Notes From the Field: Diagnostic Issues Related to Zika Virus

January 11, 2018
2:30-3:30 PM (ET)

Council of State and Territorial Epidemiologists

Webinar Housekeeping

- **Today's webinar is being recorded**
  - The webinar recording and presentation slides will be available in the webinar library on CSTE's website: http://www.cste.org/?page=WebinarLibrary
- **All lines have been muted**
- **There will be a question-and-answer session at the end of the webinar**
  - To ask a question, please use the Q&A box on the right side of your screen

Webinar Objectives

Participants will:

- Participants will understand how the biology of flaviviruses affect our ability to test for them
- Participants will be able to summarize currently available assays and their challenges
- Participants will be able to describe new approaches to testing for flaviviruses in general and Zika virus specifically
- Participants will understand the diagnostic challenges public health laboratories experienced during 2016-17 Zika response;
- Participants will be able to describe current laboratory standards regarding testing for Zika virus
- Participants will be able to restate the status of arbovirus testing in Florida before Zika;
- Participants will be able to describe how Florida was able to build the testing capability during the outbreak;
- Participants will understand the current status of Zika virus testing at the Florida laboratory

NEW YORK STATE OF OPPORTUNITY. Department of Health | Wadsworth Center
And School of Public Health, SUNY at Albany

Zika Virus

Challenges of Diagnostic Testing for Flaviviruses

Laura Kramer
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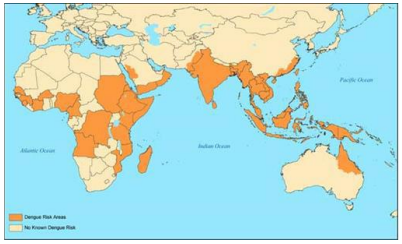
January 7, 2018
Council of State and Territorial Epidemiologists (CSTE)
Notes from the Field: Zika Laboratory Diagnostics

Outline

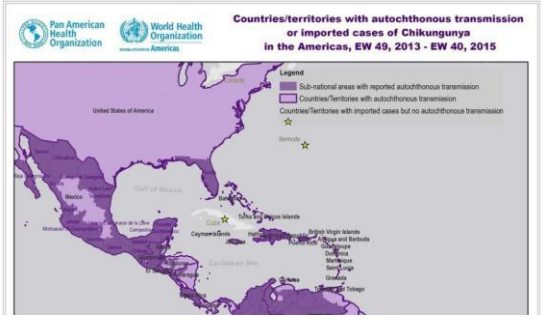
- Virus emergence
- Biology of Flaviviruses
- Diagnostic tests currently
  - available RNA detection
  - Serologic assays
- Challenges of current assays
- Summary

Current Arboviral Threats in the Americas

Dengue risk maps

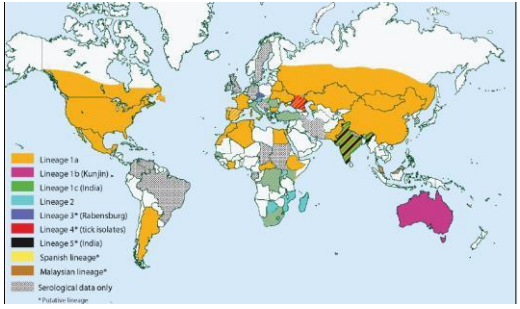


DENGUE: 1/3 of world population at risk, 30-fold increase in 50 yrs



Chikungunya

Emerged in the Americas late 2013; ≈1.8M cases



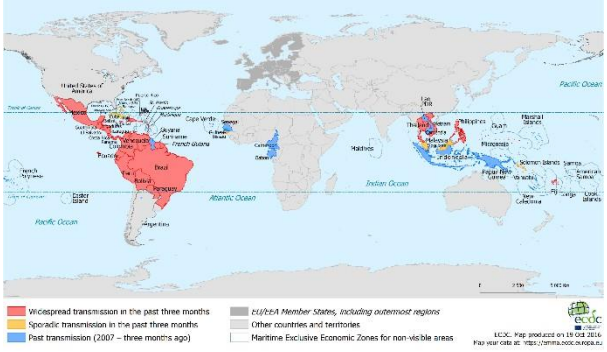
West Nile

US total cases: 43,937; 1,911 deaths (4%)

Zika

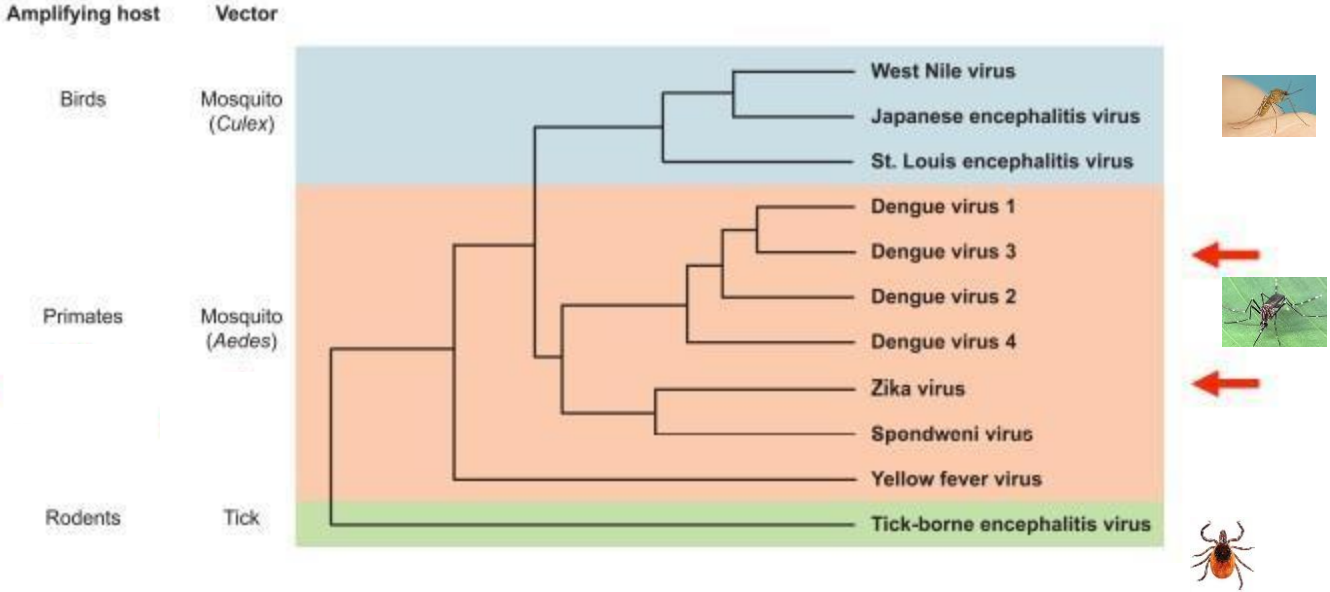
Global activity: 67 countries since 2007; 48 in the Americas to Sept 2016

583,144 suspected cases
(3715 ZCS in Americas/27 countries)



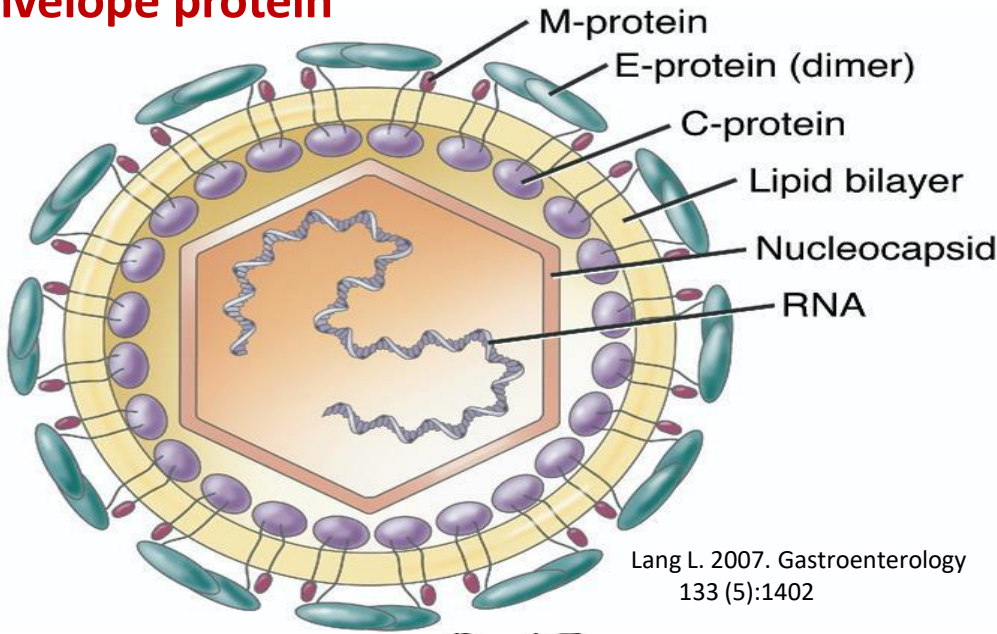
Simplified phylogeny of *Flaviviridae*: *Flavivirus* genus

