A Selective Immunoassay for the Detection of Zika Virus in Human Bodily Fluids

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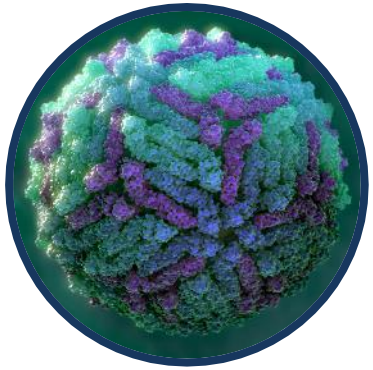
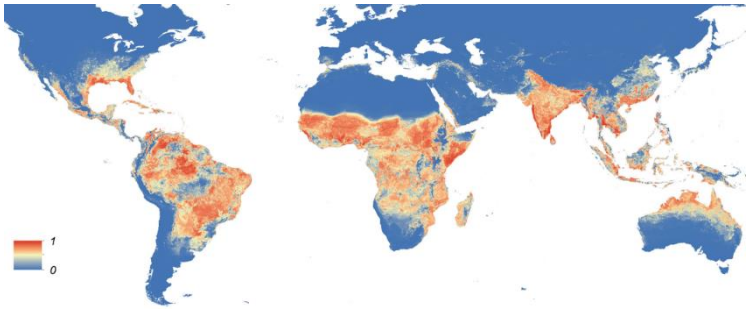
Zika Virus (ZIKV)

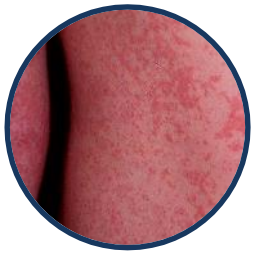


Belongs to the family of Flaviridae (Dengue Fever, Yellow Fever, West Nile Virus, Japanese Encephalitis, Chikungunya Virus)

Transmitted by Yellow Fever Mosquito (*Aedes aegypti*)



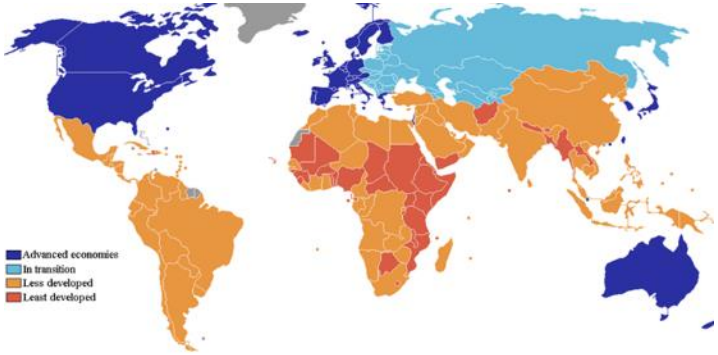
Global Yellow Fever Mosquito (*Aedes aegypti)* distribution (predicted)



