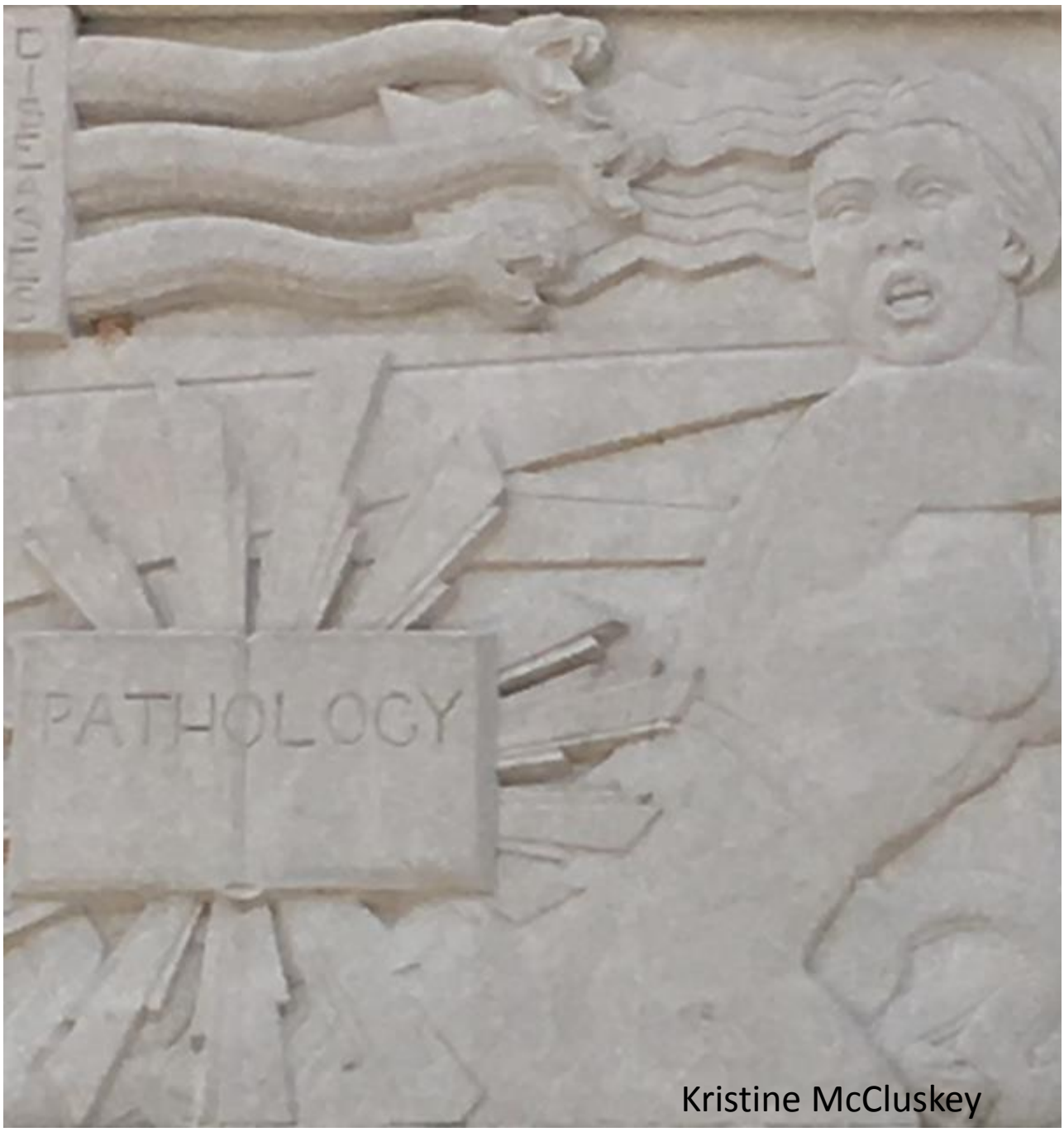# Zika Virus Tissue Protocol Purpose Defined through Algorithms in Anatomic Pathology for Trainees