Challenge: No. of closely related medically important flaviviruses

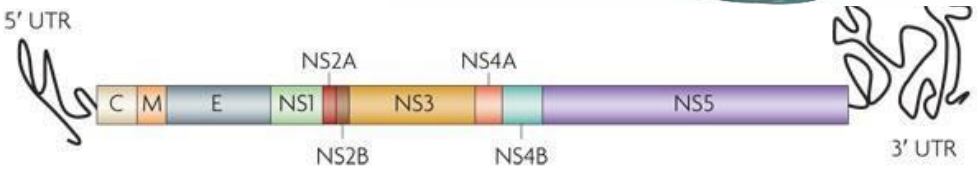


Virus structure and Flavivirus genome organization

Challenge: Single envelope protein



Lang L. 2007. Gastroenterology 133 (5):1402



Ashraf U et al. . 2015. Viruses7:219;

Challenge: Similar clinical presentations

Solution: Laboratory diagnostic assays

Features	ZIKV	DENV	CHIKV
Fever	+	+++	+++
Rash	+++	+	++
Conjunctivitis	++	-	-
Arthralgia	++	+	+++
Myalgia	+	++	+
Headache	+	++	++
Hemorrhage	-	++	-
Shock	-	+	-

Conjunctivitis

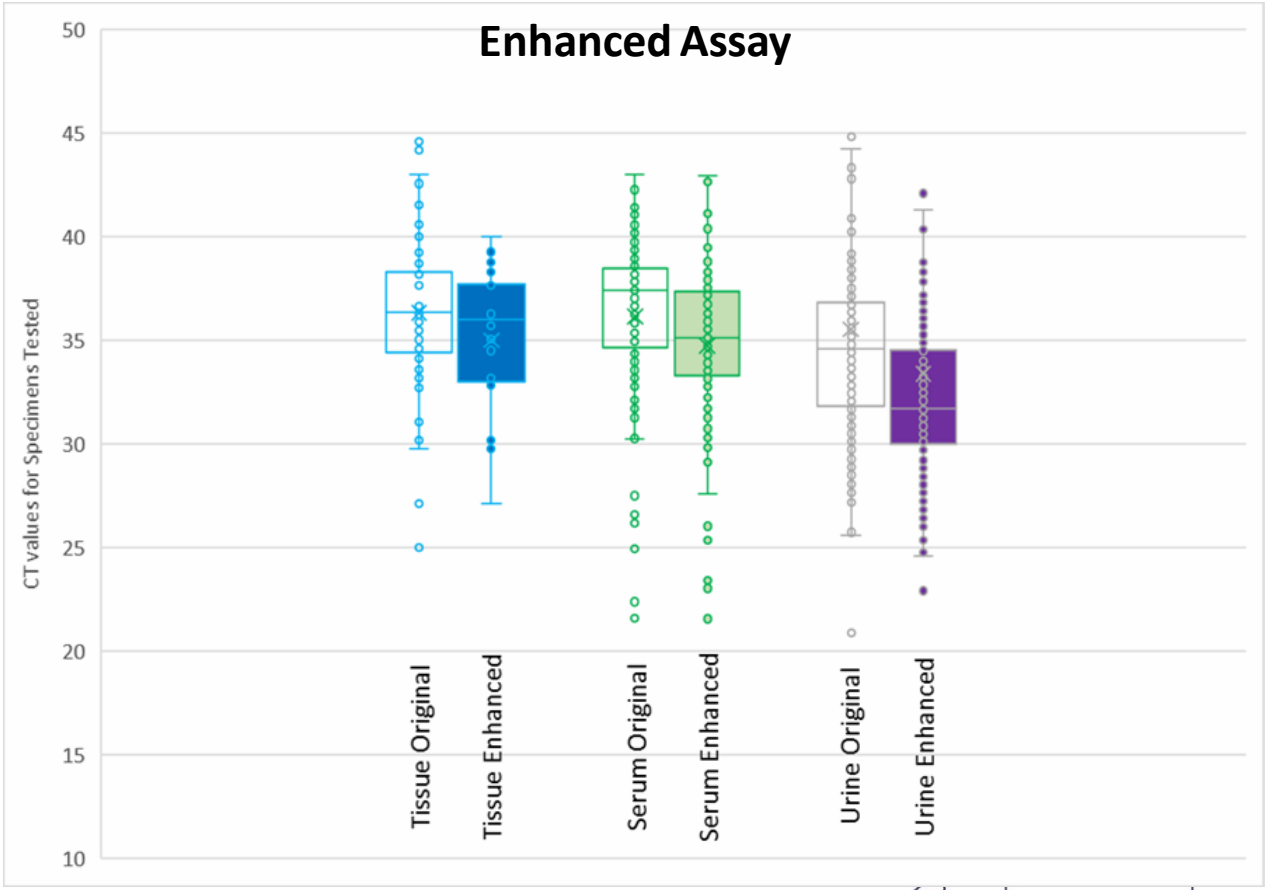
Joint pain and Swelling

Challenge: ZIKAV low level viremia, low level RNA

Solution: Enhance assay sensitivity

Extract nucleic acid on BioMerieux easyMAG
RT-PCR master mix: TaqMan Fast Virus Kit
Test nucleic acid in duplicate

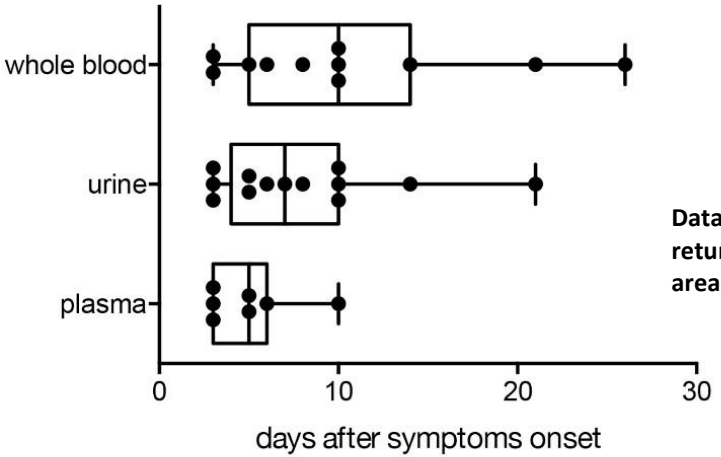
Procedure	Original	Enhanced
Extract specimen	0.25mL	1.0mL
Template volume	5µL	20µL
Reaction volume	25µL	30µL
Limit of detection in gene copies/mL		
Serum	400	70
Urine	2000	63



Note: Consistently lower CT values (higher viral load) in urine compared to serum.

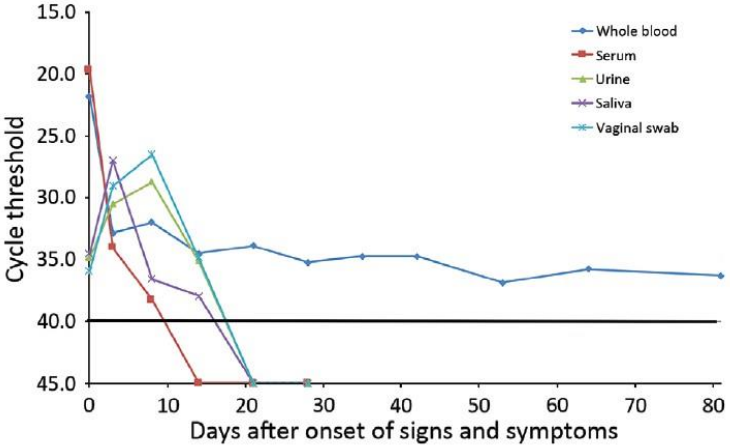
ZIKV RNA detection in whole blood, urine and plasma

Challenge: Which specimen to test



Data from 10 infected patients returning to Italy from endemic areas, February–July 2016.