Maculopapular Rash

Microcephaly

Patient with Guillain-Barré Syndrome

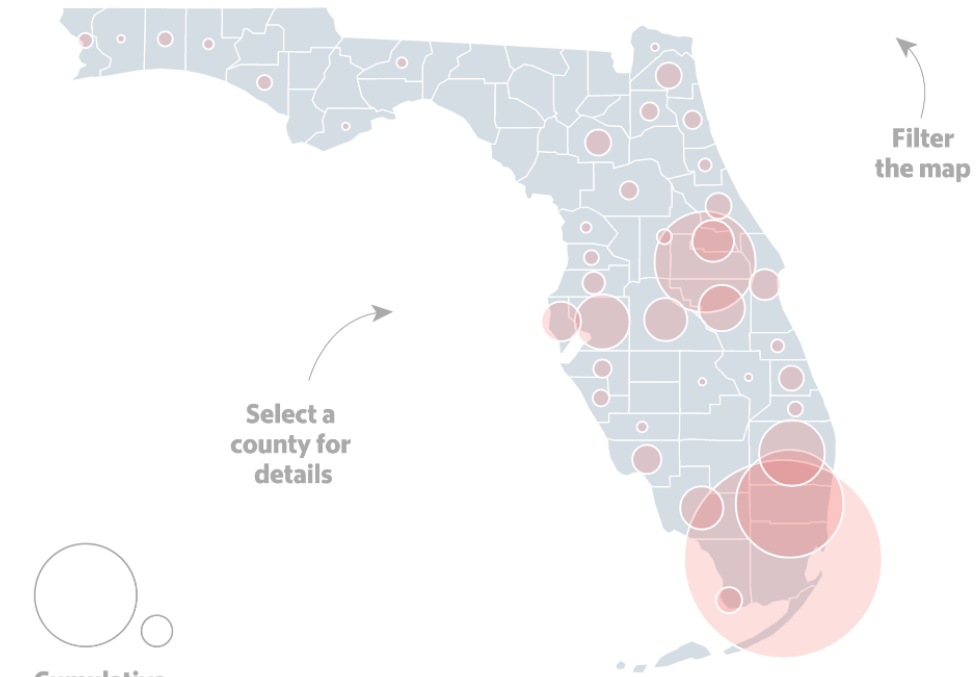


Economic development distribution

Zika Virus (ZIKV) in Florida

Florida Sep. 25, 2017

LOCAL + TRAVEL | LOCAL CASES | TRAVEL CASES



Local + Travel
1551

Local Cases
287

Travel Cases
1264

Pregnant Women
402

Unknown Origin
(Local or Travel)
80

Unknown County
(Local Cases)
9

In the news

As Zika season looms, Senate panel approves $100 million in mosquito control funding
April 26, 2017

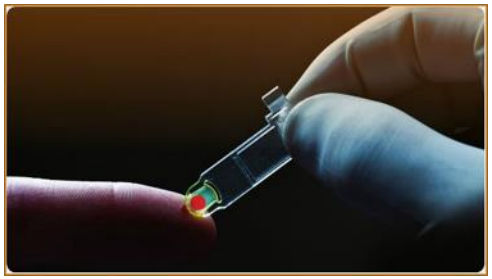
Miami Herald as of September 25, 2017.

Point of Care Testing

Point-of-care testing, also known as bedside testing, is medical diagnostic testing at or near the point of care; that is, at the time and place of the patient care.

1. 

Test ordered

2. 

Test Sample Collected

3. 

Sample is analyzed

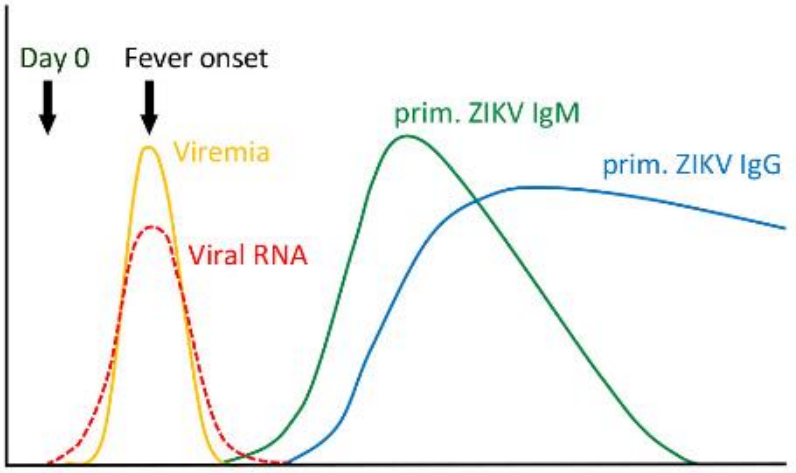
4. 

