Zika Virus:

DCLS – Public Health Lab Perspective

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May 24, 2017

DCLS
Division of Consolidated Laboratory Services

DGS
DEPARTMENT OF GENERAL SERVICES
DIVISION OF CONSOLIDATED LABORATORY SERVICES
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OBJECTIVES

1. **Recap of Zika virus testing in 2016:**
   - **Testing prior to implementation at DCLS.**
   - **DCLS implementation of Zika virus testing.**
   - **Volume of testing performed.**

2. **Discuss Zika virus testing availability:**
   - **Testing algorithm at DCLS.**
   - **Testing performed at the CDC.**

3. **Discuss Zika response challenges.**

4. **Discuss testing at public health lab vs. commercial lab.**

5. **Review new initiatives for 2017.**

ARBOVBIRUS TESTING AT DCLS (PRE-ZIKA)

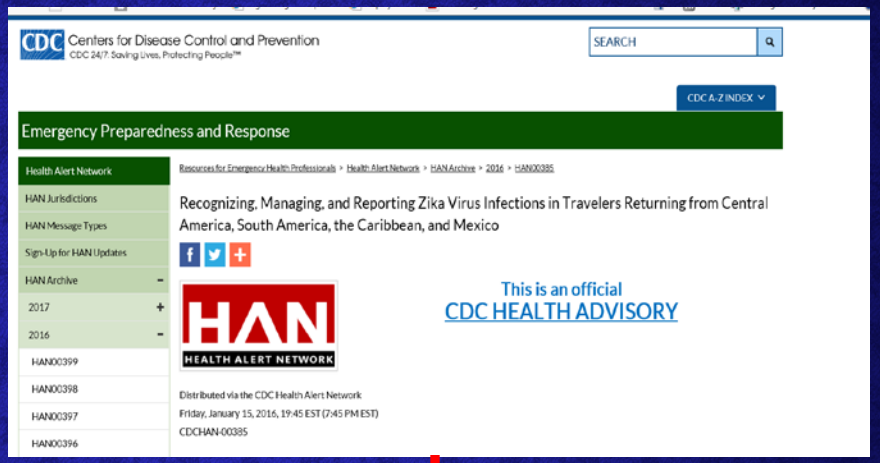
Virus	Test Method	Humans	Mosquitoes	Chickens
Chikungunya	Serology / PCR*	Yes	No	No
Dengue	PCR*	Yes	No	No
Eastern Equine Encephalitis (EEE)	Serology	Yes	No**	Yes
LaCrosse (LAC)	Serology	Yes	No	No
St. Louis Encephalitis (SLE)	Serology	Yes	No	No
West Nile Virus (WNV)	Serology	Yes	No**	Yes

***Real-time PCR testing using individual CDC lab-developed assays for each virus fully validated at DCLS late January 2016.**

**** EEE and WNV real-time PCR testing in mosquito pools was performed previously from 2000-2009.**

HOW DID THIS DYNAMIC RESPONSE BEGIN?

ZIKA VIRUS RESPONSE TIMELINE



Nov (2015)

Dec (2015)

DENV and CHIKV real-time PCR validation testing.

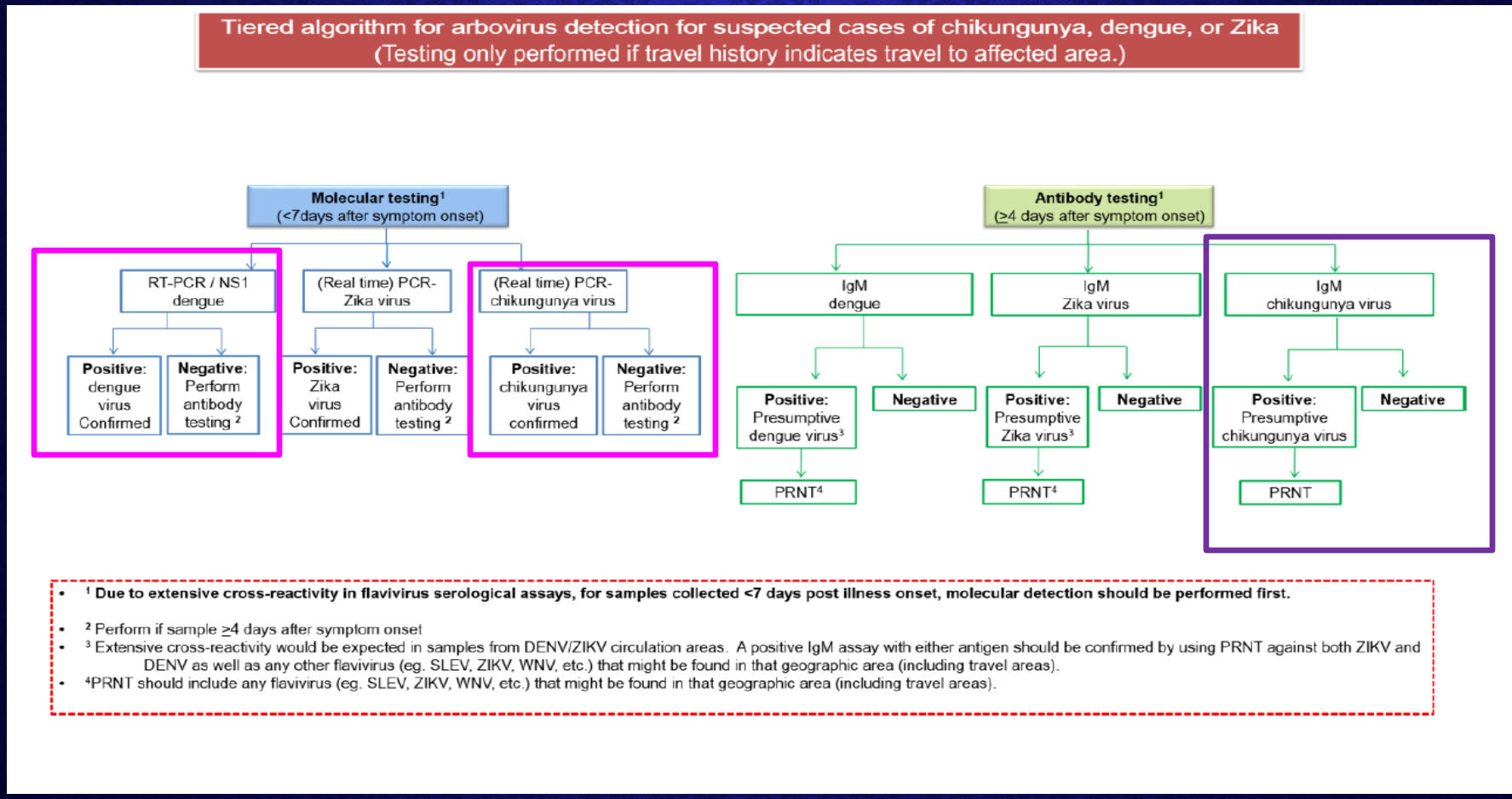
Jan (2016)

January 15, 2016:

- CDC releases testing guidance to SPHLs for Zika virus testing.

