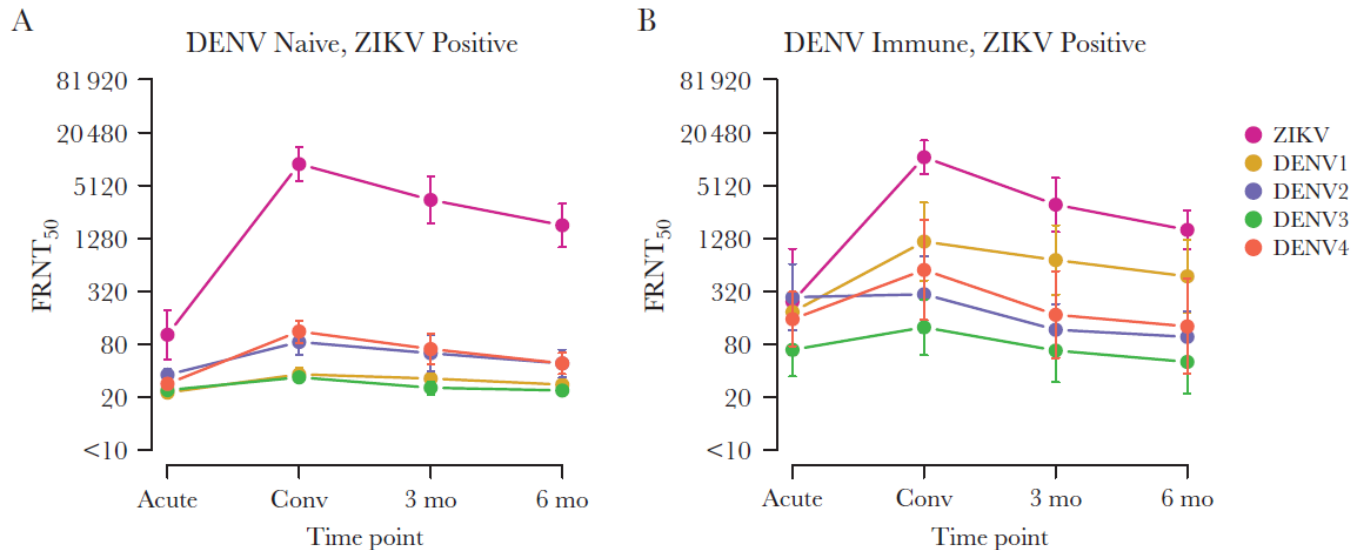
Percent confirmation of Zika IgM positive results by PRNT₍₉₀₎

PRNT ₍₉₀₎ Interpretation	US States (n=759)	USVI (n=52)	American Samoa (n=103)	Puerto Rico (n=123)
ZIKV infection	27	21	15	11
Unspecified flavivirus infection	48	62	83	84
DENV infection	9	8	1	6
Negative	19	10	1	0

Lindsey et al, 2018

Study of symptomatic and asymptomatic infections, including pregnant women

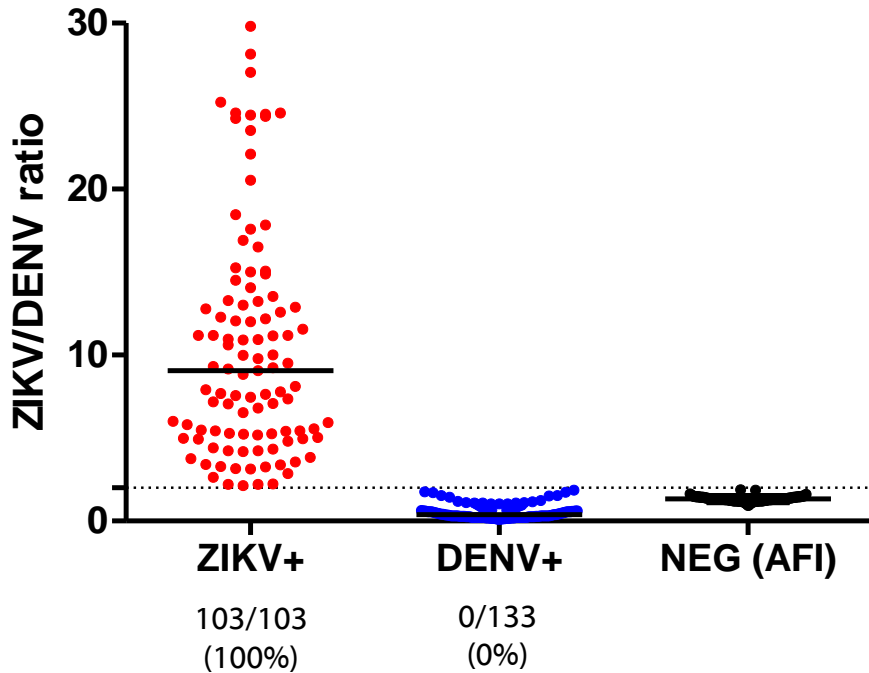
Potential of PRNT₍₅₀₎ to confirm Zika infections in people with or without previous dengue