Clinician acts on the results

Advantages

- Decreased turnaround time
- Evidence-based medical decisions in “real time”
- Elimination of specimen transport and processing time
- Reduction in duplicate test orders
- Reduction in unnecessary medications
- Reduced consumption of other expensive services/products (lab tests, pharmaceuticals)

Current Methods for ZIKV Detection



RNA NAT (RT-PCR)
Trioplex RT-PCR Assay
Concurrently detects ZIKV, DENV, CHIKV

- Gives information about active infection
- Detects low copies of viral RNA
- Sensitive and selective

- Requires trained personnel and expensive equipment

ZIKV MAC-ELISA

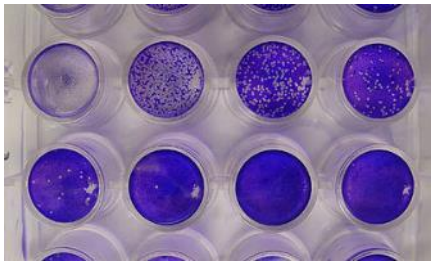
- Detects the IgM antibodies developed against ZIKV) in serum or CSF)

- Due-to cross reactivity with other flaviviruses the results may be difficult to interpret. Inconclusive results must be confirmed with plaque-reduction neutralization test

Plaque Reduction Neutralization Test (PRNT)

- Considered to be the gold standard for detecting and measuring antibodies that can neutralize the virus. It has higher sensitivity than other tests

- Relatively cumbersome and time intensive.

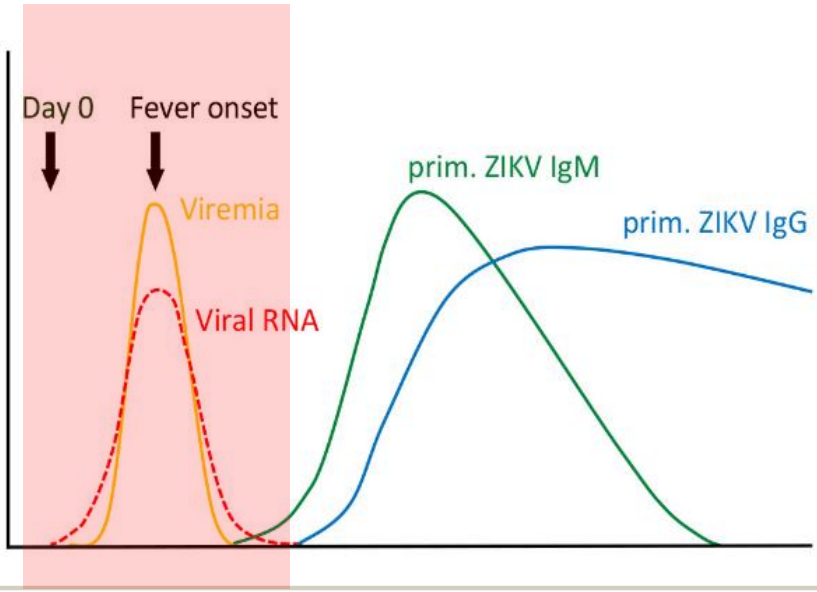


Immunoassay for ZIKV

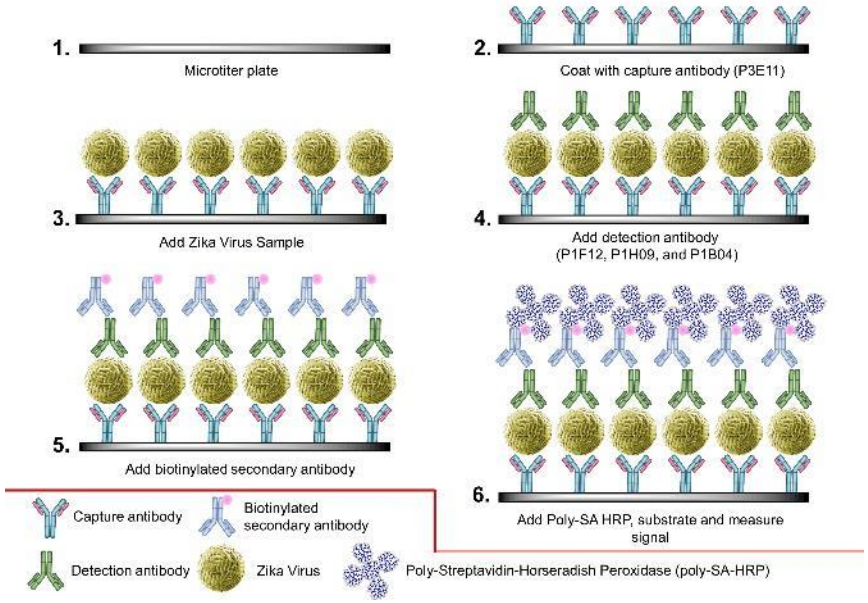
Development of two Assays:

- Clinical laboratory based assay for the direct detection of virus in bodily fluids of a patient with an active infection.
- A rapid lateral-flow type point-of-care assay.

Novelty: use of highly specific monoclonal antibodies against ZIKV that was developed by Watkins' Group.

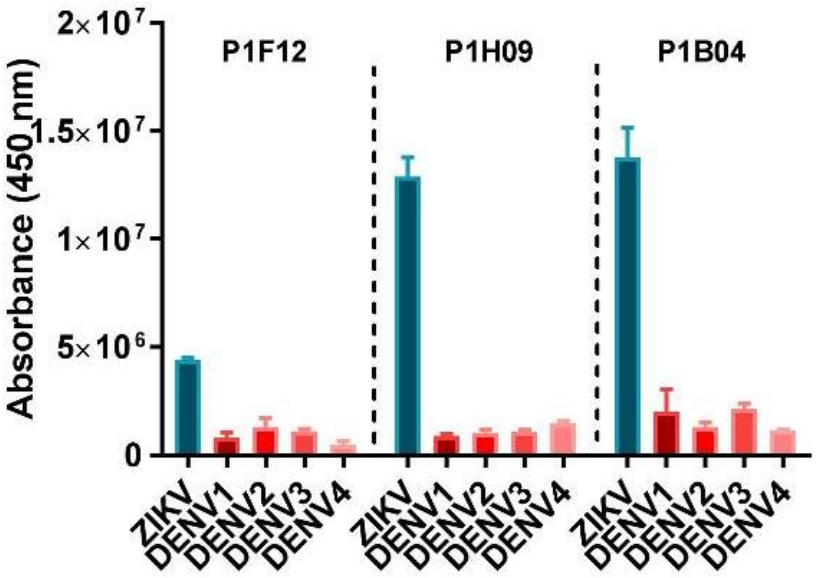


Assay Scheme



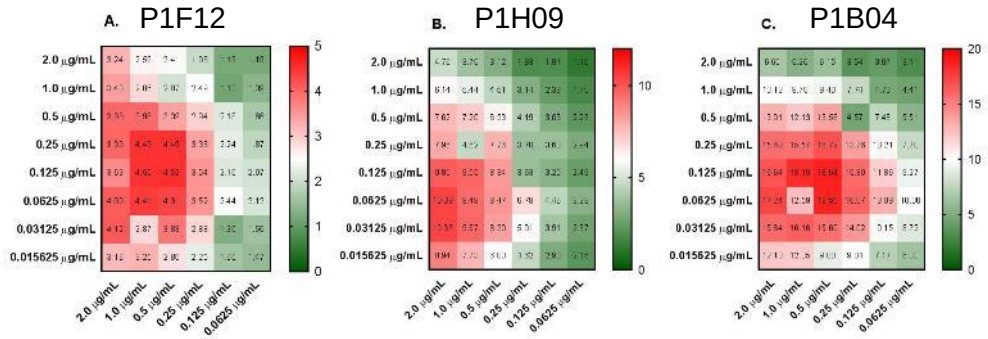
Optimization of the ZIKV Assay

Selectivity

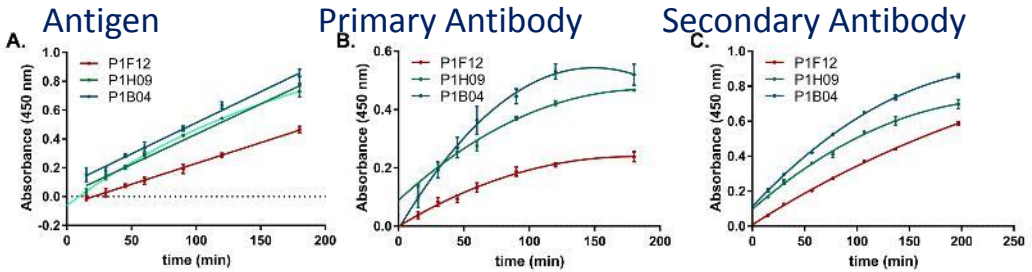


Response of the Immunoassay to the same concentrations (2.0 x 10^5 pfu/mL) of ZIKV and different DENV variants

Optimization of the Assay

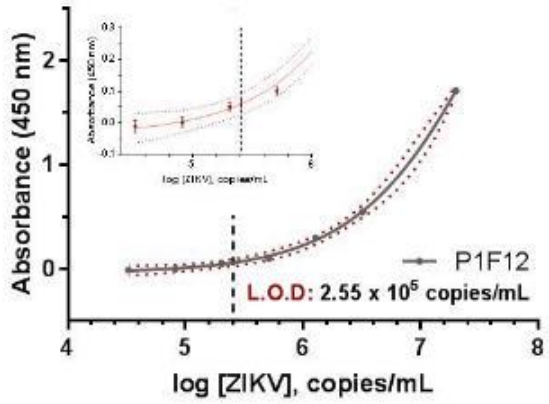


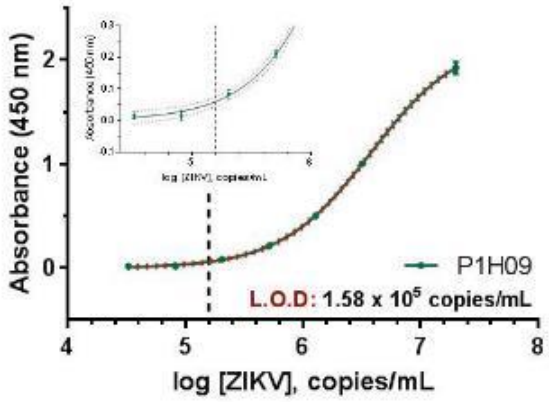
Optimization of the antibody concentrations for the primary and secondary antibody pair.