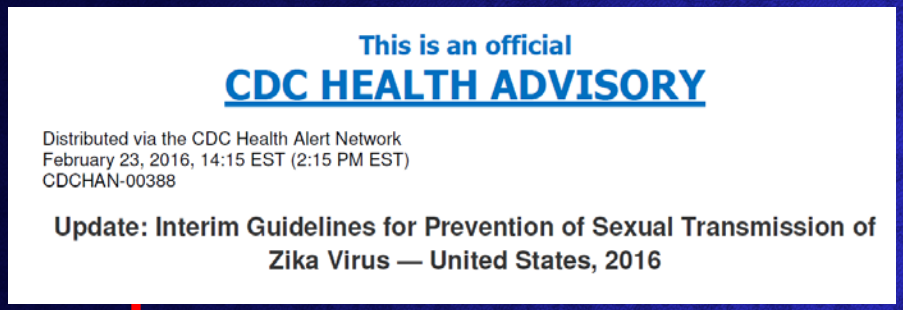
- DCLS requests Zika virus real-time PCR and IgM ELISA assay protocols from CDC.

INITIAL ZIKA VIRUS TESTING ALGORITHM



- **January 28, 2016: Version 1 of DCLS Zika, Dengue and Chikungunya Testing instructions were released.**

ZIKA VIRUS RESPONSE TIMELINE



This is an official

CDC HEALTH ADVISORY

Distributed via the CDC Health Alert Network
February 23, 2016, 14:15 EST (2:15 PM EST)
CDCHAN-00388

Update: Interim Guidelines for Prevention of Sexual Transmission of Zika Virus — United States, 2016

- **14 cases of sexual transmission reported**
- **Recommendation to test asymptomatic pregnant women with sexual exposure**

Feb
(2016)

Mar
(2016)

February 29, 2016:
LRN releases FDA Emergency Use Authorization (EUA) protocol for Zika IgM MAC ELISA

March 18, 2016:
LRN releases FDA Emergency Use Authorization (EUA) protocol for Trioplex real-time PCR assay

ZIKA VIRUS RESPONSE TIMELINE

April 7, 2016:
DCLS receives LRN approval to commence Zika IgM MAC ELISA testing.

April 20, 2016:
DCLS receives LRN approval to commence Trioplex real-time PCR testing.

Detection of Zika virus IgM neutralizing antibodies.

Simultaneous detection of Zika, Chikungunya and Dengue virus RNA in a single PCR reaction.

ZIKA VIRUS RESPONSE TIMELINE

Validation testing of Zika virus real-time PCR testing in mosquito pools

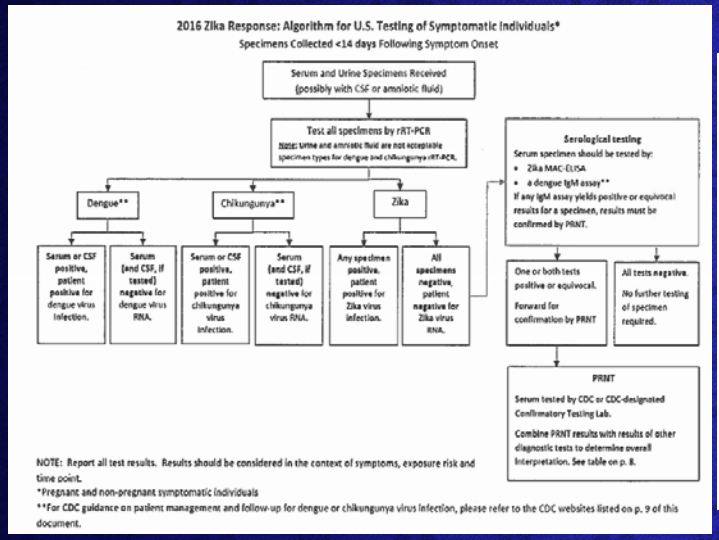
July 4, 2016:
Implementation of Zika virus real-time PCR testing in mosquito pools!

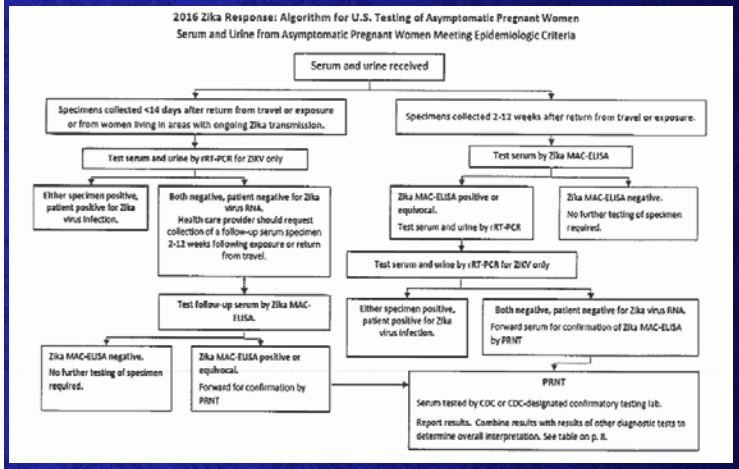
Jun (2016)

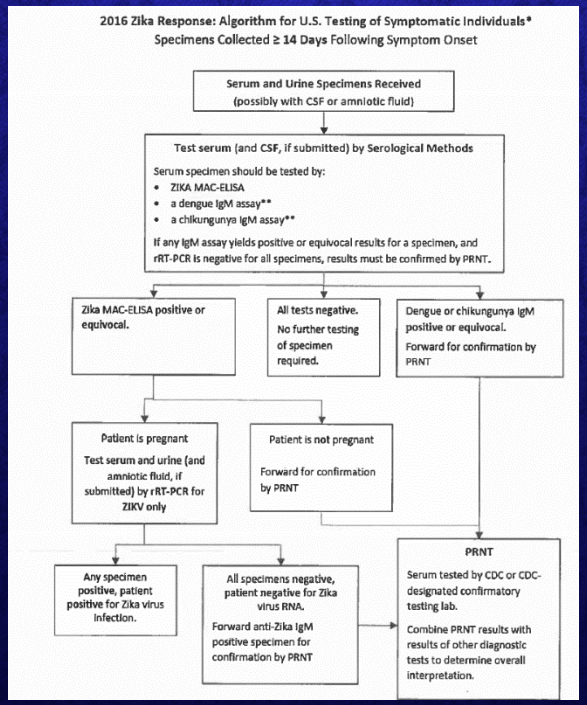
Jul (2016)

July 25, 2016:
CDC Releases Updated Testing Guidance for Symptomatic and asymptomatic pregnant and non-pregnant persons
Updated algorithm = 3 multi-tier flow charts!