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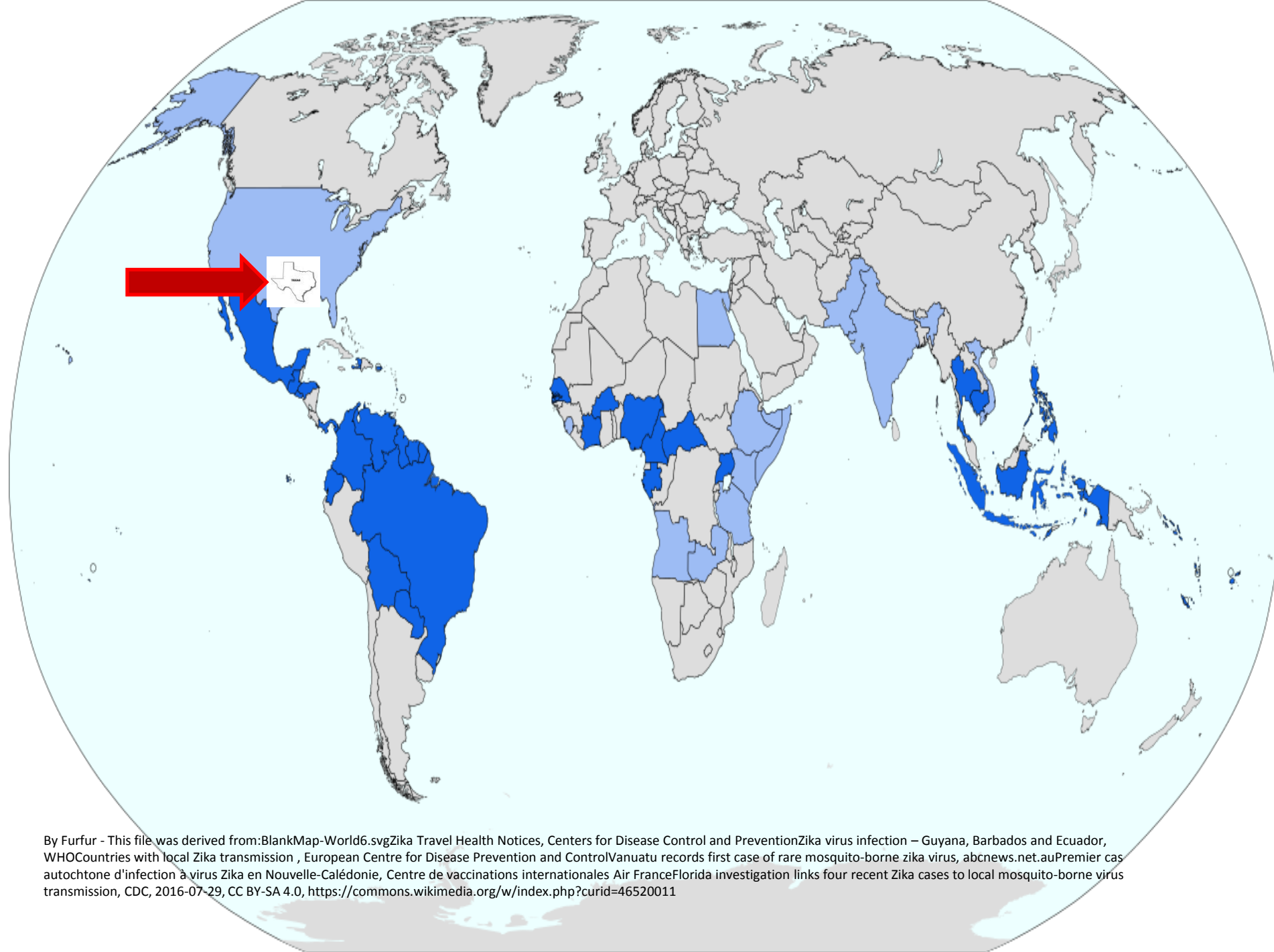August 27th, 2018

Baylor
College of
Medicine

PATHOLOGY

Kristine McCluskey

Disclosures

- No financial disclosures are present.
- This presentation contains photographs of a graphic nature.

Current US trend:

- no ZIKV testing occurs unless clinically driven
- standardized testing could incur a large expense
- Blood donations have been tested since 2016, but found to be costly[6]

TEXAS

HOUSTON

2015-2017 Texas reported 378 cases of illness due to ZIKV. These cases include 7 transmitted by mosquitoes and all others were travel associated, through sexual contact or passed from mother to child prior to birth[7].

Texas Zika Cases by County:

County (A – Ja)	2015 Cases	2016 Cases	2017 Cases
Angelina	0	2*	0
Bastrop	0	1	0
Bell	0	7	0
Bexar	0	21	4
Brazoria	0	2	1
Brazos	0	4	1
Burnet	0	1	0
Cameron	0	26†	14‡
Collin	0	8	3
Dallas	0	44*	3
Denton	0	9	1
El Paso	0	3	0
Ellis	0	1	0
Fort Bend	1	11	0
Frio	0	1	0
Galveston	0	9	0
Gray	0	1	0
Grayson	0	1	0
Gregg	0	1	0
Hamilton	0	1	0
Harris	7	74	11
Hays	0	1	0
Hidalgo	0	6	8‡
Hockley	0	1	0
Jackson	0	1	0