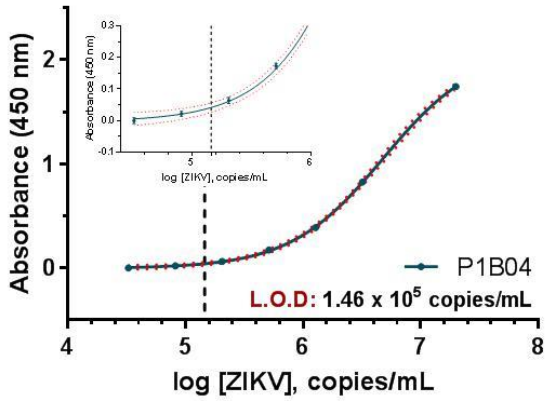


Optimization of the immunoassay incubation times

ZIKV Assay Validation



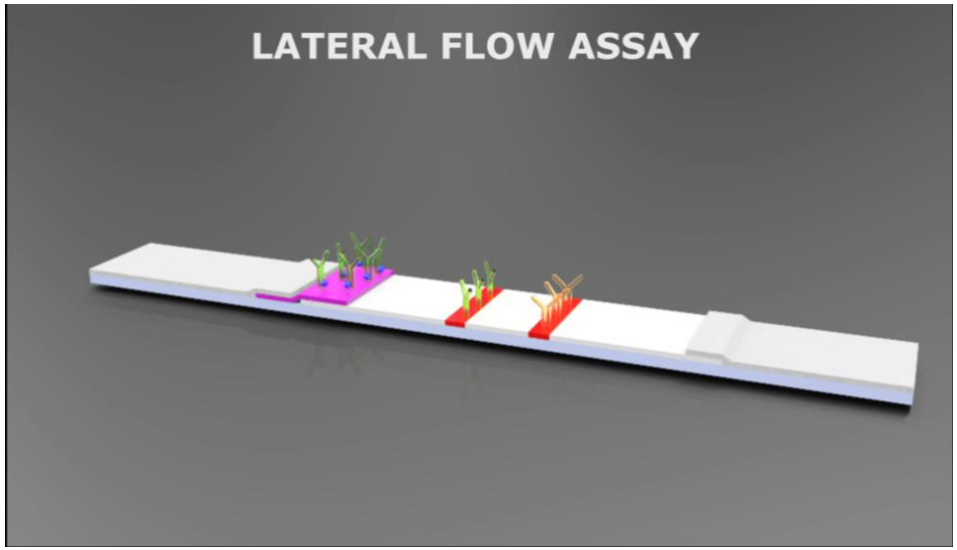


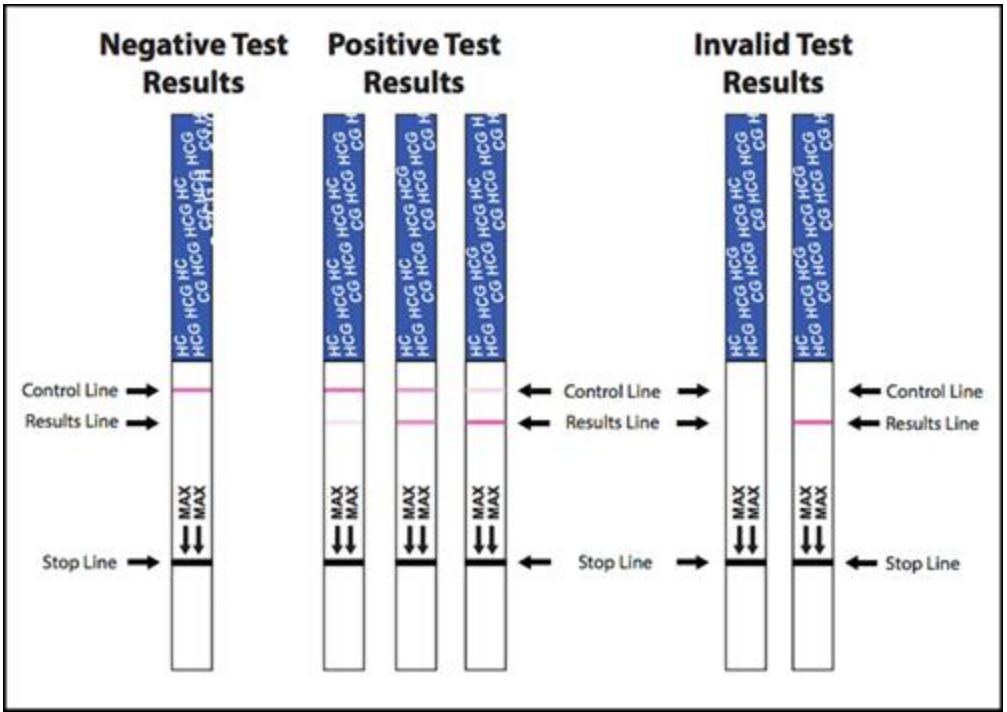


		Buffer	Urine Neat	Saliva 1:10	Serum 1:10	Whole Blood 1:10
P1F12	LOD (copies/mL)	$1.11 \pm 1.38 \times 10^5$	$8.51 \pm 5.55 \times 10^4$	$7.39 \pm 2.35 \times 10^4$	$5.81 \pm 0.39 \times 10^4$	$2.05 \pm 2.5 \times 10^5$
	LOQ (copies/mL)	$2.99 \pm 3.72 \times 10^5$	$1.70 \pm 1.53 \times 10^5$	$1.68 \pm 0.98 \times 10^5$	$1.11 \pm 0.20 \times 10^5$	$4.14 \pm 5.2 \times 10^5$
	CV Min (%)	5.48 ± 3.86	3.23 ± 1.47	8.66 ± 6.66	2.14 ± 2.74	3.33 ± 2.13
	CV Med (%)	6.07 ± 2.40	7.44 ± 7.99	4.08 ± 0.90	3.96 ± 2.90	5.80 ± 1.74
	CV Max (%)	2.10 ± 0.46	2.48 ± 1.75	1.30 ± 1.08	1.84 ± 0.46	4.70 ± 3.43
	Recovery Min (%)	90.99 ± 14.04	94.92 ± 2.15	75.89 ± 34.83	87.41 ± 6.46	92.41 ± 11.10