CDC UPDATED ZIKA TESTING ALGORITHM







CONDENSED ZIKA TESTING ALGORITHM

Commonwealth of Virginia
Department of General Services
Division of Consolidated Laboratory Services
Richmond, Virginia

Chikungunya, Dengue and Zika Virus Testing

Days between Onset & Specimen Collection OR Last Exposure	Initial Testing	Initial Testing Specimens	Reflex Testing?	Reflex Test	Reflex Testing Specimens	PRNT Confirmation?*
Symptomatic PREGNANT WOMEN						
< 14 days	Trioplex RT-PCR	serum & urine	**YES** - if both specimens are RT-PCR negative	Zika & Dengue IgM ELISA	serum	**YES** - if IgM positive or equivocal
2 - 12 weeks	Zika & Dengue IgM ELISA*	serum	**YES** - if Zika IgM positive or equivocal	Trioplex RT-PCR	serum & urine	**YES** - if both specimens RT-PCR negative
			NO - if Zika IgM negative	n/a	n/a	n/a
Asymptomatic PREGNANT WOMEN						
< 14 days	Trioplex RT-PCR	serum & urine	**NO** - collect convalescent (2 -12 weeks) specimen for Zika IgM testing	n/a	n/a	n/a
2 - 12 weeks	Zika IgM ELISA	serum	**YES** - if Zika IgM positive or equivocal	Trioplex RT-PCR	serum & urine	**YES** - if both specimens RT-PCR negative
			NO - if Zika IgM negative	n/a	n/a	n/a
Symptomatic Patients (NON-PREGNANT)						
< 14 days	Trioplex RT-PCR	serum & urine	**YES** - if both specimens are RT-PCR negative	Zika & Dengue IgM ELISA	serum	**YES** - if IgM positive or equivocal
≥ 14 days	Zika IgM ELISA	serum	n/a	n/a	n/a	**YES** - if IgM positive or equivocal
	Dengue & Chikungunya IgM ELISA		n/a	n/a	n/a	**YES** - if IgM positive or equivocal
Patients Diagnosed with Guillain-Barre Syndrome						
< 14 days of diagnosis	Trioplex RT-PCR	serum & urine	**YES** - if RT-PCR negative	Zika IgM ELISA	serum	**YES** - if IgM positive or equivocal
≥ 14 days	Zika IgM ELISA	serum	n/a	n/a	n/a	**YES** - if IgM positive or equivocal
Infants						
< 14 days from birth	Trioplex & Zika IgM ELISA	serum & urine	n/a	n/a	n/a	**YES** - if IgM positive or equivocal
≥ 14 days from birth	Zika IgM ELISA	serum	**YES** - if Zika IgM positive or equivocal	Trioplex RT-PCR	serum (urine optional)	**YES** - if RT-PCR negative only

Testing algorithm based on most current CDC guidance and recommendations. *Dengue IgM ELISA and PRNT confirmation are performed at the CDC currently.

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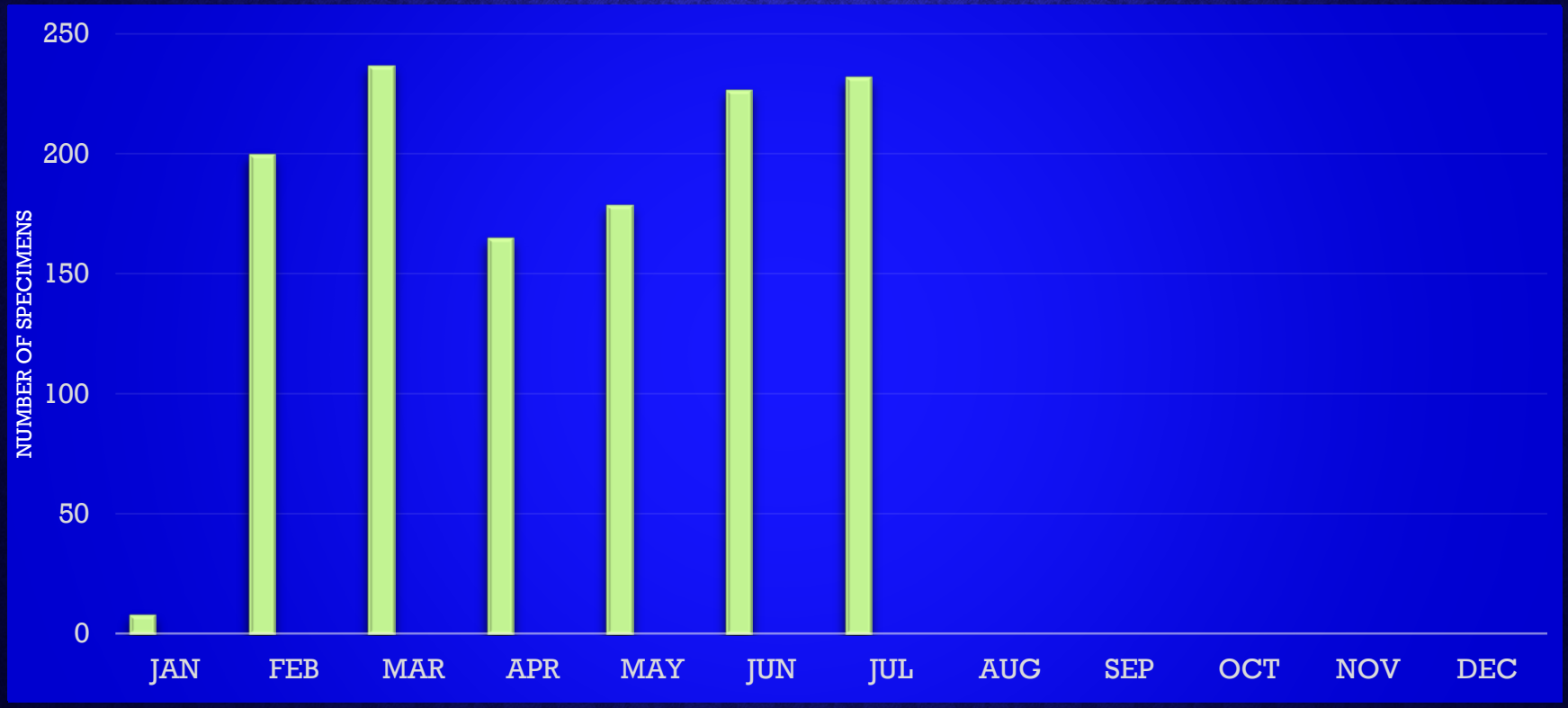
2016 SPECIMEN SUBMISSIONS (JAN – JUL)