County (Je – W)	2015 Cases	2016 Cases	2017 Cases
Jefferson	0	2	0
Jones	0	1	0
Kerr	0	0	1
Lee	0	1	0
Lubbock	0	1	1
Matagorda	0	1	0
Medina	0	1	0
Midland	0	1	0
Montgomery	0	1	0
Navarro	0	1	0
Palo Pinto	0	1	0
Parker	0	1	0
Randall	0	1	0
Rusk	0	1	0
Smith	0	1	2
Starr	0	1	0
Tarrant	0	28	1
Travis	0	18	1
Upshur	0	1	1
Val Verde	0	1	0
Walker	0	1	0
	0	6	1
Willacy	0	1	0
Williamson	0	5	1
Wise	0	1	0
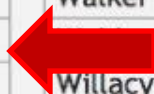
In 2015 there were a total of 8 confirmed Zika cases and 315 in 2016. There have been 55 cases reported for 2017, though that number could still change.

http://www.texaszika.org

TEXAS

HOUSTON

Since 2016, United States (US) Zika virus (ZIKV) transmission provoked our pathology residents, and pathologists' assistant students to follow fetal and placental tissue sampling protocols recommended by the Centers for Disease Control (CDC)

Hurricane Harvey
August 25th-30th, 2017

CNN.com

Unpublished data emerged at our institution suggesting viral presence in fetal and placental tissue without maternal viral positivity implying the importance of adequate training regarding prosection and sampling[4]

Current research demonstrated that ZIKV persisted in fetal tissue which could result in Congenital Zika Syndrome[3]

Unbeknownst of recent findings, learners procuring samples required repeated updated protocol review and often questioned purpose behind submission to the CDC.

In response, we designed two traditional algorithms combining our work flow and resources beginning with identifying presumed ZIKV transmitted specimens to receiving verification of ZIKV infection and the repercussions thereafter.

Methods

Survey-prior

- 1. Do you understand the Zika sampling guidelines for placentas, POCs and autopsies?
- 1-not at all, 2-somewhat, 3-yes, with complete understanding
- 2. Do you know the workflow of the sampling procedure?
- 1-not at all, 2-somewhat, 3-yes, with complete understanding
- 3. Do you understand the significance of knowing the previous history of POCs, placentas and fetal autopsies?
- 1-not at all, 2-somewhat, 3-yes, with complete understanding
- 4. Can you name the features of the Congenital Zika Syndrome?
- 1-not at all, 2-somewhat, 3-yes, with complete understanding

Observation of Learners-Prior

- 1. Are the previous histories being checked for POCs and placentas on a regular basis pertaining to Zika exposure or symptoms?
- 2. Is there an understanding of the end result to sampling?
- 3. Is proper sampling occurring?
- 4. Is there an exchange into preservation fluids in a timely fashion?

Congenital Zika Syndrome

- Microcephaly that may result in a collapsed skull
- Thin cerebral cortex
- Eye anomalies, macular scarring and focal pigmentary retinal mottling
- Congenital contractures, limited range of motion, clubfoot
- Marked early hypertonia
- Infants with normal head circumference may have brain abnormalities[1]

Typical Head Size

Typical Head Size

Baby with Typical Head Size

Baby with Microcephaly

Baby with Severe Microcephaly

https://www.cdc.gov/pregnancy/zika/testing-follow-up/zika-syndrome-birth-defects.html

The Algorithms:

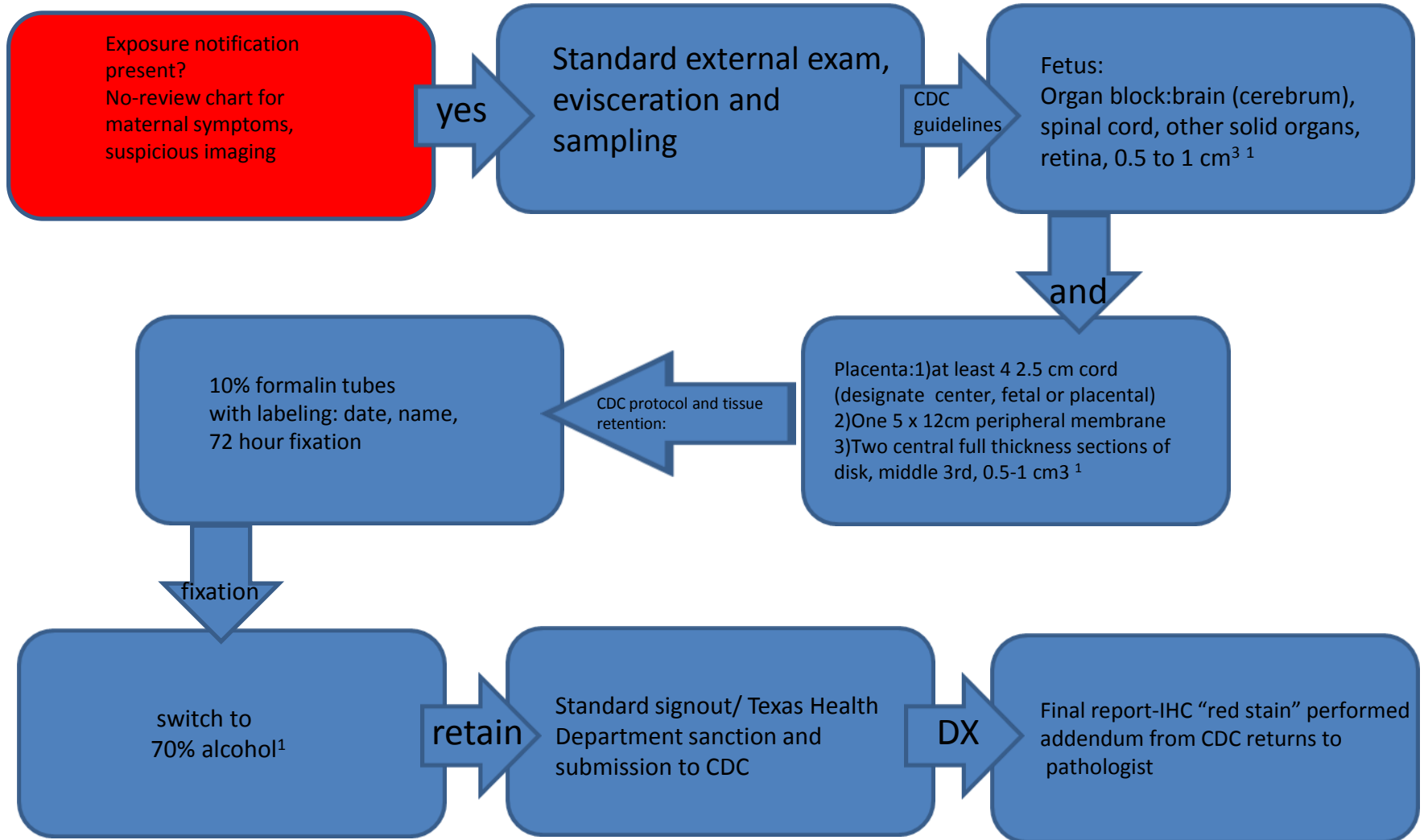# Placentas, POCs, fetuses

Exposure notification present?
No-review chart for maternal symptoms, suspicious imaging

yes →

Standard grossing procedure

CDC guidelines →

Placenta:1)at least 4 2.5 cm cord (designate center, fetal or placental) 2)One 5 x 12cm peripheral membrane 3)Two central full thickness sections of disk, middle 3rd, 0.5-1 cm3 [1]

or ↓

POC:4 sections[1]

or ←

Fetus:
Organ block:brain (cerebrum), spinal cord, other solid organs, retina, 0.5 to 1 cm^3 [1]

CDC protocol and tissue retention: ←

10% formalin tubes with labeling: date, name, 72 hour fixation

fixation ↓

switch to 70% alcohol[1]

retain →

Standard signout/ Texas Health Department sanction and submission to CDC

DX →

Final report-IHC "red stain" performed addendum from CDC returns to pathologist

Autopsy

Exposure notification present?
No-review chart for maternal symptoms, suspicious imaging

yes →

Standard external exam, evisceration and sampling

CDC guidelines →

Fetus:
Organ block:brain (cerebrum), spinal cord, other solid organs, retina, 0.5 to 1 cm^3 [1]

and ↓

Placenta:1)at least 4 2.5 cm cord (designate center, fetal or placental) 2)One 5 x 12cm peripheral membrane 3)Two central full thickness sections of disk, middle 3rd, 0.5-1 cm3 [1]

← CDC protocol and tissue retention:

10% formalin tubes with labeling: date, name, 72 hour fixation

↓ **fixation**

switch to 70% alcohol[1]