	Recovery Med (%)	86.23 ± 24.88	78.70 ± 8.84	102.42 ± 24.57	105.35 ± 57.81	92.20 ± 2.51
	Recovery Max (%)	92.20 ± 25.85	95.10 ± 20.58	112.83 ± 11.18	108.53 ± 6.44	96.71 ± 3.48
P1H09	LOD (copies/mL)	$3.81 \pm 0.72 \times 10^4$	$4.40 \pm 6.03 \times 10^4$	$5.50 \pm 5.99 \times 10^4$	$5.55 \pm 6.55 \times 10^4$	$5.50 \pm 8.1 \times 10^4$
	LOQ (copies/mL)	$5.08 \pm 1.48 \times 10^4$	$4.71 \pm 0.56 \times 10^4$	$7.11 \pm 0.06 \times 10^4$	$7.62 \pm 1.53 \times 10^4$	$7.62 \pm 1.0 \times 10^4$
	CV Min (%)	5.74 ± 7.59	4.18 ± 2.67	5.94 ± 7.15	3.61 ± 2.31	4.65 ± 2.04
	CV Med (%)	2.53 ± 1.14	13.32 ± 9.86	4.31 ± 1.98	5.13 ± 3.20	3.64 ± 1.05
	CV Max (%)	1.10 ± 0.75	1.66 ± 0.56	1.03 ± 0.71	2.90 ± 3.45	0.85 ± 0.44
	Recovery Min (%)	95.25 ± 14.16	98.90 ± 19.16	81.08 ± 31.99	75.73 ± 4.61	101.71 ± 2.60
	Recovery Med (%)	102.53 ± 29.31	97.29 ± 29.36	98.76 ± 20.24	88.57 ± 15.16	92.96 ± 4.52