ZIKA VIRUS RESPONSE TIMELINE

This is an official

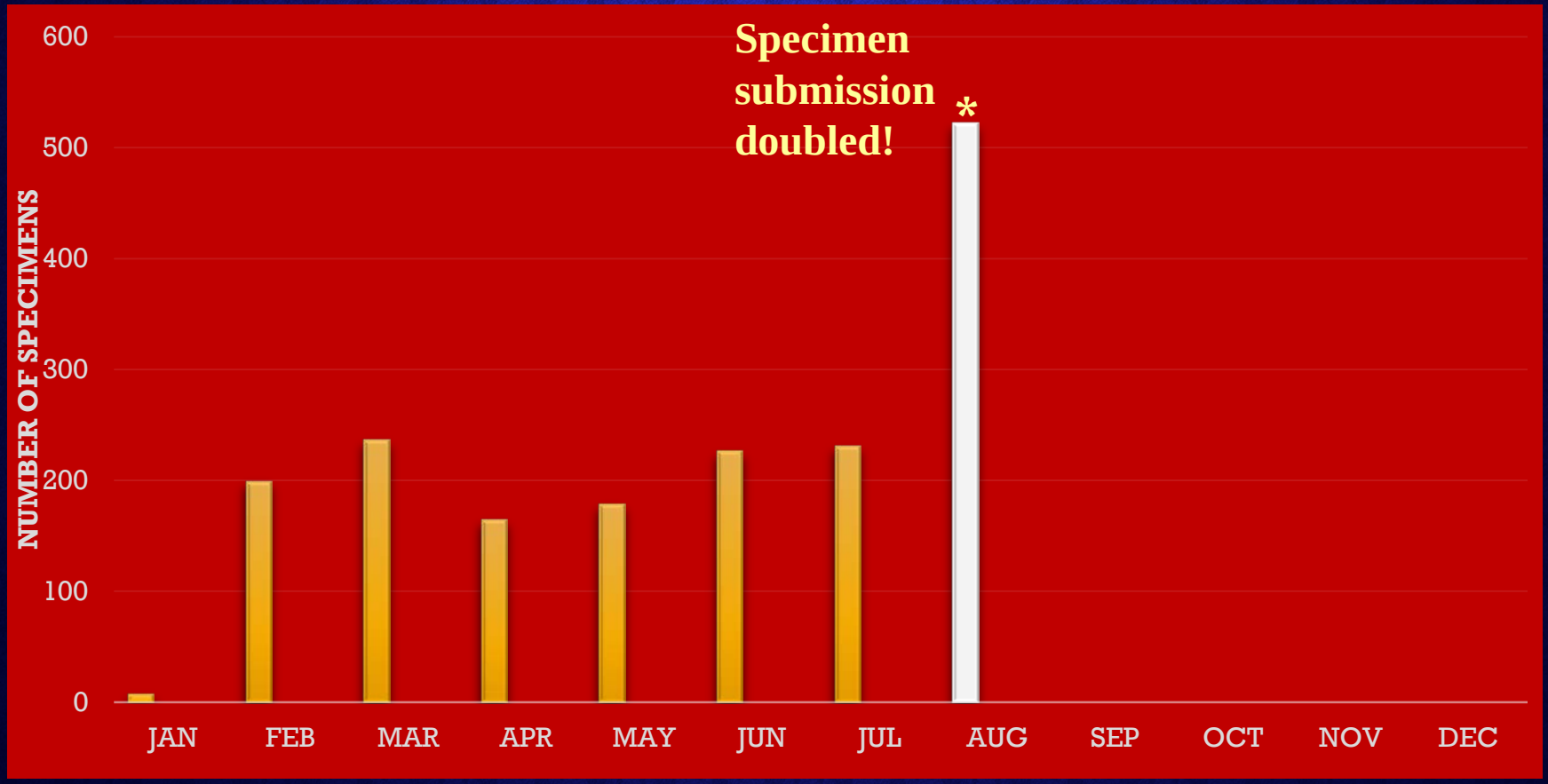
CDC HEALTH ADVISORY

Distributed via the CDC Health Alert Network
August 19, 2016, 1515 ET (3:15 PM ET)
CDCHAN-00394

CDC Expands Guidance for Travel and Testing of Pregnant Women, Women of Reproductive Age, and Their Partners for Zika Virus Infection Related to Mosquito-borne Zika Virus Transmission in Miami-Dade, Florida

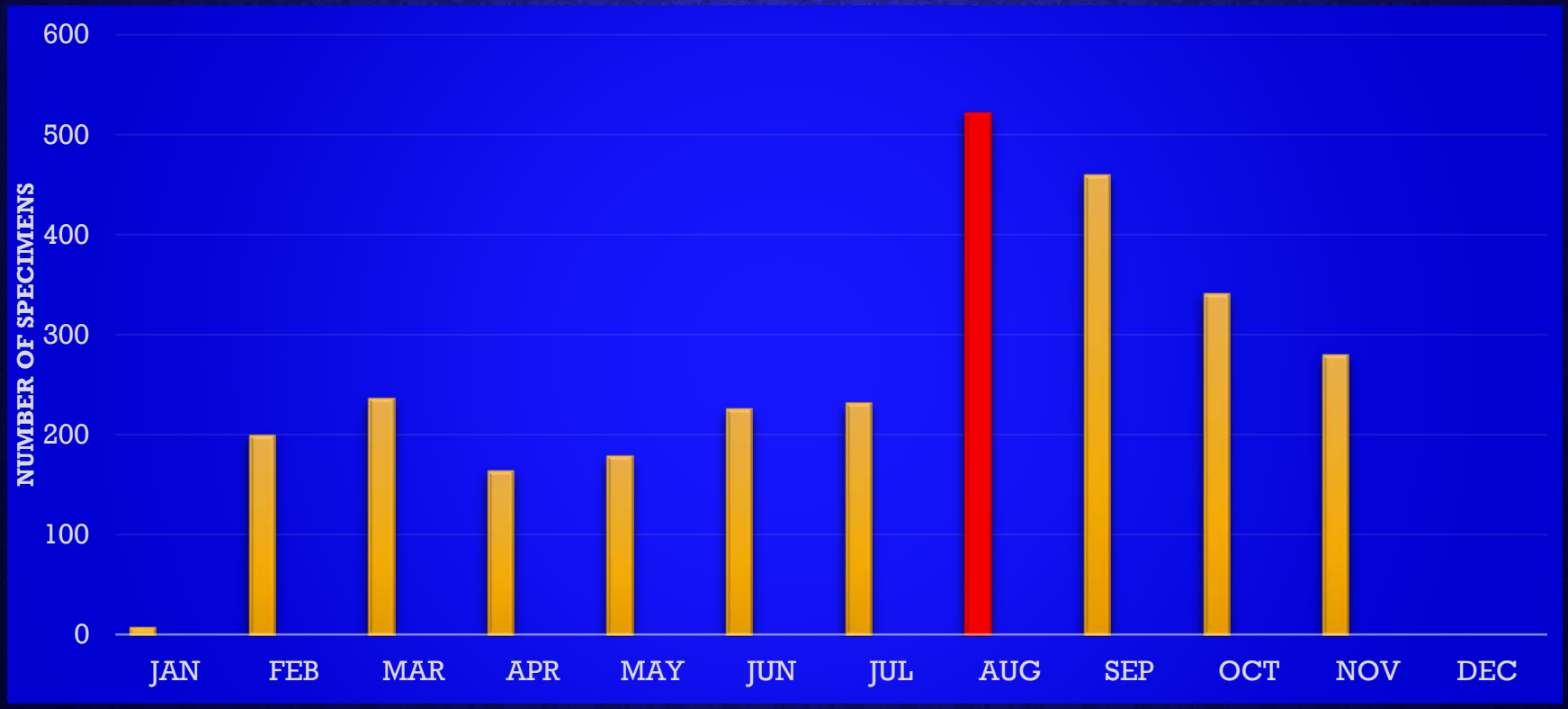
August
(2016)