From Rossini et al. 2017, J Infect 75:242-245



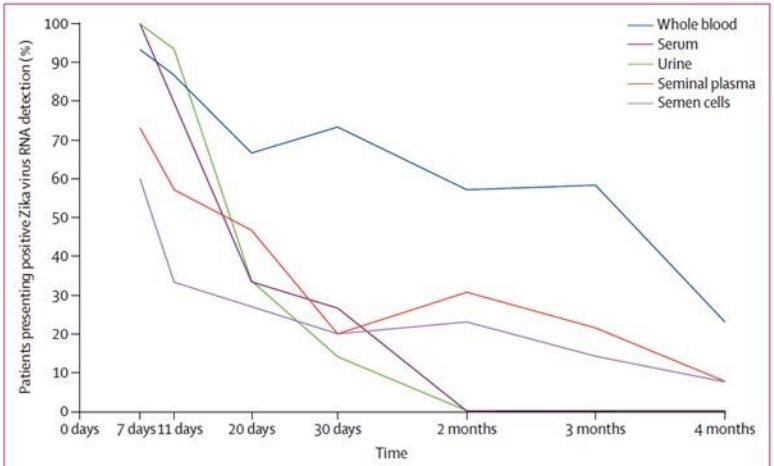
Specimens from 26yo F returned from Honduras to the US.
Black horizontal line at CT 40 is cutoff negative result.

♀

From Murray et al. 2017, Emerg Infect Dis 23:99-101

♂

Combined data from Zika virus infection in 15 patients



From Joguet et al. 2017, Lancet Infect Dis 17:1200-08

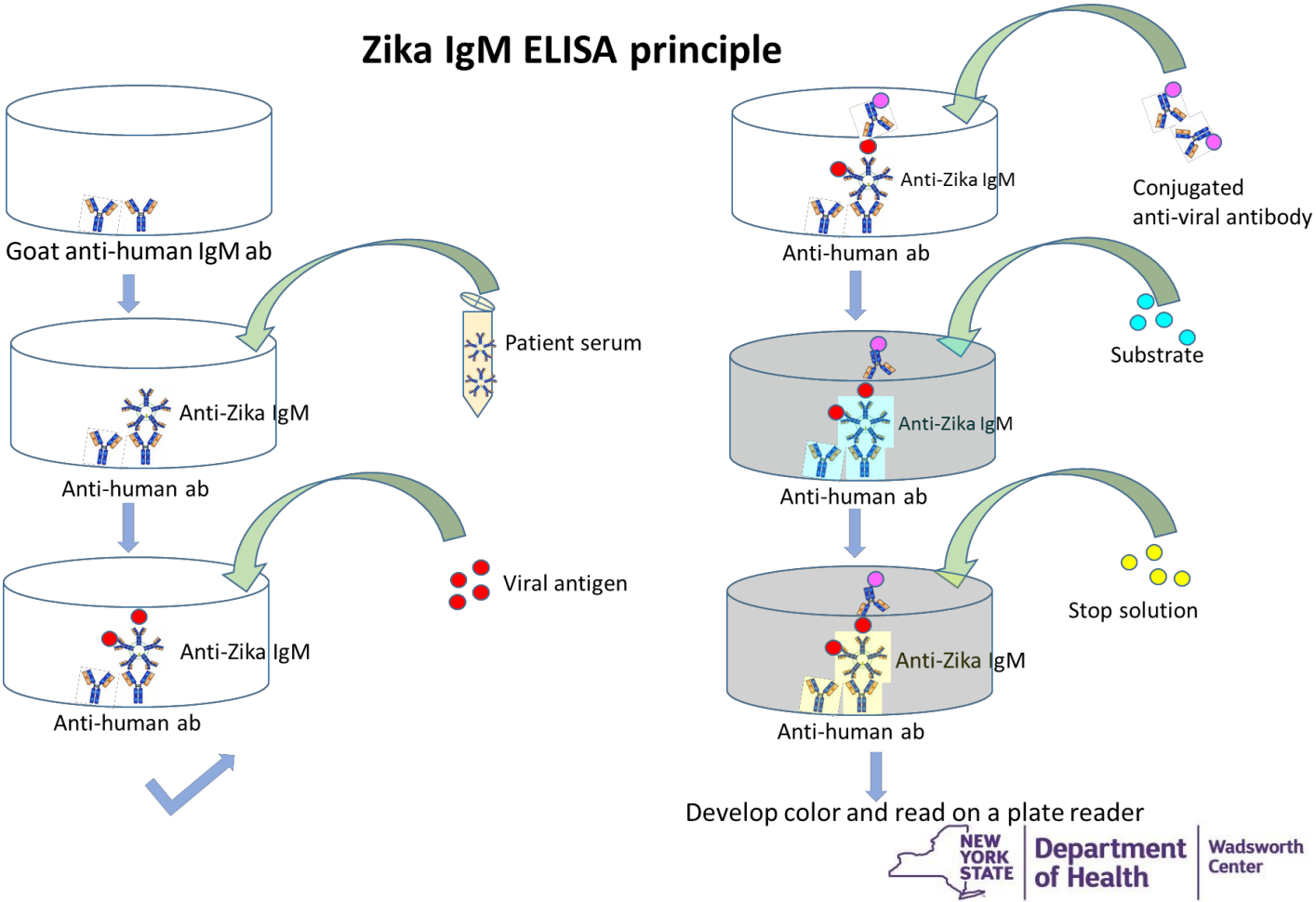
Serologic assays

Challenges: Serologic assays difficult to interpret due to cross-reactivity, secondary infection and original antigenic sin, asymptomatic infection

Solution: Development of new assays

- Standard assays - IgM ELISA
  MIA (E protein)
  PRNT
- New assays – Multiplex MIA with E, NS1, NS5 proteins

Zika IgM ELISA principle



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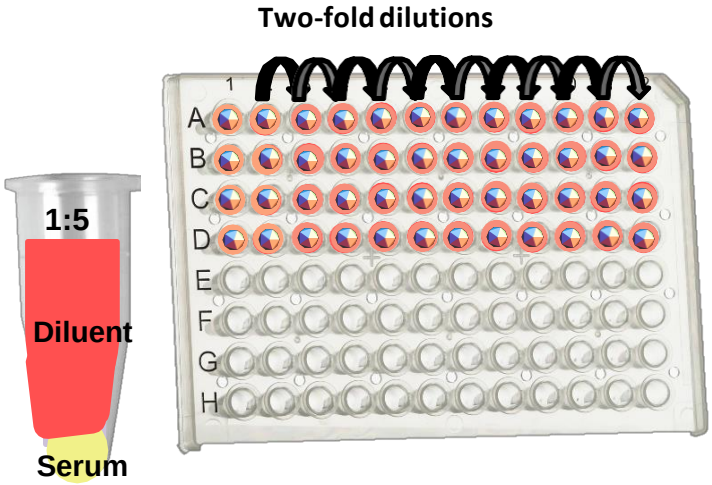
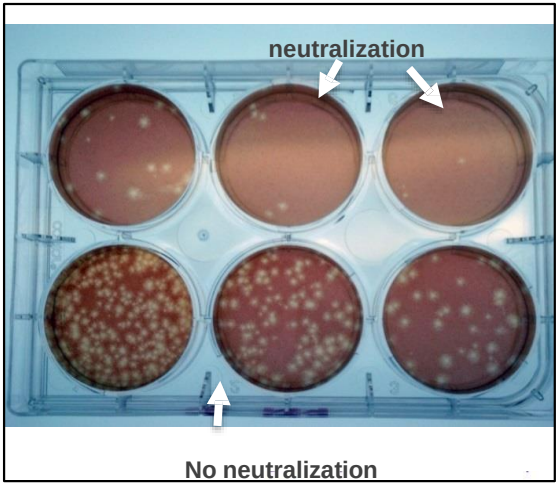
Plaque Reduction Neutralization Test (PRNT)