	Recovery Max (%)	105.87 ± 12.48	109.90 ± 3.72	118.45 ± 8.33	103.87 ± 11.93	94.18 ± 0.57
P1B04	LOD (copies/mL)	$3.73 \pm 0.71 \times 10^4$	$5.58 \pm 0.90 \times 10^4$	$4.73 \pm 0.64 \times 10^4$	$5.88 \pm 0.50 \times 10^4$	$5.01 \pm 0.8 \times 10^4$
	LOQ (copies/mL)	$4.52 \pm 1.79 \times 10^4$	$6.20 \pm 1.73 \times 10^4$	$5.20 \pm 0.94 \times 10^4$	$7.20 \pm 0.91 \times 10^4$	$6.51 \pm 0.9 \times 10^4$
	CV Min (%)	2.61 ± 1.41	7.50 ± 2.46	7.68 ± 2.46	1.64 ± 1.01	7.79 ± 3.93
	CV Med (%)	2.26 ± 2.07	4.70 ± 1.53	7.03 ± 8.34	3.59 ± 2.29	4.13 ± 2.83
	CV Max (%)	1.00 ± 0.24	0.49 ± 0.15	1.39 ± 0.78	0.95 ± 0.35	0.97 ± 0.31
	Recovery Min (%)	90.65 ± 15.31	103.89 ± 15.00	90.84 ± 15.83	83.93 ± 12.14	96.09 ± 4.98
	Recovery Med (%)	93.67 ± 15.20	103.58 ± 16.88	97.74 ± 16.55	93.55 ± 5.04	90.33 ± 5.63
	Recovery Max (%)	102.06 ± 11.07	117.02 ± 6.56	106.28 ± 5.00	95.36 ± 10.94	89.89 ± 4.96