2016 SPECIMEN SUBMISSIONS (JAN – AUG)



* Specimen submissions doubled upon notification of local transmission in Miami-Dade, FL.

2016 SPECIMEN SUBMISSIONS (JAN – NOV)



ZIKA VIRUS RESPONSE TIMELINE

CDC Guidance for Travel and Testing of Pregnant Women and Women of Reproductive Age for Zika Virus Infection Related to the Investigation for Local Mosquito-borne Zika Virus Transmission in Brownsville, Cameron County, Texas

Language: English

HAN HEALTH ALERT NETWORK

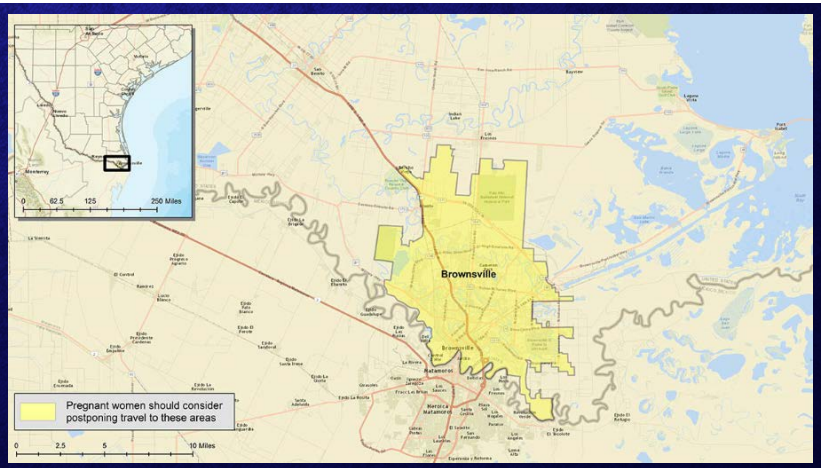
Distributed via the CDC Health Alert Network
December 14, 2016, 16:15 ET (4:15 PM ET)
CDCHAN-00399

This is an official CDC HEALTH ADVISORY

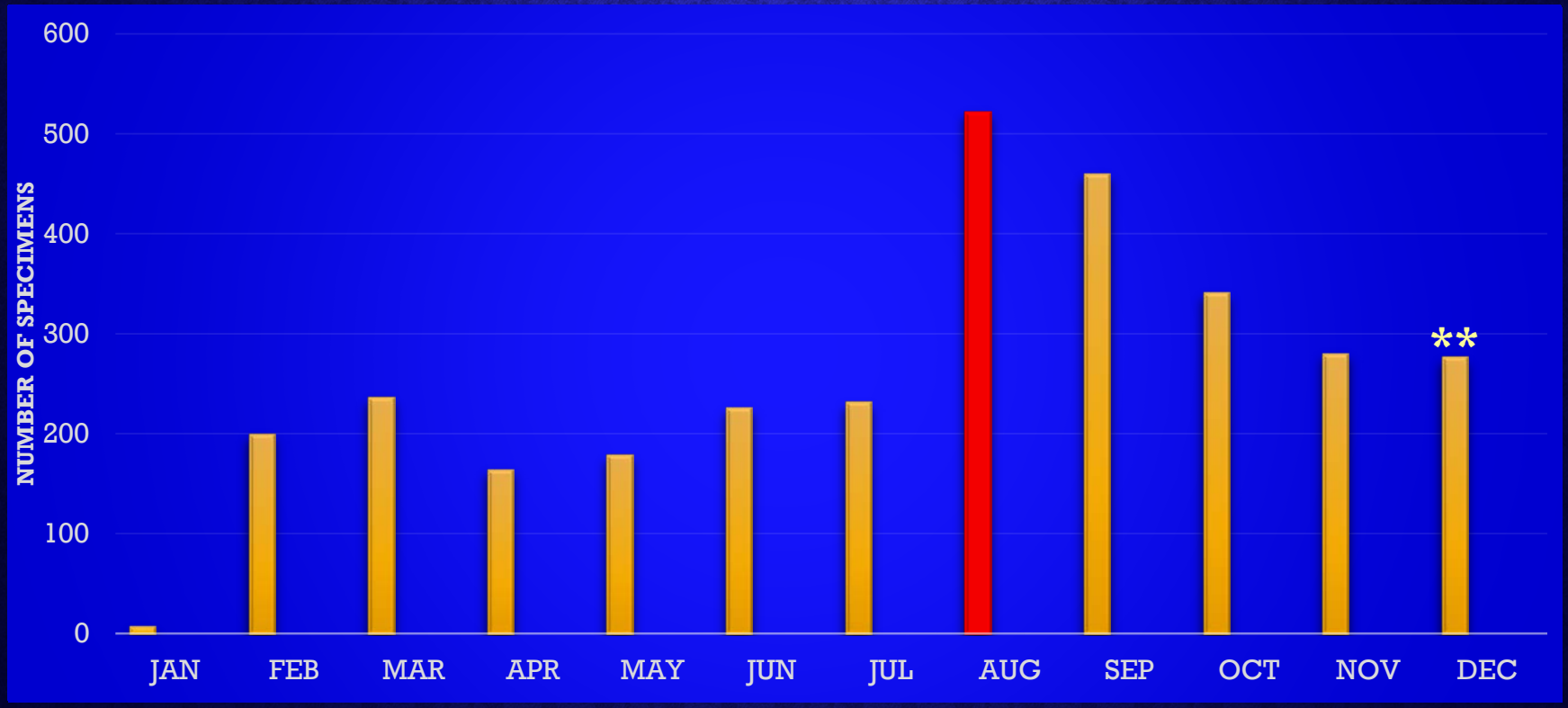
Summary

On November 28, 2016, the Texas Department of State Health Services (TDSHS) reported the first case of locally acquired mosquito-borne Zika virus infection in the city of Brownsville, Cameron County, Texas. On December 9, 2016, four additional cases in people living in proximity to the first case were reported. TDSHS continues to investigate Zika virus transmission in Brownsville.