Challenge: Lack of virus-specificity; infectious virus (biosafety); training

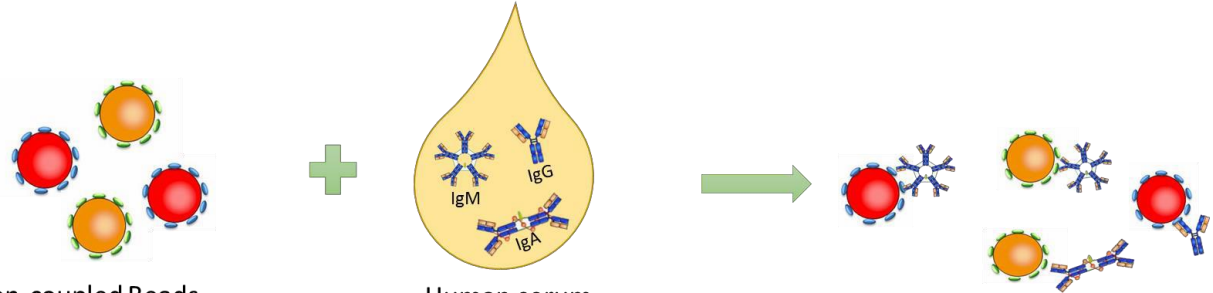


http://4ti.co.uk/files/9914/5371/6634/4ti-0760.jpg

90% reduction of virus plaques = virus neutralizing AB present



Microsphere Immunofluorescence Assay (MIA) principle



Antigen-coupled Beads
Zika NS1, NS5, Den1-4 NS1

Human serum containing IgG, IgM, IgA

Antigen specific antibodies bind to beads

Anti-human SA-PE conjugate

Read signal in Luminex
(MFI=Mean fluorescence intensity)

Wong SA et al. EBioMedicine. 2017 Feb;16:136-140

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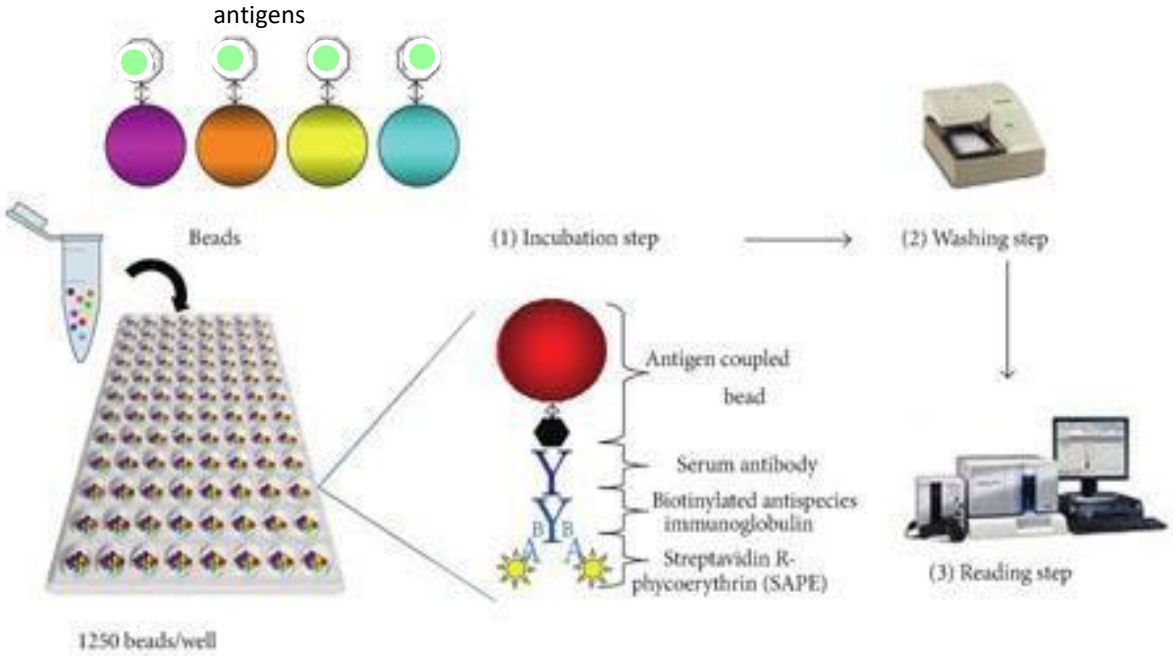


FIGURE 2: Presentation of the flavivirus microsphere immunoassay (MIA) with beads coupled to antigens

Conclusions: Need for improved Zika serologic assays

- **Challenges:**
  - Cross-reactions between DENV and ZIKAV antibody
  - Secondary infections and original antigenic sin
  - Low titer, short-lived viremia
  - Unknown exposure date / asymptomatic women
  - Problems with commercial assays
- **Need test that:**
  - Can discriminate ZIKAV from DENV antibodies (both Zika IgM ELISA as well as PRNT lack this ability)
  - Is specific for anti-ZIKAV antibodies past the IgM period (~45 days) (PREGNANT women!)
  - Rapid turnaround time, high throughput assay
  - Reasonable cost / skill ; POC

SUMMARY

- Promising new diagnostic assays with high throughput, sensitivity, specificity, but challenges remain
- Whole blood seems to be the best specimen for ZIKAV RNA detection, diagnosis, and follow-up.
- New NS1 –based assays appear to be more specific
- MIA has some advantages over ELISA - speed, throughput, reagent volume, multiplex format
- Extension of new assays to detect antibodies against other medically important arboviruses , eg CHIKV, YFV

Acknowledgments

- S Wong, K St. George, P-Y Shi*
- Andrea Furuya
- AP Dupuis, A Payne and Arbovirus Lab staff
- Amy Dean and Virology Lab staff
- Bill Lee
- Karen Kulas and Diagnostic Immunology Lab Staff
- *Funding from NYSDOH and a UTMB innovation grant to PYS

The Public Health Laboratory Perspective on the Zika Response

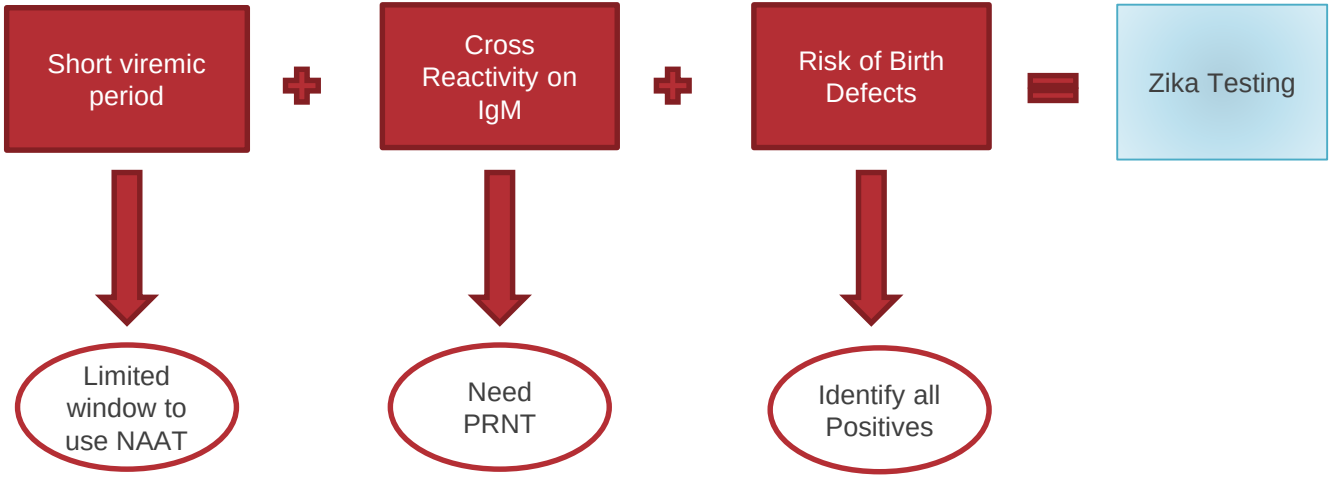
Kelly Wroblewski, MPH, MT(ASCP)

January 11, 2018

Zika Test Review

1. Nucleic Acid Amplification Test
   - Very specific
   - Most sensitive if used <14 days after symptom onset
2. IgM Antibody
   - Cross reactive with Dengue virus and other flaviviruses
   - Use up to 12 weeks from symptom onset
3. Plaque Reduction Neutralization
   - Confirms Zika Virus Infection
   - Turnaround time 7-14 days

Why is Zika Testing So Challenging?