retain →

Standard signout/ Texas Health Department sanction and submission to CDC

DX →

Final report-IHC "red stain" performed addendum from CDC returns to pathologist

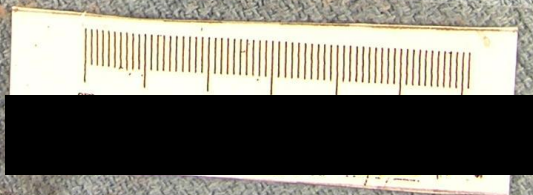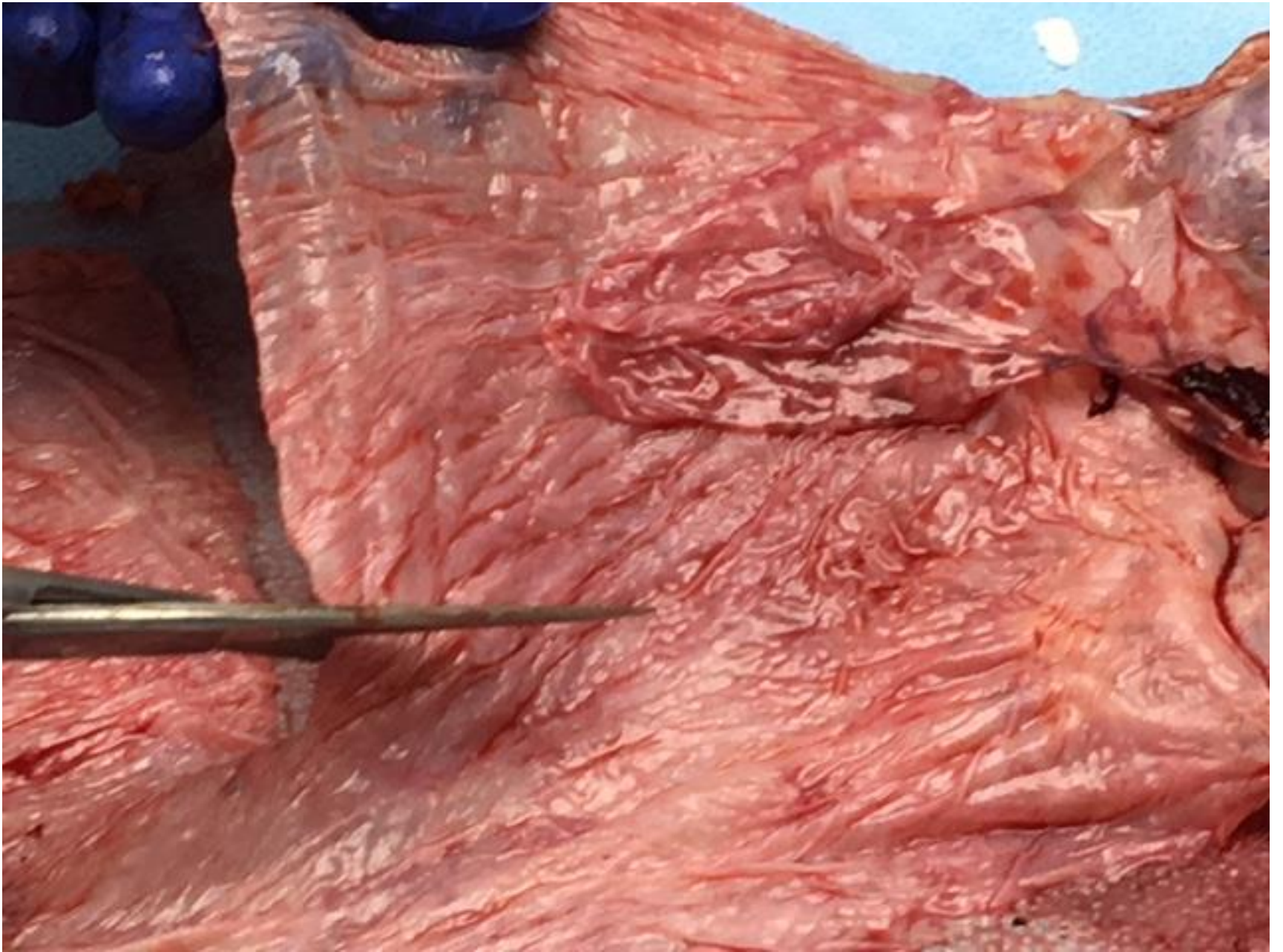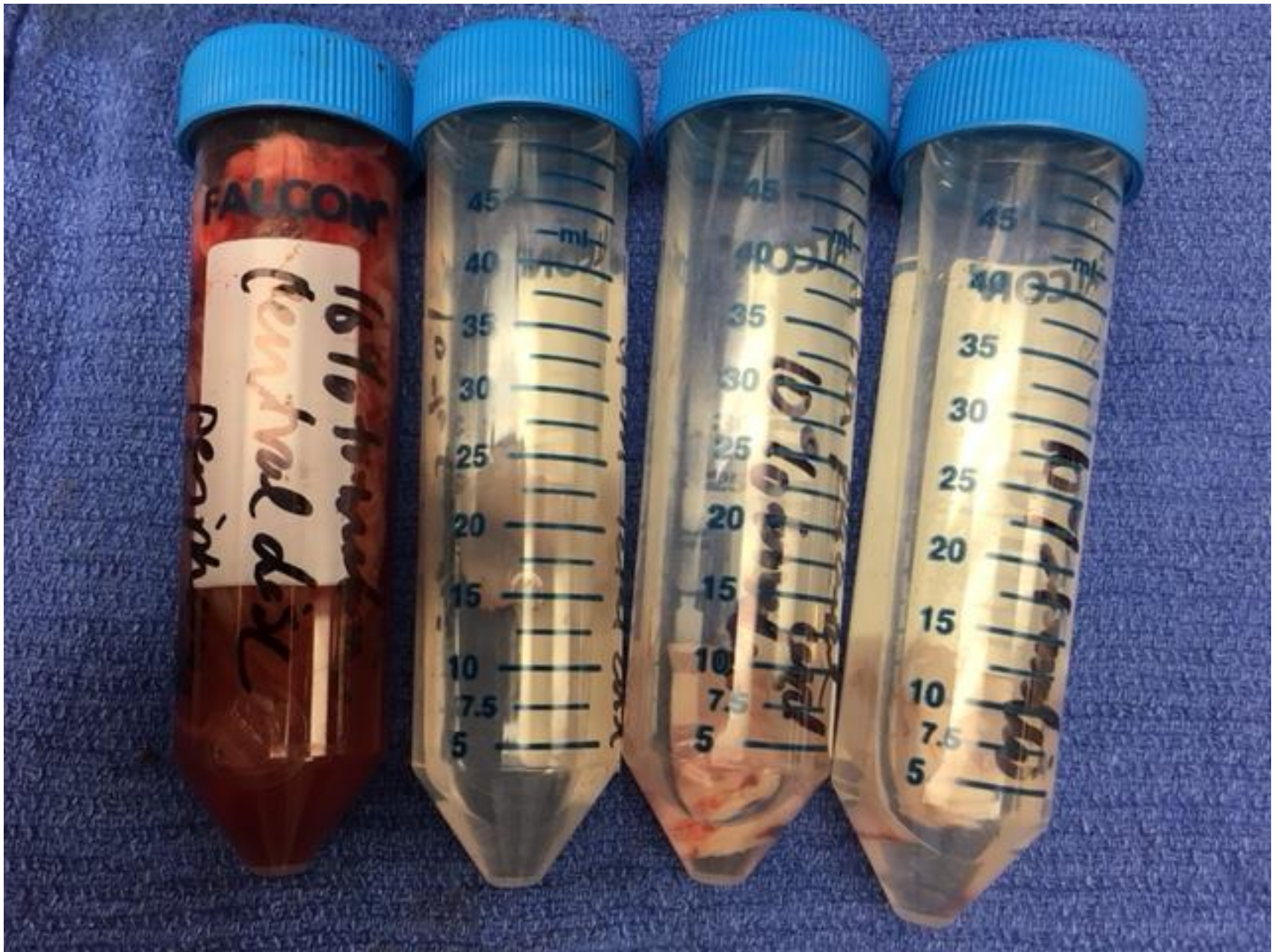https://acdbio.com/science/applications/disease-areas/zika-virus-research