Dec (2016)



2016 SPECIMEN SUBMISSIONS (JAN – DEC)

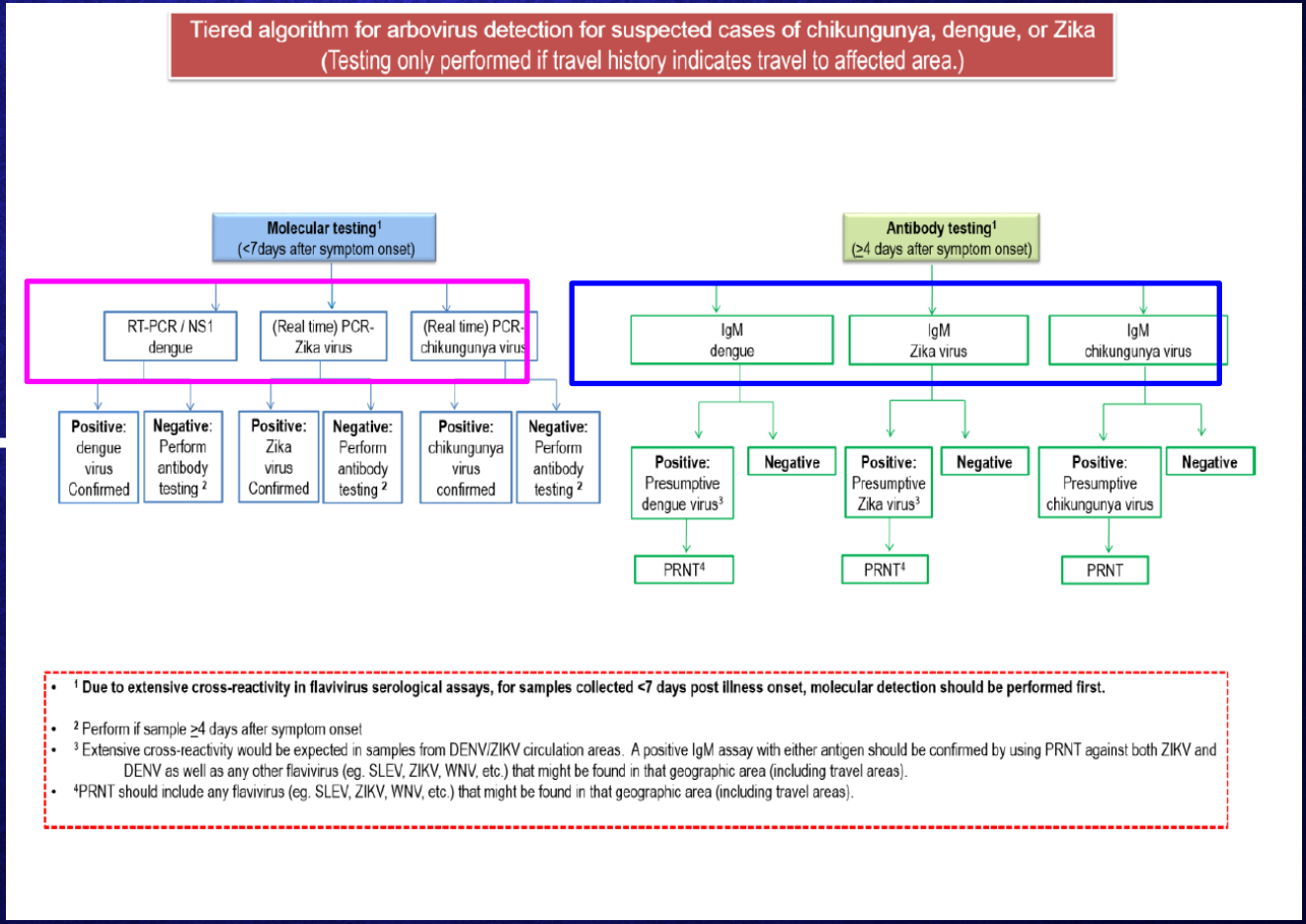


** Only 1 patient with travel to Brownsville, TX approved for testing at DCLS.

ENHANCED TESTING CAPACITY: 2017

January 27, 2017:
DCLS implements Dengue virus IgM ELISA testing (InBios Dengue ELISA kit)

Jan (2017)



ZIKA TESTING ALGORITHM - V2

Commonwealth of Virginia
Department of General Services
Division of Consolidated Laboratory Services
Richmond, Virginia

Chikungunya, Dengue and Zika Virus Testing

Days between Onset & Specimen Collection OR Last Exposure	Initial Testing	Initial Testing Specimens	Reflex Testing?	Reflex Test	Reflex Testing Specimens	PRNT Confirmation?***
Symptomatic PREGNANT WOMEN and Non-Pregnant Individuals						
< 14 days	Trioplex RT-PCR*	serum & urine	**YES** - if both specimens are RT-PCR negative	Zika, & Dengue IgM ELISA	serum	**YES** - if IgM positive or equivocal
2 - 12 weeks	Zika & Dengue IgM ELISA	serum	**YES** - if IgM positive or equivocal	Trioplex RT-PCR	serum & urine	**YES** - if both specimens RT-PCR negative
	Chikungunya IgM ELISA (2nd tier if applicable)		**NO** - if IgM negative	n/a	n/a	n/a
Asymptomatic PREGNANT WOMEN and/or Sexual Partners who traveled to a ZAA**						
< 14 days	Trioplex RT-PCR*	serum & urine	**NO** - collect convalescent (2 -12 weeks) specimen for Zika IgM testing if RT-PCR negative	n/a	n/a	n/a
2 - 12 weeks	Zika IgM ELISA	serum	**YES** - if Zika IgM positive or equivocal	Trioplex RT-PCR	serum & urine	**YES** - if both specimens RT-PCR negative
			NO - if Zika IgM negative	n/a	n/a	n/a
Patients Diagnosed with Guillain-Barre Syndrome						
< 14 days of diagnosis	Trioplex RT-PCR*	serum & urine	**YES** - if RT-PCR negative	Zika IgM ELISA	serum	**YES** - if IgM positive or equivocal
≥ 14 days	Zika IgM ELISA	serum	n/a	n/a	n/a	**YES** - if IgM positive or equivocal
Infants (Suspected congenital infection and/or born to mothers with Zika infection)						
< 3 days from birth (recommended)	Trioplex RT-PCR* & Zika IgM ELISA	serum & urine	n/a	n/a	n/a	**YES** - if IgM positive or equivocal

*** RT-PCR positive = confirmed**
**** ZAA = Zika Affected Area**
***** PRNT confirmation is performed at the CDC.**
Note: Testing algorithm based on most current CDC guidance and recommendations.