Zika and the Public Health Labs: Then and Now

Early 2016

- Waiting on EUA assays
- Less than 10 PHLs testing
- Complex 3 test algorithm
- Manual Serology Only
- Serology assay cross reacts with other flaviruses
- PRNT takes a long time
- Cumbersome reporting processes

Early 2018

- 19 assays have received EUA
- Testing widely available
- Complex 3 test algorithm
- Automated serology available
- Serology assay cross reacts with other flaviruses
- PRNT takes a long time
- Somewhat less cumbersome reporting processes…maybe

Early 2016

- Waiting on EUA assays

Early 2018

- 19 assays have received EUA

Zika Assays Available in 2018

Nucleic Acid Amplification Testing

- 14 assays have received Emergency Use Authorization
- 10 of those assays available commercially for purchase

IgM Antibody

- 5 assays have received Emergency Use Authorization
- 4 of those assays available commercially for purchase

https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm

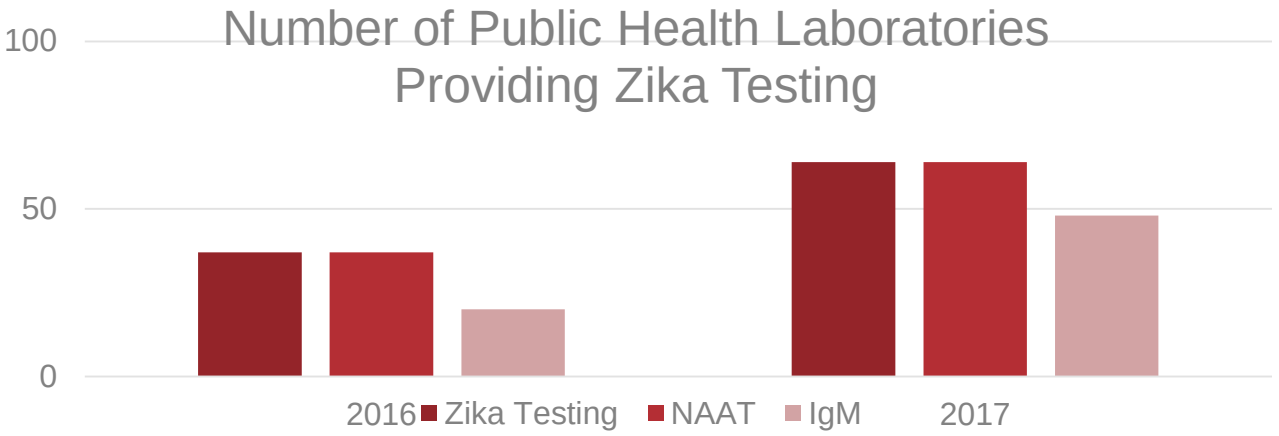
Early 2016

- Less than 10 PHLs testing

Early 2018

- Testing widely available

Zika Testing Availability



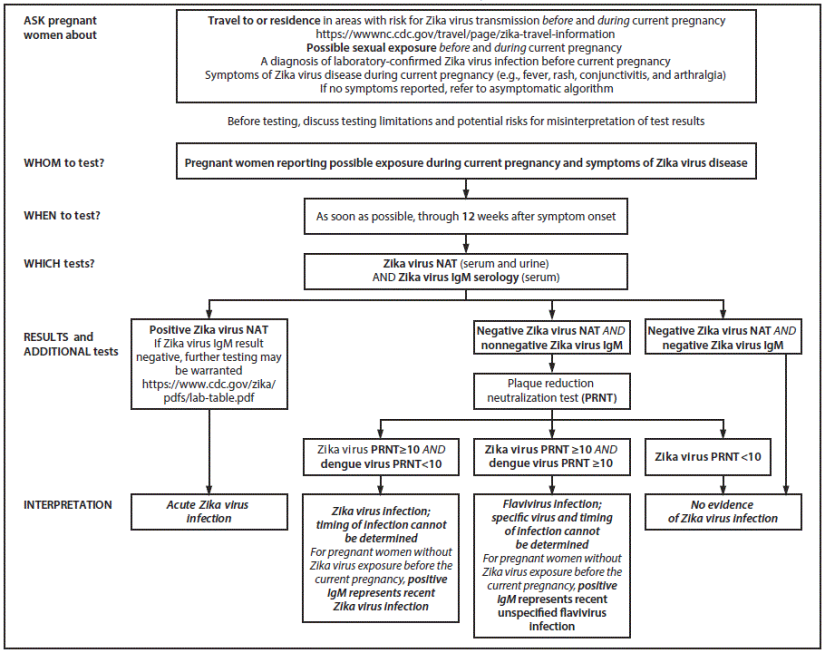
Early 2016

- Complex 3 test algorithm

Early 2018

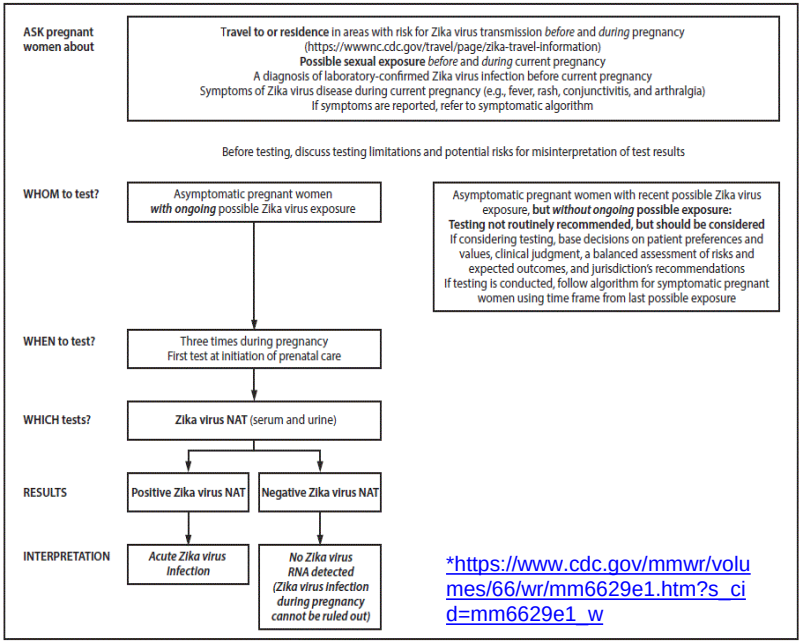
- Complex 3 test algorithm

Current Testing Algorithm: SYMPTOMATIC Pregnant Women*



*https://www.cdc.gov/mmwr/volumes/66/wr/mm6629e1.htm?s_cid=mm6629e1_w

Current Testing Algorithm: ASYMPTOMATIC Pregnant Women



*https://www.cdc.gov/mmwr/volumes/66/wr/mm6629e1.htm?s_cid=mm6629e1_w

Early 2016

- Manual Serology Only
- Serology assay cross reacts with other flaviruses

Early 2018

- Automated Serology Available
- Serology assay cross reacts with other flaviruses

APHL™

Serology Testing Today

Improved Throughput

- Automation of the Zika MAC ELISA
  - Dynex Agility and DSX
- Automated IgM Assays Available
  - Diasorin LIAISON® XL Zika Capture IgM Assay
  - Siemens ADVIA Centaur Zika test

Specificity Still an Issue

- Use of the NS-1 antigen versus the envelope protein
- Evaluations ongoing at CDC and in Public Health Laboratories