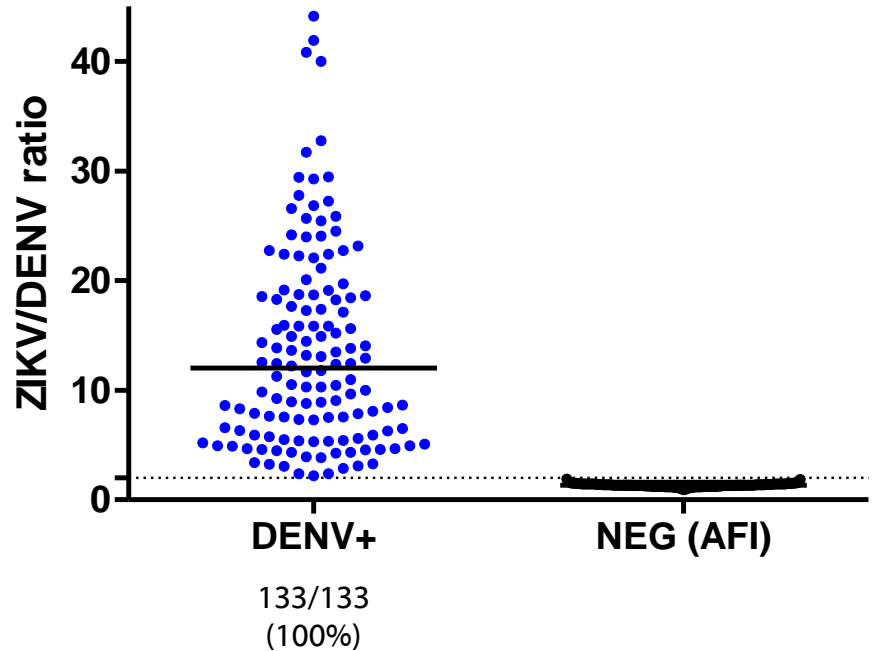
Longitudinal neutralizing antibody responses against dengue virus serotypes 1–4 (DENV1–4) and Zika virus (ZIKV) in samples obtained after ZIKV infection. (Montoya et al, 2018)

The ZIKV/DENV Duo MAC-ELISA can Discriminate ZIKV vs DENV Infections

ZIKV/DENV Duo for ZIKV



ZIKV/DENV Duo for DENV



* One DENV+ specimen was equivocal

Recent lessons on Immunoassay Development

- Dengue vs Zika ratios increase specificity of IgM tests without reducing sensitivity:
 - E.g. InBios Zika Detect test (Granger et al. 2017)
 - CDC Tri-ELISA (under development)
- NS1 antibody detection tests are more specific than E antibody detection tests, but with some reduction in sensitivity
 - E.g. EuroImmune (Lustig et al. 2017)
 - BOB ELISA, Balmaseda et al, 2017
- Differential avidity of antibodies for dengue and Zika E and NS1/NS5 antigens (Wong et al, 2017)

Increased reliance on molecular testing

- Highly sensitive during acute illness
- Approx. 60-75% of cases (Zika, dengue or chik) can be diagnosed during the first 6 days of illness
- Useful as confirmatory test for dengue or Zika IgM positive cases
- PRNT less useful in areas of flavivirus co-endemicity
- Long Zika viremias in pregnant
- Recommendation to test in 3 trimesters of pregnancy
- Able to detect pathogens in all fluids and tissues