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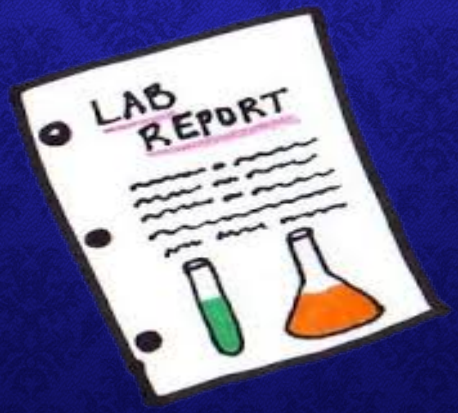
HOW ARE TEST RESULTS REPORTED?



REPORTING LANGUAGE:
TRIOPLEX ASSAY

Trioplex positive results = CONFIRMATORY

Zika virus RNA **detected** OR **not detected** by real-time PCR.
Dengue virus RNA **detected** OR **not detected** by real-time PCR.
Chikungunya virus RNA **detected** OR **not detected** by real-time PCR.
Inconclusive: Trioplex RT-PCR results for the presence of Zika, Chikungunya and Dengue virus RNA were inconclusive, which may occur in the case of inadequate specimen. These results should not be considered negative, as results for the specimen quality control were not able to be obtained. Please recollect and submit another sample for further testing.

REPORTING LANGUAGE:
ZIKA MAC ELISA ASSAY

IgM positive results = PRESUMPTIVE POSITIVE

Presumptive Positive: Serological evidence of possible recent Zika virus infection identified by Zika MAC-ELISA. Additional testing is required. Sample will be forwarded to CDC.
Equivocal: Zika MAC-ELISA results were equivocal for the presence of anti-Zika virus antibodies. This result should not be considered negative or presumptive positive. Additional testing is required. Sample will be forwarded to CDC.
Negative: No evidence of recent Zika virus infection detected by Zika MAC-ELISA. No additional testing is required.
Negative: No evidence of recent Zika virus infection detected by Zika MAC-ELISA. This result reflects testing performed during the acute phase of illness. Please submit a convalescent sample collected more than 14 days post symptom onset for additional testing.
Inconclusive: Attributes of the specimen interfered with the Zika MAC-ELISA and prevent a conclusive result interpretation. Please submit a sample collected more than 14 days post symptom onset for additional testing.

REPORTING LANGUAGE:
DENGUE ELISA ASSAY

IgM positive results = PRESUMPTIVE POSITIVE

Dengue IgM ELISA Negative: No detectable Dengue virus IgM antibody. This result does not rule out Dengue virus (DENV) infection. An additional sample collected within 7-14 days after the previous sample draw date should be submitted if an early infection is suspected.
Dengue IgM ELISA Equivocal: Dengue Virus IgM antibody cannot be determined. Additional testing is required. Sample will be forwarded to the CDC.
Dengue IgM ELISA Positive: The presence of detectable Dengue virus IgM antibody indicates presumptive infection with Dengue virus (DENV). This result must be confirmed by an alternate method. Sample will be forwarded to the CDC. A positive IgM result may not indicate a recent infection as IgM may persist several months after infection.

REPORTING LANGUAGE:
CHIKUNGUNYA ELISA ASSAY

IgM positive results = PRESUMPTIVE POSITIVE

Chikungunya IgM Positive: IgM antibodies specific to Chikungunya virus detected by MAC ELISA assay.
Chikungunya IgM Negative (<8 days): IgM antibodies specific to Chikungunya virus not detected by MAC ELISA assay. Please submit a convalescent specimen.
Chikungunya IgM Negative (>8 days): IgM antibodies specific to Chikungunya virus not detected by MAC ELISA assay. No additional testing required.
Nonspecific: Nonspecific reactivity was noted in the MAC ELISA assay that detects IgM specific to Chikungunya virus, thus the result is uninterpretable.

ZIKA VIRUS TESTING SUMMARY - 2016

- Total specimens received at DCLS = 3,030 (2,021 patients).
  - 1562 pregnant women (77%)
  - 86 infants (4%)
  - 183 men (9%)

- Total specimens tested at DCLS:
  - Zika IgM ELISA = 1,524
    - 47 presumptive positive
    - 16 equivocal
    - 5 inconclusive
  - Chikungunya IgM ELISA = 673
    - 16 presumptive positive
    - 43 equivocal
    - 6 non-specific reactivity
  - Trioplex real-time PCR = 856
    - 59 specimens (44 patients) = Zika virus RNA detected
    - 1 specimen (1 patient) = Dengue virus RNA detected
    - 1 specimen (1 patient) = Chikungunya virus RNA detected

- Total number of mosquito pools tested = ~840 (~34,000 mosquitoes).
- Total number of specimens tested for other states (n = 2) = 595.

CHALLENGES

1. **Evolving testing algorithms and approval criteria.**
   - **Threshold for acute vs. post-acute?**
   - **Revision to algorithm coming soon??**

2. **Complex testing algorithm.**
   - **Reflex testing vs no reflex testing?**
   - **When to perform Dengue and Chikungunya IgM testing?**

3. **Extended TAT for confirmatory results from the CDC (2-3 months).**

4. **IgM ELISA testing cross-reactivity.**
   - **Flavivirus infection vs. Dengue virus infection vs. Zika virus infection**

5. **Additional validation studies following LRN testing modifications.**

CHALLENGES

6. **Submission errors:**
   - Missing date of onset and/or date of specimen collection.
   - Missing travel location and return dates.
   - Missing complete submitter information.
   - Mom's submission forms submitted with infant's specimens.
   - Identifying fetal tissue as fixed or fresh.
   - Infant name discrepancies between specimen and forms (hospital name
   - identifier vs. legal name given by parents)

7. **LIMS upgrade delayed; manual reports for all results for the first 6 months.**