Survey-post

- 1. Were the algorithms easy to follow?
- 1-not at all, 2-somewhat, 3-yes, with complete understanding
- 2. Are you more confident handling placentas/fetus for Zika when using the algorithms?
- 1-not at all, 2-somewhat, 3-yes, with more confidence
- 3. Do you think the algorithms was helpful when grossing placentas/fetuses?
- 1-not at all, 2-somewhat, 3-yes, completely helpful
- 4. Did the algorithms give you a better understanding of the Zika protocol and how tissue is handled beyond sampling?
- 1-not at all, 2-somewhat, 3-yes, with complete understanding

Observation of Learners-Post

- 1. Are the previous histories being checked for POCs and placentas on a regular basis pertaining to Zika exposure or symptoms?
- 2. Is there an understanding of the end result to sampling?
- 3. Is proper sampling occurring?
- 4. Is there an exchange into preservation fluids in a timely fashion?
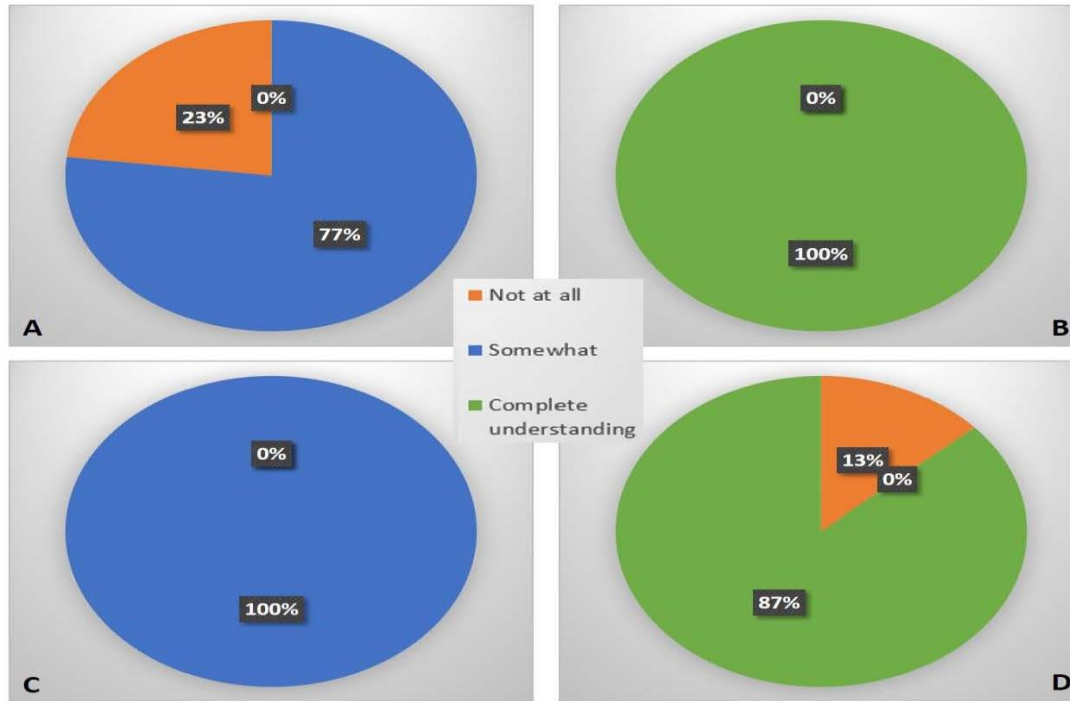
Results
N=13, 6 month period

Figure 1. Assessment of knowledge and grossing technique prior and post Zika specimen algorithm training. (A. Percentage of understanding Zika sampling guideline prior to algorithm; B. Percentage of understanding Zika sampling guideline post to algorithm; C. Percentage of proper sampling completed prior to algorithm; D. Percentage of proper sampling completed post to algorithm)
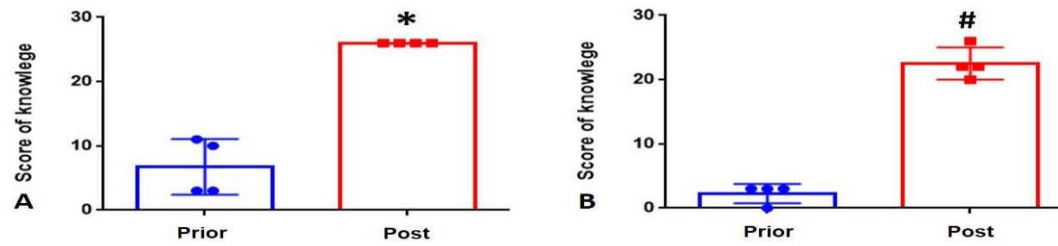
Figure 2. Average score of knowledge and grossing technique prior and post Zika specimen algorithm training. (A. Score of knowledge prior and post algorithm; B. Score of grossing technique prior and post algorithm; * P<0.01; # P<0.001)

Assessment of trainee's knowledge about Zika infection and sampling guideline	Prior/Post algorithm training N=13, 3 PA students and 10 residents			Prior/Post Score of Knowledge	P Value
	Not at all	Somewhat	Yes with complete Understanding		
Do you understand the Zika Sampling guidelines for placenta, POCs and autopsies?	76.9%/0% (0/0)	23.1%/0% (3/0)	0%/100% (0/26)	(3/26)	< 0.0001
Do you follow the workflow of the sampling procedure?	76.9%/0% (0/0)	23.1%/0% (3/0)	0%/100% (0/26)	(3/26)	< 0.0001
Do you understand the significance of knowing the previous history of POCs, placenta and fetal autopsies?	15.4%/0% (0/0)	84.6%/0% (11/0)	0%/100% (0/26)	(11/26)	< 0.0001
Can you name the features of the congenital Zika syndrome?	23.1%/0% (0/0)	76.9%/0% (10/0)	0%/100% (0/26)	(10/26)	< 0.0001
Average score of knowledge	N/A	N/A	N/A	(6.75/26)	0.003

Table 1. Assessment of trainee's knowledge about Zika infection and sampling guideline prior and post algorithm training. (Scoring system: Not at all=0; Somewhat=1; Complete understanding=2)

Dates of specimen receipt	POC (Products of Conception)	Fetus with POC	Fetal autopsy	placenta	Zika exposure Yes/no	Sent to CDC	CDC result

POC, Placenta, Autopsy Count

year	Number of POCs, placentas, fetuses (w/o autopsy)	Number of fetal autopsies	Results from CDC-specimens	Results from CDC-autopsy
2016 12 months	725 23 possible ZIKV 3.17%	15 0 possible ZIKV 0%	5 sent, 3 returned= negative or canceled-over fixation	0 sent
2017 12 months	587 22 possible ZIKV Post HH 3.75%	3 2 possible ZIKV 66.67%	7 sent, 5 returned= negative	2 sent, 2 results= negative
2018- 5 months	121 (5/2018) Post HH 7 possible ZIKV 5.78%	3 1 possible ZIKV 33.33%	4 sent=3 negative and one rejected due to autolysis of tissue	1 sent= negative