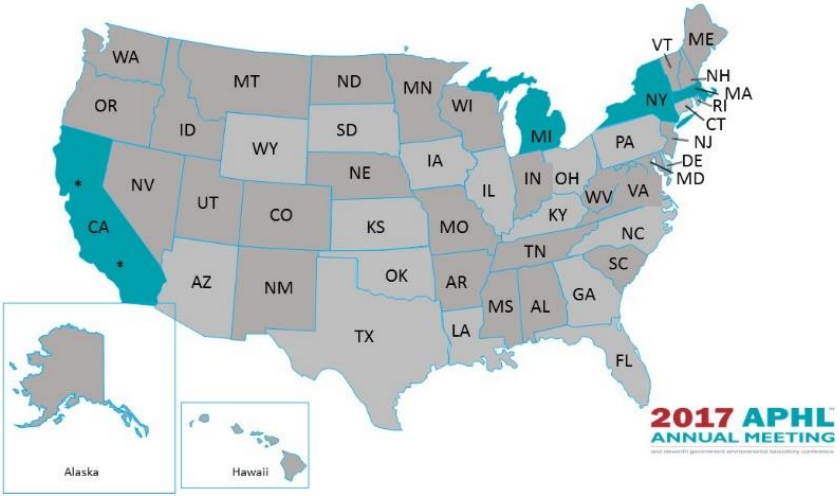
Early 2016

- PRNT takes a long time

Early 2018

- PRNT takes a long time

PRNT Reference Centers



Early 2016

- Cumbersome reporting processes

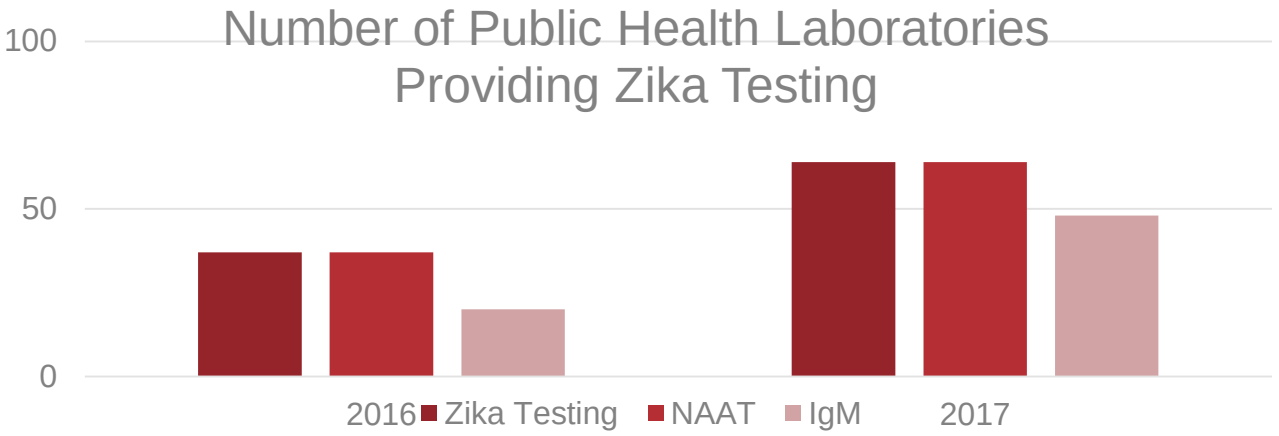
Early 2018

- Somewhat less cumbersome reporting processes…maybe

Electronic Ordering and Reporting

- Working with CMS and the HHS Office of the National Coordinator for Health Information Technology
- Currently working on requirements gathering with a handful of states
  - Looking at new use cases to support epi needs
- Will focus on piloting in Texas

Zika Testing Availability



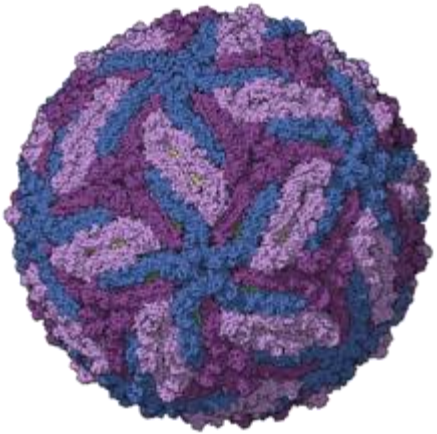
ZIKA IN FLORIDA 2016: THE LABORATORY RESPONSE TO AN EMERGING PATHOGEN

CSTE's Zika Virus Prevention and Control Project
January 11, 2018

Andrew Cannons
Laboratory Director
Bureau of Public Health Laboratories (BPHL)
Tampa

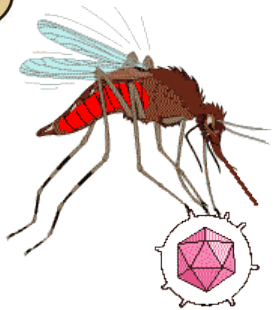
Outline

- The Laboratory Response
  - Arbovirology in Florida
  - Timeline
  - Testing Capability
  - Challenges
  - Successes
  - Status of the Lab in 2017
- Conclusions



Arbovirology Testing in Florida

- Many years of arbo testing
- Clinical (human, animal) and environmental (mosquito pools)
- Chicken surveillance program
- Arboviruses in FL:
  - West Nile virus
  - Dengue
  - Eastern Equine Encephalitis virus
  - Chikungunya virus
  - St Louis Encephalitis virus



Arbovirology Testing in Florida

- Major player in testing birds during the West Nile virus outbreak of 2001
- Identified Dengue outbreaks in Florida Keys (2009) and St. Lucie/Martin Counties (2013)
- BPHL identified first local case of Chikungunya in US in 2014
- Typically test over 50,000 chicken samples per year as part of the surveillance program
- Test horses, wild birds and other exotic animals (e.g. dolphins), in addition to clinical samples

Zika Tests – PCR and ELISA

PCR Test

- PCR will detect Zika virus <14 days after symptom onset
- Use of Lab Developed Test (LDT)
- Detects presence of Zika RNA
- Switched to CDC Trioplex
  - Detects Zika RNA, PLUS Dengue and Chikungunya (serum only)

MAC ELISA Test

- Used as back up to negative PCR, and for asymptomatic priority samples
- Detects immune response to infection by a flavivirus
- Not Zika specific
- Need for follow up testing of positives with plaque reduction neutralization test (PRNT)

Zika Testing – Algorithm

- Specimens collected from all symptomatic individuals < 14 days after the onset of symptoms
  - PCR on serum/urine
  - Negative PCR, test serum for anti-Zika IgM
- Serum collected from symptomatic individuals presenting ≥ 14 days following symptom onset
  - Test serum for anti-Zika IgM
- Asymptomatic pregnant women meeting epidemiological criteria for testing
  - PCR test if exposed (travel etc.) <14 days
  - Anti-Zika IgM if PCR negative
  - Anti-Zika IgM 2-12 weeks following possible exposure

BPHL Response

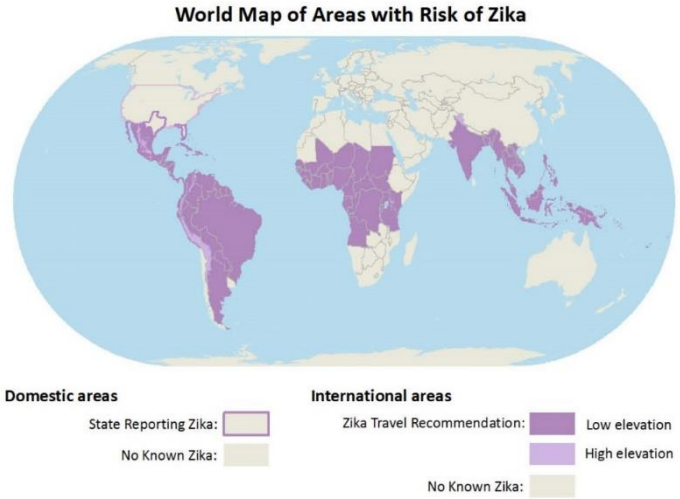
Testing at BPHL

- BPHL-Jacksonville and BPHL-Tampa
  - Zika PCR (LDT) and MAC ELISA Test by January 2016
  - Switched to Trioplex, December 2016
- BPHL-Miami
  - Zika PCR Test (Trioplex) July 2016