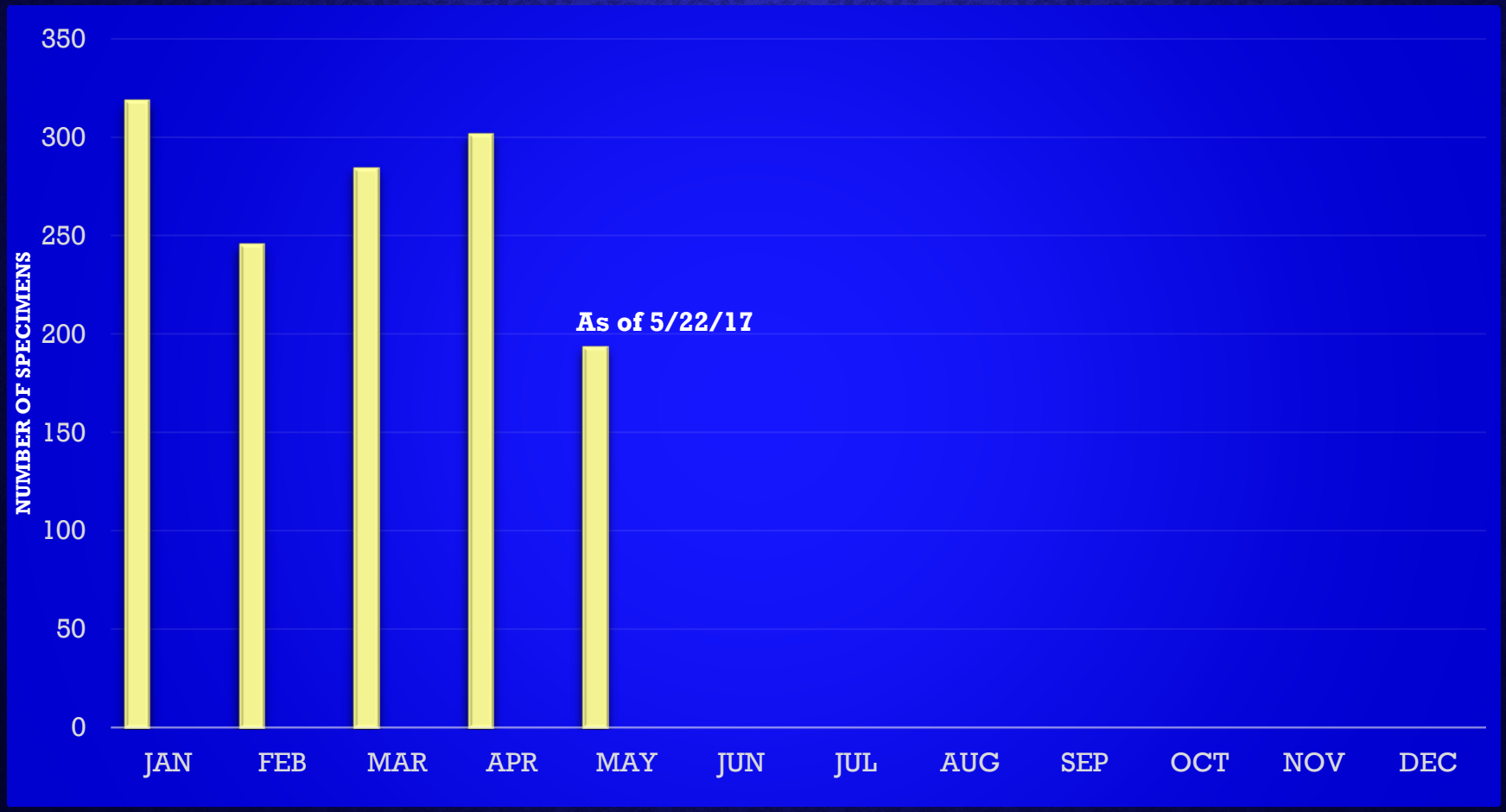
8. **Results reporting: DCLS reporting policy to provide results to submitter on DCLS test request form only.**
   - **What if physician is not listed as the submitter?**

9. **Specimen storage – lots and lots of serum and urine specimens.**

AND

10. **Daily high volume submission of specimens CONTINUES!**

2017 SPECIMEN SUBMISSIONS



CDC ZIKA TESTING

CDC – Fort Collins, CO: Division of Vector Borne Diseases

- Arbovirus testing:
  - WNV, EEE, Powassan, Zika, Chikungunya, Dengue, SLE, EEE, Yellow Fever, Japanese Encephalitis
- Test methods:
  - Real-time PCR
  - IgM ELISA
  - Plaque Reduction Neutralization Test (PRNT)
- Acceptable specimen types (no pre-approval):
  - Serum
  - Urine*
  - CSF*
  - Fresh/frozen tissue*
  - Amniotic fluid*
  - Other specimen types*

CDC – Atlanta, GA: Infectious Disease Pathology Branch

- Arbovirus testing:
  - Zika
- Test methods:
  - Real-time PCR
- Acceptable specimen types (pre-approval required):
  - Fixed fetal tissue
- Mom and infant metadata:
  - Mom's exposure/travel histor
  - Illness onset
  - Mom and child test results
  - EDD
  - Pregnancy outcome / gestational age
  - Ultrasound results
  - birth anthropometric measurements / physical findings
  - Additional infant test results

ZIKA VIRUS TESTING AT PUBLIC HEALTH VS COMMERCIAL LAB: PHL PERSPECTIVE

- **Benefits**:
  - **Surge support testing resource.**
  - **Capacity for high-throughput serological testing.**
  - **Routine testing of non-priority individuals (pre-pregnancy screening, non-pregnant w/exposure or travel, etc.).**

- **Challenges:**
  - **Reproducibility of commercial lab test results:**
    - **Technical error?**
    - **sample degradation (freeze thaw, transport)?**
    - **LOD?**
    - **Reagent/kit variability (e.g. serological testing reagents)?**
  - **Submission errors when referring samples to PHL.**
  - **Communication**

WHAT'S NEW IN 2017?

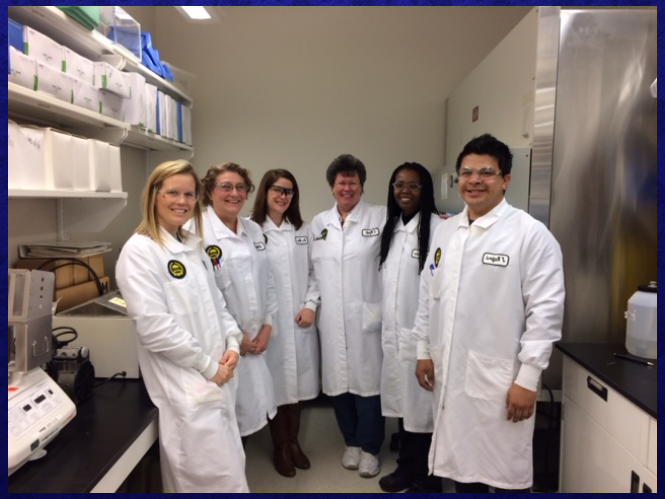
- **Changes to the CDC testing algorithm – coming soon?!!!**
- **Validations:**
  - **CSF, whole blood and amniotic fluid specifor real-time PCR testing.**
  - **Automated nucleic acid extraction platforms.**
  - **Quant Studio Dx real-time PCR platform.**
- **Verification of single-plex assays using Trioplex reagents.**
- **Yellow Fever preparedness.**
  - **APHL plans to work with CDC to distribute protocols on a national level.**
- **Real-time PCR testing of WNV and EEE in mosquito pools.**

DCLS ZIKA RESPONSE TEAMS

Sample Receipt/Accessioning (SSS)

Trioplex Testing - MDC

Serological Testing – I/V



Mosquito Testing - MDC