Characteristics of the CDC-Trioplex RT-PCR assay

- Detects DENV (no subtyping), CHIKV and ZIKV
- Recommended for serum collected 0-7 DPO
- Recommended for urine, amniotic fluid and CSF (paired with serum)
- Contains internal control
- Adapted and validated for widely available equipment

Comparative analytical sensitivity of Zika Vvirus NATs

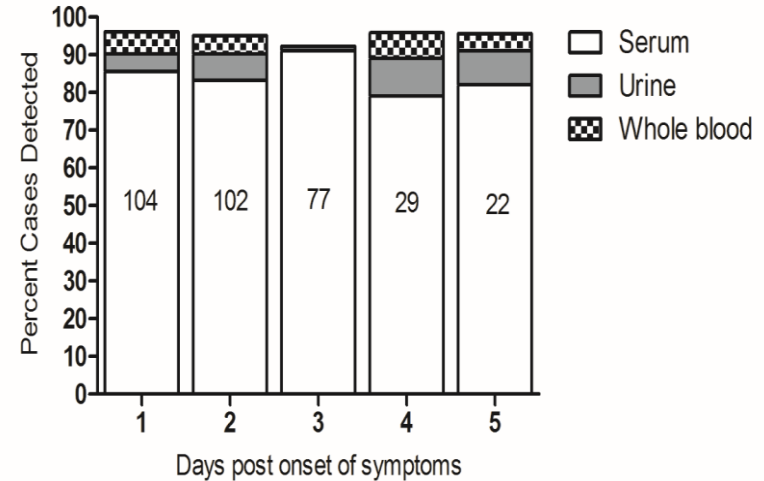
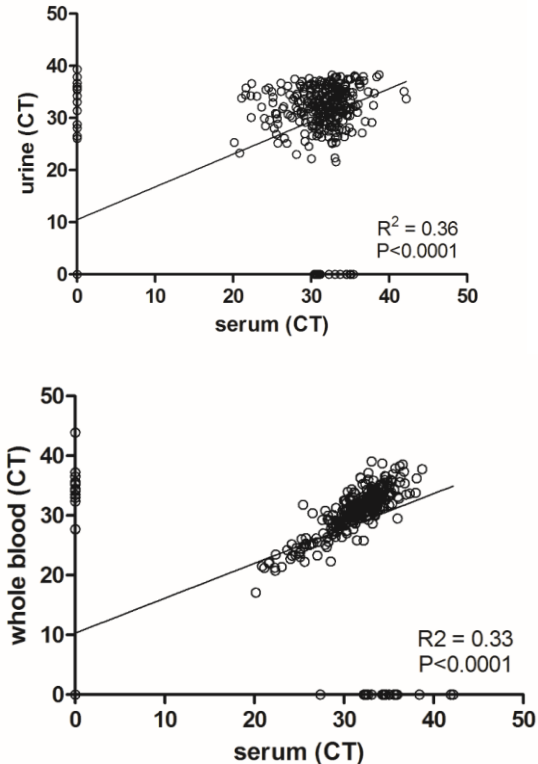
Relative analytical sensitivity of donor nucleic acid amplification technology screening and diagnostic real-time polymerase chain reaction assays for detection of Zika virus RNA

Stone et al, Transfusion (2016)