- Data obtained by spiking clean bodily fluids with ZIKV
- Representative calibration curves with individual L.O.D.
- Data in table are an average of at least three separate experiments

Point of Care ZIKV Test





Preparation of the Conjugate

Sonicate 1% CNB Soln
30 x 1 sec (1 on, 2 off)

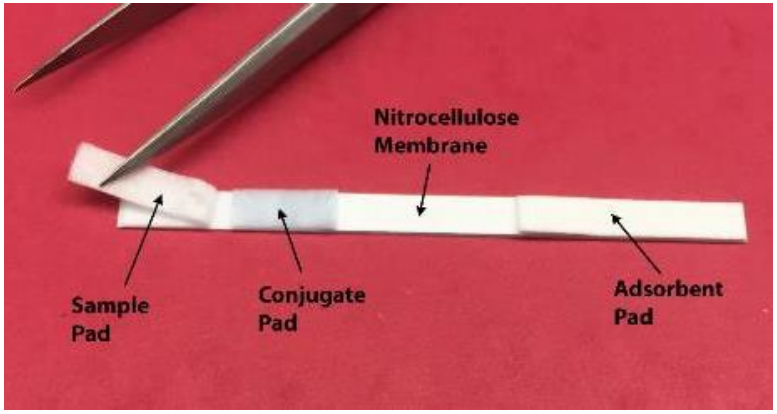
0.5 mg/mL
Ab in 120 µL
Conjugation Buffer

60 µL 1% CNB in 180 µL
Conjugation Buffer

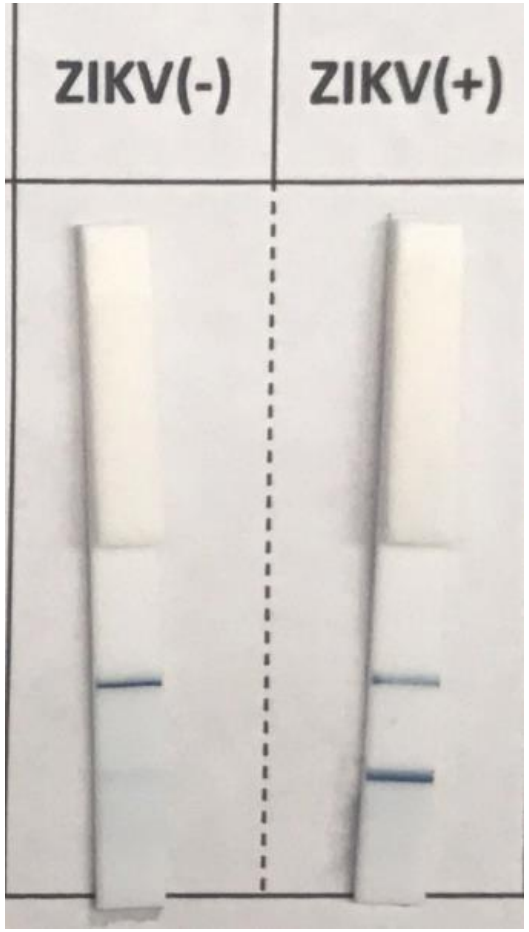
Incubate 2 h while mixing at 40 °C

1. Add the reaction mixture to blocking buffer, Incubate 2 h while mixing at 40 °C
2. Centrifuge 20 min at 14,400 xg at 20 °C
3. Wash with wash buffer
4. Vortex and Sonicate to resuspend in wash buffer

Point-of-Care ZIKV Test



Detection of ZIKV in Buffer



Detection of ZIKV in Urine

Team

Florida Department of Health (FLDOH) Zika Initiative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