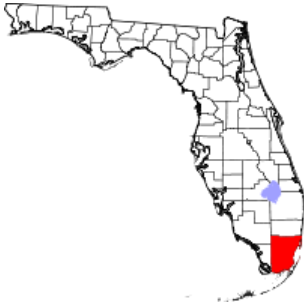
The Laboratory Response

Florida Timeline

- Zika PCR test on board in July 2015
- MAC ELISA test on board January 2016
- Testing samples from international travelers begins January 2016
- First PCR positive traveler identified in January 2016
- Emergency Declaration February 2016

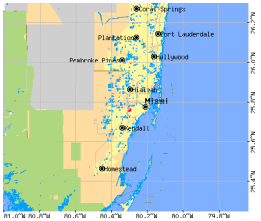


The Laboratory Response

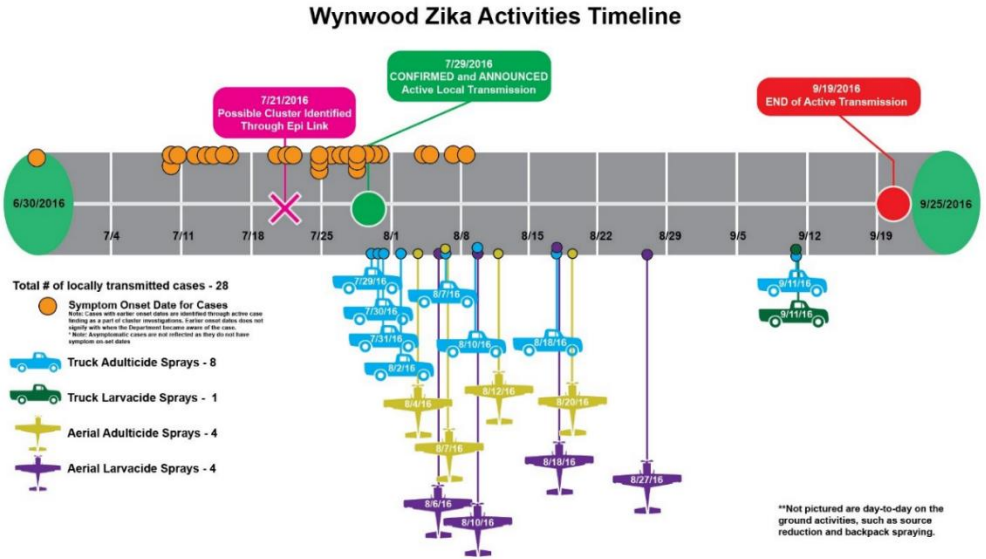


First Local Investigation, July 2016

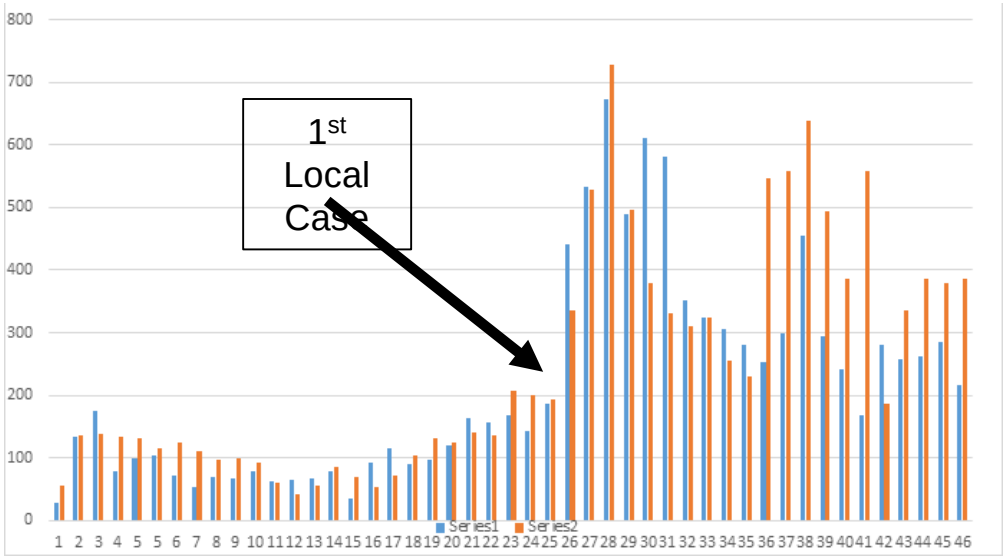
- Identified in Wynwood area of Miami
- PCR detection of Zika virus
- Subsequent testing of friends and family
- Urine surveys of inhabitants of box (area possibly served by mosquito, 150 meters) to identify other possible exposures



The Laboratory Response



The Laboratory Response Testing in 2016



The Laboratory Response

- August 2016
  - Free Zika testing at county health departments for all pregnant women in Florida
  - Approximately 200,000 births per year in Florida
  - Would overwhelm testing in BPHL very quickly
  - Contracted with LabCorp to help with testing asymptomatic pregnant women for Zika IgM

The Laboratory Response Challenges

Instrumentation:

- At the beginning of 2016 BPHL was not geared for Zika surge testing
- Equipment not high throughput:
  - Added later in the year:
    - PCR extraction capacity increased – added MagnaPure 96 robots in each laboratory
    - ELISA testing increased – added additional plate washers and readers in each laboratory

The Laboratory Response Challenges

Staffing:

- Shortage of Zika testing staff in all three laboratories
  - Internal surge, by moving staff around to help out
  - Had to meet state & CLIA licensure regulations
  - External surge – deployment of CDC staff (not licensed)

The Laboratory Response Challenges

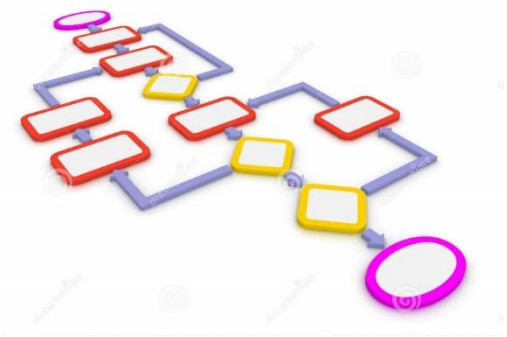
Sample Volume & Integrity:

- Samples arriving from new submitters
- Mislabeled/unlabeled samples
- Poor condition – cracked, leaking
- Reached out to submitters to improve packaging and shipping

The Laboratory Response Challenges

Testing algorithm – a moving target:

- CDC and Florida made changes to the testing algorithm as more was known
- Testing staff had to accommodate and implement changes rapidly
- BPHL had to ensure sample submitters knew of changes



The Laboratory Response Challenges

Data management:

- Complex accessioning
  - Multiple test options depending on patient history
  - Data on submission form often missing (pregnancy, travel, symptom onset)
  - Laboratory Information Management system needed updating as needs changed

The Laboratory Response Challenges

Data management (asymptomatic pregnant women):

- Samples from new partners (hospital labs, clinics)
- Limited information on submission forms
- Where to send laboratory results?

The Laboratory Response Successes

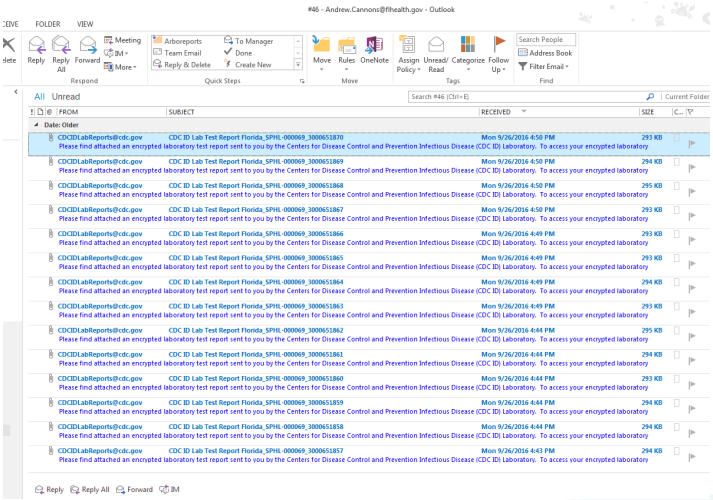
Surge capability:

- BPHL surged internally
  - Each laboratory moved staff around
  - Samples could be redirected to each laboratory in BPHL
  - BPHL-Miami was brought on board for PCR testing when local cases identified
  - CDC deployed staff
- External surge
  - Use of CDC for surge samples – approximately 1500 samples sent over a 6 week period)

The Laboratory Response Challenge/Success

Sending Samples to CDC:

- Initially challenging
  - Added work during accessioning
  - Repackaging samples for shipment
  - Data input/generation of data file for CDC
  - Result and reporting (email nightmare!)