Supernatant		Donor screening NAT, % ^a *	CDC PR Trioplex-LI, %	CDC PR Trioplex-HI, %	CDC FC 1087-LI, %	CDC FC 108-HI, %	BSRI/UC Davis, %	FDA, %
cp/mL	PFU/mL	N = 7	N = 6	N = 4	N = 3	N = 3	N = 20	N = 12
4.4E+04	5.76E+01	100	100	100	100	100	100	100
1.4E+04	1.82E+01	100	100	NA	100	100	100	100
4.4E+03	5.76E+00	100	100	NA	100	100	100	100
1.4E+03	1.82E+00	100	100	100	100	100	100	67
4.4E+02	5.76E-01	100	100	100	100	100	90	67
1.4E+02	1.82E-01	100	17	100	33	100	60	25
4.4E+01	5.76E-02	100	17	100	0	100	15	8
1.4E+01	1.82E-02	86	0	0	0	33	10	8
4.4E+00	5.76E-03	71	17	0	0	0	15	0
1.4E+00	1.82E-03	43	0	0	0	0	0	0
4.4E-01	5.76E-04	0	0	0	0	0	0	0
	95% LOD	17.9 [5.6, 38.1]	1529 [362, 3829]	28.8 [17.3, 37.9]	205 [80, 337]	20.3 [8, 33.6]	1102 [466, 2053]	4918 [1596, 10,660]
	50% LOD	2.5 [1.3, 4.9]	123 [55.4, 273]	24.8 [17, 36.1]	152 [77.2, 301]	15.1 [7.7, 30.2]	81.7 [52.5, 127]	321 [179, 578]

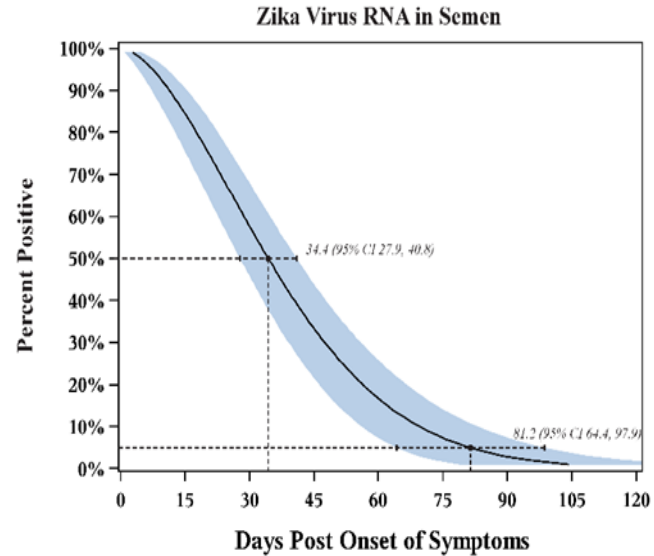
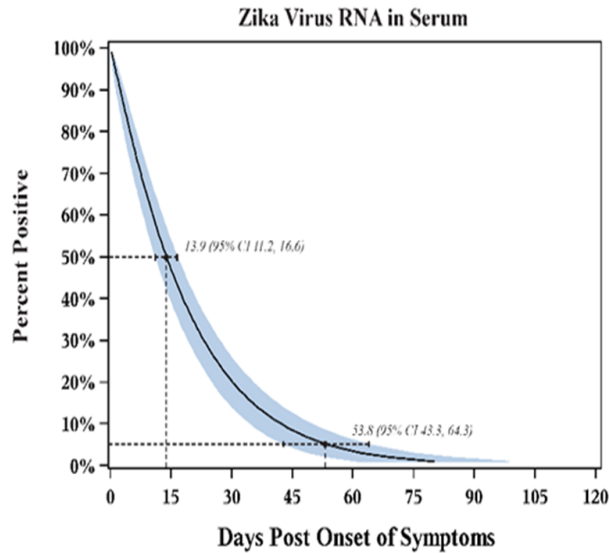
* Donor screening NAT includes the RMS and Hologic NAT assays; CDC Puerto Rico (PR) low-input (LI) assay results were combined for Singleplex and Trioplex versions of the Trioplex assay (Trioplex-LI).

Utility of the CDC Trioplex RT-PCR by sample types



Santiago et al, 2018

Persistence of Zika virus in body fluids



Paz-Bailey et al, 2017

Possible Routs for Test Development

1- Symptomatic Patients:

- Increase sensitivity of multiplexed (dengue/chik/Zika) antigen- or nucleic acid-based tests
- Increase specificity of IgM tests (E.g. Duo ELISA for Zika/dengue detection and differentiation)

2- Asymptomatic or post-symptomatic:

- Specific dengue and Zika IgG detection
 - Screening for asymptomatic pregnant women
 - Applications for vaccine evaluations and effectiveness

Acknowledgments

- CDC Dengue Branch
 - Surveillance and Research Lab
 - Epidemiology Team
- CDC Arbovirus Diseases Branch
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