Factors to consider

- Placentas not all sent to pathology
- Patient dependent for exposure notification or asymptomatic, prenatal imaging unremarkable
- Prenatal care non-existent
- US does not test all pregnant mothers
- Autopsy number decline due to administrative constraints at our institution

Reasons for decline

- Advertisement: billboards, TV and radio messages
- Mosquito spray in neighborhoods
- Patient awareness and education-Prenatal care includes travel advisories and precautionary instructions
- Lastly…..

CDC's Current Stats 2015-2018

- US states=5716 symptomatic cases reported
- 5430 travel associated
- 231 presumed mosquito transmission
- 55 sexual/laboratory/person-person[9]

Laboratory Confirmed
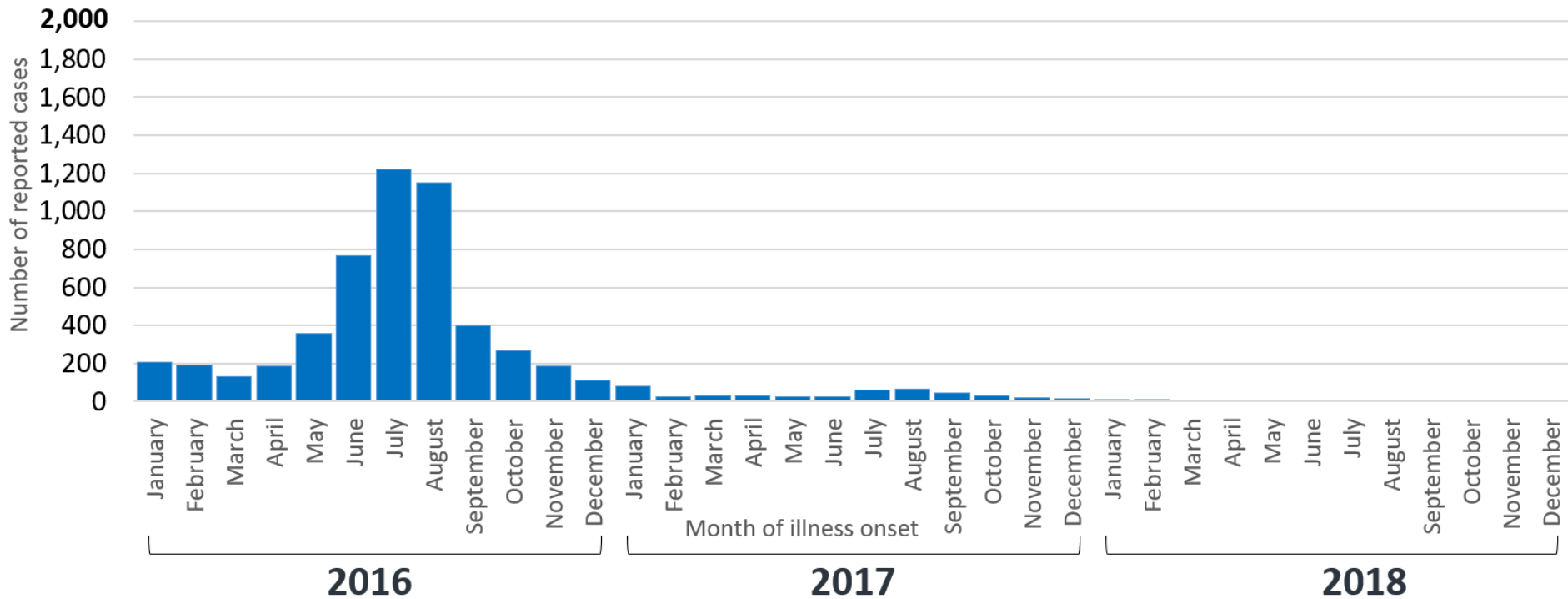
www.cdc.gov/zika/reporting/case-counts.html

Conclusion

- Our algorithms became indispensable learning devices for our trainees and will remain as a dynamic teaching tool . By generating a well-defined, customized, condensed ZIKV transmitted tissue protocol specific to our institution to include current emerging discoveries, gross inspectors and autopsy prosectors will remain updated and continue their vital role in continuity of care for those afflicted by ZIKV. Even though the disease population has decreased in Texas due to several possible factors, we can be prepared for future outbreak.

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- 9.https://www.cdc.gov/zika/reporting/case-counts.html
- 10.https://www.cdc.gov/zika/laboratories/test-specimens-tissues.html

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