The Laboratory Response Challenge/Success

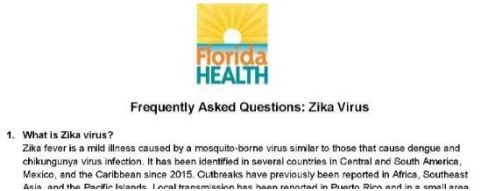
Sending Samples to CDC:

- Challenges became successes:
  - Utilized additional staff with accessioning and repackaging to CDC
  - Utilized dedicated personnel for data input and generation of data file for CDC
  - Developed macro for downloading results

The Laboratory Response Successes

Guidance documents for those submitting samples:

- Developed guidance documents for sample submission
  - Help with what sample types, how to package, how and where to ship
- Developed FAQ documents for those submitting samples
  - Answered the commonly asked questions



Frequently Asked Questions: Zika Virus

1. **What is Zika virus?**
   Zika fever is a mild illness caused by a mosquito-borne virus similar to those that cause dengue and chikungunya virus infection. It has been identified in several countries in Central and South America, Mexico, and the Caribbean since 2015. Outbreaks have previously been reported in Africa, Southeast Asia, and the Pacific Islands. Local transmission has been reported in Puerto Rico and in a small area

Florida Department of Health

Zika Virus Laboratory Diagnosis	Focus Area: Collection, Packaging and Shipping of Laboratory Specimens

Guidance document number 2016-02

Zika Virus Diagnostic Specimen Collection, Packaging and Shipping Guidance for Laboratories and County Health Departments

Version 4.0 February 28, 2017

Summary:

- Authorization: Contact your local county health department for authorization prior to

The Laboratory Response Successes

Communications:

- FL Department of Health utilized an Incident Command Structure and formed an Incident Management Team early on in the outbreak response
- Excellent communications between BPHL, Bureau of Epidemiology and Bureau of Preparedness and Response
- Regular conference calls between all parties
- BPHL held their own daily “huddle” calls

The Laboratory Response Successes

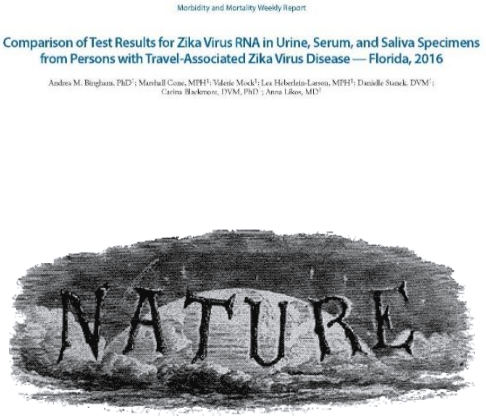
Maintaining A Happy Workforce:

- Worked long hours
- Maintaining a Zika–free zone
  - Food, drink, puzzles, games
- T-shirt competition

The Laboratory Response Successes

Outreach and Publications:

- MMWR publication
  - Use of urine as an additional sample type
    - Virus retained longer than in serum
    - CDC added urine to the Trioplex EUA
- Worked with researchers in Florida to provide extracted nucleic acid for whole genome sequencing
  - Significant studies transmission of Zika virus in Florida
    - At least two Nature publications


Morbidity and Mortality Weekly Report

Comparison of Test Results for Zika Virus RNA in Urine, Serum, and Saliva Specimens from Persons with Travel-Associated Zika Virus Disease — Florida, 2016

Andrea M. Bingham, PhD[1]; Marshall Cone, MPH[1]; Valerie Mock[1]; Lea Hoberlein-Lareau, MPH[1]; Danielle Stanek, DVM[1]; Carina Blackmore, DVM, PhD[1]; Anna Likos, MD[1]

NATURE

The Laboratory Response Successes

Other:

- Commercial laboratories to help with increase in testing – LabCorp, Quest
- Additional staff recruited at all three BPHLs
  - All trained (and licensed)
  - Young staff in public health
- Flexible staff
  - Adjust to changes in testing algorithm
  - Adjust to changes in work
  - Making on the fly changes to practices (e.g. sample accessioning)

BPHL-Tampa:
2016: Average age 44
Median 40
2017: Average age 40
Median 32

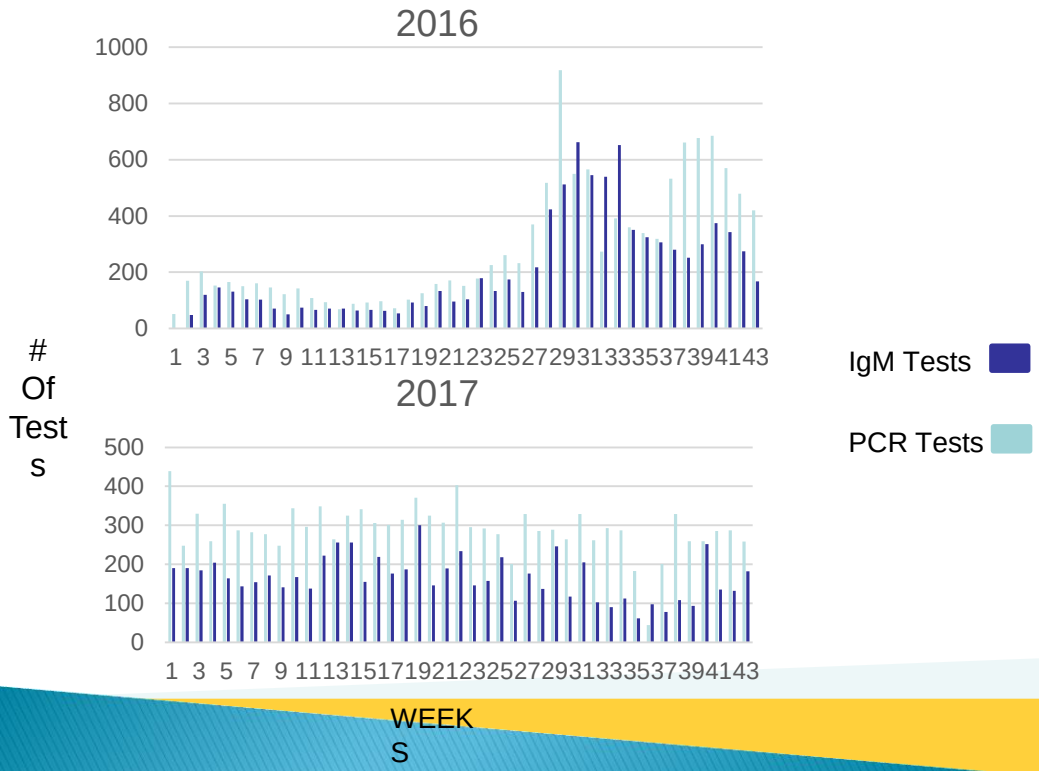
Status of the Laboratory Testing in 2017

- Staff
  - Increased staffing in all three BPHLs for Zika testing
- Equipment
  - Better equipped than 2016
  - Equipment for high throughput (PCR & ELISA)
- Testing Capability
  - Able to manage testing numbers in all three BPHLS
    - PCR at Jacksonville, Miami, Tampa
    - IgM ELISA at Jacksonville, Tampa
    - PRNT at Tampa
- Reporting
  - Improvements in LIMS

Testing Numbers, 2016 versus 2017

2016:
1,115 cases reported in Florida (218 local)

2017:
80 cases reported in Florida (1 local)



Conclusions

- 2016 was a very busy year for BPHL
- Zika testing involved all three laboratories
- Just about all staff in all three laboratories were involved!
- Keys to success:
  - Preparedness, Communication, Flexibility, Troubleshoot often!
- 2017
  - BPHL well prepared for testing
  - Held a face to face meeting with partners (BOE and BPR) to identify successes and challenges

Contact Information

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Thank You

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